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Variation among dengue virus-1 lineages affects the neutralization breadth of antibody responses elicited by infection and vaccination

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Abstract

Dengue virus (DENV) genetic diversity within serotypes may influence the breadth and durability of vaccine-induced immunity. We developed a panel of DENV-1 reporter virus particles encompassing ~ 74% of known global envelope protein diversity and evaluated neutralization using sera from tetravalent vaccine recipients, experimental DENV-1 challenge participants, and individuals with monotypic natural infection. Neutralization varied substantially among DENV-1 lineages and differed by exposure history. Several variants showed reduced neutralization following vaccination despite high overall antibody titers. Subsequent DENV-1 challenge broadened responses, which remained elevated through 180 days, whereas challenge of unvaccinated individuals produced lower potency and more lineage-specific decreases over time. Natural infection produced comparatively uniform neutralization across the panel. We used antigenic cartography to identify specific envelope amino acid positions associated with increased antigenic distance and reduced neutralization across vaccine and challenge sera. These findings demonstrate substantial within-serotype antigenic variation and support using diverse lineages to assess DENV vaccine-induced immunity.

Introduction

Dengue virus (DENV), a member of the Orthoflavivirus genus, is the causative agent of a significant arthropod-borne disease that infects 96–390 million individuals annually (1, 2). DENV is transmitted by *Aedes aegypti* (primary) and *Aedes albopictus* (secondary) vectors, which are predominantly found in tropical and subtropical regions (3, 4). Primary exposure to one of the four DENV serotypes has often been associated with asymptomatic or milder infections, including fever, rash, and muscle or joint pain, while sequential exposure to a heterologous serotype increases the risk of severe DENV, which is associated with plasma leakage, organ failure, shock, or even death (5–7). However, recent evidence suggests that severe DENV also occurs in a significant portion of primary infections (7, 8).

Long-term immunity to DENV infection is induced in large part by neutralizing antibodies that recognize viral surface proteins, particularly the envelope protein (9–13). The envelope protein contains three structural domains: DI is involved in viral stability, DII contains the fusion loop and is involved in maturation/assembly, and DIII is the main target of neutralizing antibodies and plays a role in host-cell receptor binding (14–17). Neutralizing antibodies targeting the envelope protein can limit subsequent infection by blocking viral attachment, entry, or fusion, making the envelope protein antigenic structure a key determinant of immune protection. However, DENV is genetically and antigenically diverse, with substantial variation both between serotypes and among viruses within the same serotype that circulate across different geographic regions and time periods (18–20). Prior exposure history strongly shapes neutralizing antibody responses, so viral diversity may influence susceptibility to reinfection, the introduction and expansion of new strains, and the dynamics of dengue outbreaks in previously infected populations. Understanding how DENV genetic diversity intersects with antibody-mediated recognition is therefore critical for defining durable immunity and predicting patterns of viral spread.

DENVs are separated into 4 serotypes, initially based on antigenic differences. Each serotype has historically been broken down into geographically distinct genotypes based on phylogenetic analysis. However, due to the need for finer phylogenetic resolution in the setting of extensive and overlapping geographic expansion of DENV lineages, a new classification scheme was recently defined (21). This scheme divides genotypes into major and minor lineages and uses a standardized nomenclature that includes the serotype (1–4), genotype (Roman numeral), major lineage (A-Z), and minor lineage (1–9) (21).

DENV-1 has contributed to recent outbreaks across multiple endemic regions and has undergone substantial geographic expansion beyond the regions historically associated with individual genotypes (22–25). Recent phylogeographic analyses have identified multiple DENV-1 lineages circulating in Africa, including relatively recent introductions from Asia associated with regional outbreaks (26). The continued movement and expansion of DENV-1 lineages increases the likelihood that populations will encounter genetically distinct viruses, yet the extent to which this diversity translates into antigenic variation remains poorly understood.

The increasing prevalence of DENV-1 and the high genetic diversity between DENV-1 lineages led us to question the extent to which DENV-1 genetic variability might influence antigenicity. A previous study examining antigenic variation among DENV-2 genotypes using monoclonal antibodies found that neutralization efficiency of viruses from different genotypes could differ by more than 20-fold (27). Additionally, studies evaluating neutralizing antibody responses to diverse DENV-2 genotypes reported significant differences in neutralization efficiency across viruses using sera from natural infection, experimental infection, and vaccination (28). Together, these studies challenge early assumptions that genotypes are insufficiently divergent to impact susceptibility to neutralizing antibodies. Rather, current data suggest that immunity from a single representative virus within a serotype may not confer lasting protection against all viruses in that serotype, which could significantly impact long-term vaccine efficacy. This idea is further supported by recent studies that report a high prevalence of homotypic reinfections in endemic areas (29).

DENV vaccines have been in development for over a decade, with Sanofi Pasteur's Dengvaxia being the first FDA- and WHO-approved vaccine. Notably, this vaccine is approved only for people with pre-existing immunity from natural infection due to the increased severity of primary infection in some cases (30). Dengvaxia used only one representative virus from each serotype with a yellow fever virus backbone and had variable vaccine efficacy (VE) and seroconversion (SC) for DENV-1 (VE: 50.3%, SC:33%), DENV-2 (VE:42.3%, SC:48%), DENV-3 (VE:74%, SC:80%), and DENV-4 (VE:77.4%, SC:85%) (31, 32). These results indicate that Dengvaxia performed poorly when tested against DENV-1 and DENV-2, and it is unknown whether genetic diversity and neutralizing antibody susceptibility vary among specific lineages. More recent vaccines still use a single strain as a representative to cover the full antigenic breadth of a serotype, while shifting toward more endogenous components in each new formulation (33–36). Notably, the NIH TV003 tetravalent vaccine, also containing one representative virus from each serotype, showed neutralization differences greater than 10-fold among diverse DENV-2 viruses (28). This

difference in efficacy could also be impacted by the choice of reference strains, which for most current vaccines were isolated prior to 1980 and do not reflect contemporary strains. These results directly challenge the assumption that primary exposure produces lifelong or similar levels of immunity to all strains within the same serotype (37).

In this study, we investigated the correlation between genetic diversity within DENV-1 and antigenic variation in humoral immune responses following natural infection and experimental infection. The experimental infections were performed in the context of a TDENV-PIV/LAV vaccination study. Like other vaccine formulations, TDENV-PIV/LAV (tetravalent DENV purified inactive vaccine and live-attenuated booster vaccine) contains one representative virus from each serotype along with all DENV endogenous structural and non-structural components (38–41). To evaluate the DENV-1 antigenic landscape, we generated a panel of recombinant DENV-1 reporter virus particles representing ~ 74% of global envelope protein diversity, each variant with its endogenous prM. Using this panel, we characterized the neutralization profiles of serum from naturally infected individuals as well as vaccinated and unvaccinated U.S. dengue-naïve individuals challenged with DENV-1 (strain 45AZ5). By defining how neutralizing antibody responses extend across genetically diverse DENV-1 lineages, this study provides a framework for evaluating within-serotype antigenic variation and its potential relevance to future DENV vaccine evaluation and design.

Methods

Ethics statement

All human biospecimens used in this study were collected from research participants who first provided informed consent. Samples from individuals with natural infection in Colombia were collected under two successive protocols (Comité Bioética de Universidad Tecnológica de Pereira, Acta No. 22 Punto 03, Numeral 02, del 28 de Noviembre de 2016; and Comité de Ética en Investigación, Institución Universitaria Visión de las Américas, Acta No 88 del 23 de Junio de 2021) and Emory IRB protocol#: 2024P007464. De-identified samples from a DENV-1 human challenge study (NCT04786457) performed at the University of Maryland were shared for secondary analysis. Vaccinated participants were primed and boosted with tetravalent vaccine under two sequential studies (NCT02239614 and NCT03141138) performed at the Walter Reed Army Institute of Research.

Sample set characteristics

Experimental human challenge samples: Longitudinal plasma samples were made available from a DENV-1 challenge study performed in a dengue non-endemic area of the United States, obtained at Day 0, 28, 90, and 180 following challenge with attenuated DENV-1 (strain 45AZ5, derived from WestPac-74). The virus was attenuated by passaging in FRhL-2 cells and mutagenized with 5-azacytidine (42). Participants in this trial were healthy, flavivirus-naïve adults (“Control group”) or healthy adults that

previously received a tetravalent heterologous prime-boost vaccination regimen with purified inactivated virus (PIV) prime followed by a live attenuated virus (LAV) boost (TDENV-PIV/LAV; “vaccine group”) (Supplemental Fig. 1). The DENV-1 challenge occurred 27–65 months after the LAV booster dose. All plasma were heat-inactivated at 56°C for 30 minutes before clarification by centrifugation.

Natural infection samples from Colombia: Serum samples were collected from patients in a prospective cohort, an acute febrile illness (AFI) study, in Risaralda between July 2019 and April 2022. In the AFI study, patients residing in the Central Western Metropolitan Area of Risaralda were recruited at the San Pedro y San Pablo Hospital if they presented with fever less than 7 days post symptom onset. A trained nurse was responsible for informed consent and assent, verifying the inclusion and exclusion criteria. Clinical assessment and classification were performed by the attending physician, and data were obtained from clinical records and a structured questionnaire.

A separate set of serum samples from patients in Valle del Cauca were obtained from August 2021 to December 2022 at a medical center located north of the city. These were residual clinical samples from patients diagnosed with dengue fever (DFC) by clinical laboratory testing.

Serum from both Colombian departments was screened using a Dengue Duo rapid test. Monotypic serum was selected using a focus reduction neutralization test at two dilutions (1:200 and 1:2000) across the four dengue serotypes. We selected sera reactive only to DENV-1, which comprised the natural infection cohort for further analysis in this study. The monotypic sera did not have detectable viremia, and we had no record of the first exposure time point.

DENV-1 phylogenetic analysis and strain selection

To explore the global DENV-1 genetic landscape, all DENV-1 full coding sequences (CDS) from 1956 to 2023 were downloaded from the National Center for Biotechnology Information (NCBI), and their genotypes were verified using Genome Detective v3.83 (43). We extracted the envelope amino acid sequence from each strain, removed duplicate sequences, and generated a multiple sequence alignment using the MAFFT v7.490 plugin in Geneious Prime 2026.0.1. This dataset was used to construct a maximum likelihood (ML) phylogenetic tree using IQ-TREE v1.6.12 (44) with the following parameters: 1500 ultrafast bootstraps and 1000 replicates for the SH-like approximate likelihood ratio test. The best-fit model for the CDS dataset was FLAVI + I+R3, as determined by the Bayesian Information Criterion using the ModelFinder software (45). The ML trees were rooted to the midpoint and visualized in iTOL (46). The exported trees were subjected to genetic partitioning using PARNAS (47) with the diversity option to isolate the best representatives across the envelope protein's global diversity. Specifically, the diversity option shows the number of sequences that are needed to cover a range of diversity across the tree. Branch lengths were used as distances, and no additional weighting or distance thresholds were applied. Representative envelope sequences were selected, along with their corresponding prM sequences, for downstream analysis.

Viruses and cell lines

Vero E6 and HEK293T cells were obtained from ATCC. All cell lines were regularly tested for mycoplasma contamination; cell lines were authenticated by visual observations of cell morphology. All cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Corning, cat # 15-013-CV), containing high glucose (4.5 g/L), 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, sodium pyruvate, and nonessential amino acids. All cells were incubated at 37°C in 5% CO_2.

Plasmids of reporter virus particles (RVPs) for DENV-2 (strain Tonga/74, GenBank: AY744147), DENV-1 (strain WestPac-74 derivative, GenBank: DVU88535.1), and pWNII-rep-GZ have been previously described and were obtained from the research groups of Drs. Stephen S. Whitehead and Theodore C. Pierson (48–50).

Cloning of C/prM/E expression plasmids

For the expression of the flavivirus structural proteins, we designed C/prM/E ORF sequences with flanking EcoRI and NotI restriction sites at the 5' and 3' ends, respectively. The sequences were codon-optimized for human expression and synthesized by Twist Biosciences (South San Francisco, CA). The ORFs were inserted into the pTwist + CMV expression plasmid. For the chimeric WNV-C plasmid constructs, the WNV-II strain 956 capsid (NCBI Reference Sequence: NC_001563.2) was used as described previously (48). The constructs have an extended Kozak sequence (5' GCCGCCACC 3') upstream of the capsid start site and six stop codons at the end of the envelope sequence.

DENV reporter virus particle production

HEK-293 T cells were co-transfected with the West Nile Virus (WNV)-replicon plasmid and the respective structural gene-expressing plasmids using PEI reagent (Promega, Madison, WI) (48, 49, 51, 52). The ratio of replicon plasmid to structural plasmid was 1:1.5; 1 µg replicon plasmid and 1.5 µg structural gene plasmid. 7.5 µL of PEI (1mg/mL) was used per 2.5 µg of DNA, which was incubated in OPTI-MEM. Transfected cells were incubated at 37°C in 5% CO2 for 4 hours, after which the medium was replaced with low-glucose DMEM supplemented with 2% FBS and 2 mM L-glutamine. After the initial medium change, the RVP-containing supernatant was harvested in 24-h intervals and replaced with fresh low-glucose DMEM (Corning) supplemented with 2% FBS and 2 mM L-glutamine. Supernatants containing RVPs were harvested, passed through 0.45 µm filters, aliquoted (supplemented with 10 mM HEPES), and stored at -80°C.

RVP titration

We determined the infectious units of RVP-containing supernatants by serially diluting them in low-glucose DMEM containing 2% FBS. 1.3×10^4 Vero E6 Progen cells plated in 96-well plates were infected with two-fold serially diluted RVPs and incubated for 24 h at 37°C, 5% CO_2. Cells were washed with DPBS, fixed with 4% paraformaldehyde, then washed three times with DPBS, and the infectivity of each serial dilution was determined by quantifying GFP-positive cells using a Cytation 7 cell imager (Agilent Technologies, Santa Clara, CA).

Neutralization assay

To measure RVP neutralization by monoclonal antibodies or human sera, Vero E6 cells were seeded at 10,000 cells per well in 96-well plates 24 h prior to neutralization assays. Serum samples were initially diluted 1:30 in low-glucose DMEM and serially diluted at 1:2 to a maximum serum dilution of 1:4480. RVPs were diluted to ~ 10^4 infectious units per mL, a concentration that maintains the equilibrium state of neutralization and would not violate mass action, creating a reaction that is not driven by the affinity of the antibody to the antigen (53). 50 µL of RVP was mixed with 50 µL of serially diluted antibody/sera. The antibody-virus mix was incubated at 37°C for 1 hour with slow agitation. Media on the Vero E6 cells was then replaced with 50 uL of antibody-virus mix and incubated for 24 hours at 37°C. Cells were subsequently fixed with 4% paraformaldehyde for 15 minutes, washed with DPBS, and stained with DAPI (5.7 mM) at a 1:2000 dilution. Cells were imaged on the Cytation 7 imager, and infection was determined by GFP expression using CellProfiler (54). Maximum relative infection was determined by measuring the number of infected cells in the absence of antibody. The NT50 was calculated using nonlinear regression analysis (GraphPad Prism 10, sigmoidal 4-parameter logistic model). To determine statistical differences in RVP neutralization susceptibility across specific cohorts, we performed Friedman’s test and Dunn's multiple comparisons test using the NT50 values for each prospective RVP:antibody pair, with an alpha of 0.05.

Antigenic cartography

Antigenic maps were generated using the Racmacs package v1.2.9 (55) in R v2026.01.0 (56). NT50 values for each RVP:antibody pair were used to populate the titer distance table. From the distance table, Racmacs uses multidimensional scaling and the Limited-memory Broyden–Fletcher–Goldfarb–Shanno (L-BFGS) gradient-based optimization algorithm to generate an antigenic map that minimizes the error between the predicted map and the titer distance table (57). To generate the maps, 1,000 optimizations and options (list(ignore_disconnected = TRUE)) were used. Titers below the limit of detection (NT50 < 50) were set to “50” in the titer table to minimize stress due to low-titer variability.

Data Availability

The code and data underlying antigenic cartography analyses are publicly available on https://github.com/Piantadosi-Lab/Variation-among-dengue-virus-1-lineages-affects-the-neutralization-breadth-.git

Results

Selecting a panel of DENV-1 variants that represent global genetic diversity

To identify variants that represent the global genetic diversity of the DENV-1 serotype, we performed a phylogenetic analysis of genetically distinct DENV-1 envelope protein sequences circulating from 1960 to 2022 (n = 624). This dataset contained representatives from all six DENV-1 genotypes: genotype I (n = 355), genotype II (n = 2), genotype III (n = 92), genotype IV(n = 37), genotype V (n = 129), and genotype VI (n = 10) (Supplemental Table 1). This dataset also encompassed all 26 major lineages (21). We used PARNAS (47) to select a subset of DENV-1 sequences that best represented the diversity within the full dataset. Our resulting panel of 20 sequences represented 20 of the 26 major lineages and covered ~ 74% of the known global DENV-1 envelope protein diversity (Fig. 1). Increasing coverage beyond 74% would require disproportionately more sequences; for example, 86% coverage would require an additional 60 sequences.

To evaluate the amino acid sequence variation in envelope and prM proteins among the 20 selected DENV-1 variants, we created a multiple-sequence alignment with WestPac-74 derivative strain as the reference. The envelope protein amino acid sequences in our panel differed by an average of 13 amino acids (range 5–27) from the reference (Fig. 2 **and Supplemental Fig. 2A**). Variants 1I_H and 1I_K were the most divergent, with 27 and 26 amino acid substitutions, respectively. Substitutions were found in all three envelope protein domains: DI (n = 13), DII (n = 31), and DIII (n = 26). There were 12 amino acid positions with 5 or more substitutions across our panel, and 7 of these positions (a.a. 88, 202, 203, 297, 324, 339, and 380) carried substitutions across at least 6 of the sequences. Most of these sites were on the envelope surface and aligned with known regions of high sequence entropy (**Supplemental Fig. 2B**).

The prM sequences differed on average by 4 amino acids (range 1–10) from the reference (**Supplemental Fig. 2C**). Variants 1I_J and 1I_B were the most divergent, with 10 and 6 amino acid substitutions in prM, respectively.

Generation and validation of the DENV-1 variant reporter virus particle (RVP) panel

We next generated a panel of reporter virus particles (RVPs) using a previously described system (48, 49). Each RVP contained the full envelope and prM sequence of the representative DENV-1 variant, with the WNV-II capsid sequence (strain 956; NCBI reference NC_001563) that was previously shown to increase RVP production efficiency (48). Structural proteins were complemented with a WNV-II replicon (strain 956), including a GFP reporter to evaluate infection (**Supplemental Fig. 3A)** (48, 49). Before using RVPs for neutralization assays, each RVP was titered, and those with titers lower than ~ 10^3 infectious units per mL were concentrated by filter centrifugation and re-titered to equalize input titers across RVPs (**Supplemental Fig. 3B**). Notably, for three variants (MT397277 [1I_J], KJ755855 [1I_H], and MN933667

[1I_K]), we did not observe detectable RVP titers. Sequences from these three variants had the largest number of nonsynonymous mutations (range 14–27) in the envelope and prM proteins compared to the WestPac-74 derivative reference strain but did not contain premature stop codons (Fig. 2). These three sequences exhibited long branch lengths (at least 0.01 substitutions per site) in our phylogenetic tree (Fig. 1). Together, these findings suggest that extensive within-host mutations, sequencing artifacts, or sequence-specific incompatibilities with the RVP production system resulted in nonfunctional RVPs. Therefore, we modified the original phylogenetic tree by excluding all sequences with a branch length of 0.01 or higher (**Supplemental Fig. 3C**). Based on the new phylogenetic analysis, our remaining 17 variants captured 69% of the total amino acid diversity in the DENV-1 envelope tree.

We next assessed whether each RVP expressed antigens properly for use in a neutralization assay by measuring neutralization by the pan-flavivirus monoclonal antibody (mAb) EDE C10. This mAb successfully neutralized all DENV-1 RVPs with low NT50 values corresponding to concentrations of 2–11 ng/mL, which is consistent with established values for live DENV-1 neutralization by focus-reduction neutralization tests (~ 15 ng/mL) (**Supplemental Fig. 3D)** (58). These results suggested that our RVP panel was suitable for investigating neutralization differences across DENV-1 variants.

DENV-1 lineage-specific neutralization after experimental human challenge infection

To evaluate the potency and breadth of antibody responses to DENV-1 generated in controlled human challenge experiments in unvaccinated and vaccinated individuals, we evaluated neutralization of our DENV-1 RVP panel using sera from the TDENV-PIV/LAV vaccine trial (Fig. 3) (38).

We first evaluated the control cohort, in which unvaccinated, Orthoflavivirus-naïve individuals were challenged with an attenuated DENV-1 strain 45AZ5 (major lineage 1IV_B.1) derived from the WestPac-74 (reference strain), and sera were collected pre-challenge on day 0 and post-challenge on days 28, 90, and 180, as previously described (40). In this control-challenged cohort, the peak RNA levels ranged from 6.07×10^6 to 4.76×10^7 genome equivalents (GE) per mL across participants. We tested the sera against our panel of 17 DENV-1 variants, as well as DENV-2 and WNV controls (Fig. 3A **and Supplemental Fig. 4**). From these neutralization profiles, we evaluated (1) potency, represented by the NT50, which is the dilution of serum needed to neutralize 50 percent of the virus, (2) breadth – the ability of a serum sample to neutralize genetically distinct viruses, and (3) the degree to which a particular RVP was neutralized across sera from multiple individuals.

The average potency (NT50) of serum from this cohort against the DENV-1 RVP panel varied by day post-challenge: pre-challenge = 55 (NT50 range 50–142), day 28 = 389 (range 50-1513), day 90 = 211 (range 50–552), and day 180 = 168 (range 50–639) **(Supplemental Fig. 5A**). Importantly, NT50 titers were comparable with the original study (40). Consistent with previous studies evaluating anti-DENV humoral responses, sera collected at the early time point after challenge (day 28) had the highest potency across all RVPs (59, 60), and the median neutralization potency waned by day 180. Notably, sera from one participant (P8) had a substantially higher potency than the other three.

To determine breadth, we applied a heuristic NT50 threshold of 100, representing a two-fold increase over the baseline signal, and counted the number of RVPs that were neutralized at this threshold by each serum sample (Fig. 3, **red line**). For the following participant breadth results, we focused on day 180, the latest time point available, which would be most representative of the long-term response. Participant 5 had the lowest breadth, with NT50 values for 10 of 17 RVPs falling below the threshold (**Supplemental Fig. 5A**). Participants 1 and 7 had NT50 values below the threshold for 7 and 8 of the 17 RVPs, respectively. By contrast, participant 8 showed the highest breadth, with all NT50 values above 100.

We next evaluated differences in neutralization for individual RVPs within our panel, when measured across all serum samples. Not surprisingly, DENV-1 lineage 1IV_B.1 [WestPac-74 derivative] was typically the most potently neutralized (Fig. 3A, circled dots), as this was the challenge virus. Although the neutralization profiles were not entirely consistent across participants, we did identify three RVPs that were below the NT50 = 100 threshold in 3 out of 4 participants (1III_A, 1V_E, and 1V_G) (**Supplemental Fig. 5A)**, suggesting that they may have important antigenic differences compared to the challenge virus.

To further explore antigenic variation between RVPs, we created an antigenic map based on NT50 values for each RVP tested against sera from each participant (Fig. 4A **and Supplemental Fig. 5B and 5E**). Our analysis again primarily focused on Day 180 as the representative of a long-term response. At Day 180, the antigenic map centered around the challenge virus (lineage 1IV_B), while control RVPs DENV-2 and WNV were most distant. To quantify relative antigenic distances, we used this map to measure the distance in antigenic units (AU) between each RVP and each patient serum, then calculated the average value for each RVP (Fig. 4A-C **and Supplemental Fig. 5B-D and 5E-G**). These analyses showed that the majority of DENV-1 RVPs fell within 2 AU from the sera, while a subset mapped to more than 2 AU from the sera. Consistent with our NT50 data showing waning neutralization over time (Fig. 3A), the number of RVPs that mapped beyond 2 AU increased between day 28 and day 180 (Fig. 4C **(day 180) and Supplemental Fig. 5D (day 28), 5G (day 90)**). Notably, major lineage 1V_G was the most antigenically distant strain at both 90 and 180 days post-challenge, but not day 28, suggesting antigenic divergence may increase for specific variants as the immune response matures. Overall, these results demonstrate that substantial antigenic heterogeneity exists within DENV-1 and highlight lineage-dependent variation in the neutralization profiles of antibodies elicited by DENV-1 challenge.

Comparing DENV-1 antibody responses between vaccination and attenuated human challenge

Current tetravalent DENV vaccines include a single representative strain from each serotype, raising the question of how broadly vaccine-elicited antibodies neutralize genetically diverse DENV-1 lineages. Thus, we asked what the DENV-1 neutralizing response is in individuals vaccinated with a tetravalent dengue vaccine and whether this response would be broader and stronger following DENV-1 challenge in vaccinated individuals compared to unvaccinated, Orthoflavivirus-naïve individuals. TDENV-PIV, a tetravalent DENV purified inactivated vaccine, was administered to Orthoflavivirus-naïve individuals,

followed by the live attenuated tetravalent vaccine booster 28–180 days later (**Supplemental Fig. 1**) (40). For the DENV-1 component, both the inactivated vaccine and booster contained strain 45AZ5, major lineage 1IV_B.1. Participants were challenged with DENV-1 27–65 months after the LAV booster. Serum samples were collected on day 0 (pre-challenge) and post-challenge at days 28, 90, and 180 and tested against each RVP (**Supplemental Fig. 6**).

The average potency (NT50) of serum from the vaccinated cohort against the DENV-1 RVP panel prior to challenge was 403 (NT50 range 50–4480). After DENV-1 challenge, potencies increased substantially: day 28 = 3057 (range 72-4480), day 90 = 1790 (range 50–4480), and day 180 = 760 (range 50-4480) (Fig. 3B **and Supplemental Fig. 7A**). Consistent with typical antibody response kinetics and our previous findings in the unvaccinated challenged cohort, we found that sera collected on day 28 had the highest potency across all RVPs, and there was a reduction in potency by day 180. Notably, serum from one participant (P16) showed substantially lower potency than the other four at all post-challenge collection time points.

Breadth analysis using an NT50 cutoff of 100 showed variability in the sera from vaccinated individuals collected before challenge infection (day 0, which was 27–65 months after vaccination), with between 2 and 8 RVPs per participant falling below the threshold (**Supplemental Fig. 7A**). P16 had the lowest pre-challenge breadth, with 8 of 17 RVPs below the threshold. While most participants had uniformly high breadth post-challenge, P16 had lower breadth, with 5 of 17 NT50 values falling below the threshold (Fig. 3B, **red line**). Clinically, P16 exhibited no viremia or signs/symptoms indicative of acute infection post-challenge; further, they had no detectable changes in microneutralization titers. However, an increase in pre-challenge IFN-γ levels was observed, suggesting that P16 may have had altered baseline immunity that impacted vaccine-elicited immunity and challenge infection, leading to drastic reductions in NAb titers compared to the other vaccinated participants after exposure to the challenge (40). P16 highlights the variability in vaccine-induced immunity across individuals.

Examining differences in neutralization for individual RVPs within our panel across all serum samples from the vaccinated-challenged cohort, we found that 1IV_B.1 [WestPac-74 derivative] was again among the most susceptible to neutralization both before and after DENV-1 challenge (Fig. 4B, circled dots). Testing sera collected before challenge at Day 0, which represents only vaccine-induced neutralizing antibodies, revealed 4 RVPs that were below NT50 = 100 in at least 2 participant sera, and two RVPs (1I_B and 1I_E.1) that were below the threshold in 3 or more sera (Fig. 3B **and Supplemental Fig. 7A**). Notably, 1I_E.1 and 1I_B were below the threshold in at least 2 participants for both the vaccinated cohort at day 0 and the unvaccinated/challenge cohort, while 1V_G and 1V_E were only below the threshold among 3 participants in the unvaccinated/challenge cohort at day 180 (**Supplemental Fig. 5A).** At day 180 post-challenge, within vaccinated individuals exposed to experimental challenge, we identified five RVPs that were below the NT50 = 100 threshold; however, these were all in P16. Among the other four participants, NT50 values for all RVPs remained above 100 at all post-challenge timepoints tested (days 28, 90, and 180), though we still observed a general decrease in neutralization over time. This waning is less than the decrease observed for the unvaccinated/challenge cohort

because the vaccinated participants had a primary and secondary immune response. These results suggest that TDENV-PIV/LAV vaccination leads to variable responses to DENV-1 lineages, including less potent neutralizing immunity to certain lineages, particularly from genotype I. Additionally, challenge infection after vaccination significantly increases neutralization potency and breadth after vaccination.

To further explore the DENV-1 antigenic differences in the vaccinated/challenged cohort, we generated antigenic maps focusing on two time points: pre-challenge, capturing the long-term reactivity of the vaccine-induced response, and again on Day 180 post-challenge to parallel analysis from the unvaccinated/challenged cohort (Fig. 4D, 4G). At the pre-challenge time point, the antigenic map showed much greater dispersion in antigenic distances than the Day 180 profile from the unvaccinated/challenged group (Fig. 4D **compared to 4A**). The antigenic distances between RVPs and sera were substantially larger, with only a small group of RVPs below 2 AU and many around 4.5 AU (Fig. 4E). Notably, two major lineages of genotype I, 1I_E.1 and 1I_B, were the most antigenically distant, each with average antigenic distances similar to the WNV control (Fig. 4F). Unsurprisingly, DENV-2, which is a component of the vaccine, mapped closer to the sera. Together, these results indicate there is substantial variability in the vaccine-induced antibody responses between DENV-1 lineages.

For the vaccinated/challenged cohort at Day 180 after challenge, antigenic distances were lower than pre-challenge, but higher than the antigenic distances in the unvaccinated/challenged cohort at Day 180 (Fig. 4G **compared to 4A**). Most RVPs had an average antigenic distance above 2 AU, with several showing average distances greater than WNV, except for day 28 (**Supplemental Fig. 7B-D**). Notably, 1I_E.1 and 1I_B had the greatest AU values among all RVPs at day 180, similar to the pre-challenge results (Fig. 4H, 4I); this was also true at day 90 (**Supplemental Fig. 7E-G**). Together, these results suggest that TDENV-PIV/LAV leads to varying responses across DENV-1 lineages, inducing less potent neutralizing antibodies to certain lineages, particularly from genotype I. Additionally, after experimental challenge infection, while average potency significantly increases, the antigenic distance remains high compared to unvaccinated participants who only received the challenge virus.

Comparing DENV-1 antibody responses between vaccination, attenuated human challenge and natural infection

Previous studies have suggested that antibodies elicited by natural infection have broader reactivity than those in sera from vaccinated populations (28, 61). To better assess how antibody responses elicited by the DENV-1 attenuated challenge strain compare to those generated by natural infection, we evaluated sera from individuals naturally exposed to circulating DENV in an endemic setting. Natural infection sera were collected from two Colombian departments, Valle del Cauca (rural and urban regions) and Risaralda (a western metropolitan area), from individuals with clinically suspected DENV infection based on physician diagnosis and symptom assessment. At the time of collection, DENV-1 was the primary circulating serotype in Colombia (62). From this cohort (392 samples), we identified nine monotypic sera

that were reactive only to DENV-1 and did not show evidence of active DENV infection, for testing against our RVP panel. We also included an Orthoflavivirus-naïve participant as a control (P133).

The average potency of sera from this cohort against the DENV-1 RVP panel was 300 (range: 77-1770) (Fig. 5A **and Supplemental Fig. 8**). Most sera showed high breadth, with sera from only two participants having NAb titers below the NT50 = 100 threshold (one RVP for P151 and five RVPs for P152). Compared to the unvaccinated/challenge cohort at 180 days post-challenge, average potency and breadth were slightly higher for sera from natural infection (Fig. 5E). However, potency and breadth after challenge in the vaccine/challenge cohort were significantly higher than the other two cohorts (Wilcoxon rank-sum tests, BH-adjusted, $p < 2x10^{-16}$).

Antigenic mapping of sera and RVPs from natural infection showed a general arrangement of RVPs that paralleled the unvaccinated/challenged cohort (Fig. 5B). However, the antigenic distances were lower and less heterogeneous (Fig. 5C-D). Notably, the majority of DENV-1 RVPs had density distributions under the 2 AU distance threshold, except for 1I_B and 1III_B, which were slightly above 2 AU. 1I_B also had high antigenic distances in the vaccinated/challenged cohort. Together, these data indicate that sera from natural infection may have broader responses and slightly higher potency against our DENV-1 RVP panel compared to unvaccinated/challenged individuals.

Amino acid differences in high-antigenic-distance RVPs across vaccinated and unvaccinated cohorts.

We next sought to identify the amino acid substitutions present in the RVPs with high antigenic distance. Because no threshold exists for defining meaningful antigenic distances in DENV, we applied conservative heuristic cutoffs (2 AU and then a more stringent 3 AU) to identify RVPs with high antigenic divergence from the vaccine/challenge virus. In the unvaccinated/challenge cohort at day 180, three RVPs (1IV_C, 1III_A, and 1V_E) had an average antigenic distance of greater than 2 AU, and each RVP had a separate, single lineage-specific amino acid substitution compared to the sequence of the challenge virus (Fig. 6A). In the vaccinated/challenged cohort at day 0, 10 DENV-1 RVPs had average AU values over 2, and only one (1I_E.1) had an average AU over 3 (Fig. 6B); this RVP (1I_E.1) had two unique amino acid substitutions, in domain I (N52D) and III (V312L). Similarly, in the vaccinated/challenged cohort at day 180 post-challenge, four RVPs had an average AU of greater than 2, and 1I_E.1 again had an average distance of greater than 3 AU (Fig. 6C). Notably, we did not find evidence for shared amino acid substitutions across RVPs with high antigenic distance. We also did not find much evidence for RVPs with consistently high antigenic distance across cohorts, except for lineage 1I_B, which had high antigenic distance in both the vaccinated/challenge and the natural infection cohort (Figs. 4, 5 & 7). These results demonstrate that the context of exposure to the same antigen, in this case DENV-1 45AZ5 presented alone (through experimental infection) or alongside DENV-2/3/4 antigens (through tetravalent vaccination) impacts the resulting DENV-1-reactive antibody profile. These neutralizing antibody profile differences, as demonstrated here, resulted in differential recognition of other DENV-1 strain antigens. Which DENV-1 strain amino acid substitutions contribute to this neutralization-based antigenic diversity, and whether they are clinically meaningful, remain to be uncovered.

Discussion

Developing an effective tetravalent DENV vaccine remains a major challenge for controlling dengue outbreaks and disease. Recent vaccine development has focused primarily on achieving balanced immune responses across the four serotypes via live-attenuated tetravalent vaccine formulations, and the breadth of protection against genetically diverse lineages within each serotype has received less attention. Here, we investigated antigenic diversity within DENV-1, a serotype that has shown increased prevalence in recent years (25, 63–65). We generated a panel of RVPs representing much of the known global amino acid sequence diversity of DENV-1 and evaluated their neutralization by sera collected following monotypic natural infection, tetravalent vaccination, and experimental DENV-1 challenge in vaccinated and unvaccinated individuals. We observed substantial variation in susceptibility to neutralization among DENV-1 lineages, indicating that some variants are less susceptible to humoral immunity elicited by prior infection or vaccination. We also identified amino acid substitutions associated with reduced neutralization by sera from vaccine recipients and DENV-1 human challenge participants, highlighting residues that could contribute to immune escape. Together, these findings provide a detailed map of antigenic diversity within DENV-1 and underscore the importance of accounting for within-serotype variation when evaluating the breadth and durability of vaccine-induced immunity.

The primary goal of this study was to determine how well antibody responses elicited by defined DENV exposures neutralize the broader genetic diversity within DENV-1. Previous studies evaluating neutralization following vaccination or natural infection have typically examined only a limited number of genotypes within each serotype and have often included the same reference strain used for vaccination or experimental challenge (66–68). In our study, neutralization potency was generally high against the reference strain but was markedly reduced against several other lineages in the panel. Thus, reliance on a limited number of reference strains may overestimate the breadth of neutralization within a serotype.

To expand the scope of this analysis, we included 17 DENV-1 lineages representing approximately 69% of the known global envelope protein diversity. Among currently available unique envelope protein sequences, genotype I was the most highly represented, followed by genotype V; together, these genotypes accounted for 78% of unique sequences in our dataset (Fig. 1). Genotype I has primarily been isolated from the Asia-Pacific region and was also recently detected in East Africa (69). Its geographic expansion and persistence, including during periods of co-circulation with other genotypes, may reflect increased transmission fitness arising from advantages in viral replication or entry (69). Genotype V is most prevalent in South America and has been associated with more severe clinical outcomes (69). However, the apparent distribution and relative abundance of these genotypes are also influenced by regional differences in genomic surveillance. Therefore, although our analysis identified DENV-1 lineages representing the currently known global diversity of DENV-1, the panel may not capture the full extent of DENV-1 diversity circulating globally.

We observed that genetic relatedness among DENV-1 lineages did not correspond to antigenic similarity. Closely related RVPs did not cluster together in antigenic maps generated using sera from monotypic natural infection or experimental challenge, indicating that a limited number of amino acid differences may substantially alter antibody recognition. Conversely, more genetically divergent representatives were not always antigenically distinct. Although genotype I lineages were among the most genetically distant from the vaccine lineage, several genotype I RVPs were efficiently neutralized and showed low antigenic distances from sera collected following vaccination or experimental challenge. In contrast, lineage 1IV_C, which is genetically more closely related to the vaccine and challenge lineage, consistently mapped farther from participant sera. These findings suggest that antigenic phenotype is determined less by the total number of amino acid differences between lineages than by the identity and structural context of specific substitutions. Changes at a small number of antibody-accessible or structurally important residues may have a disproportionate effect on neutralization, whereas broader sequence divergence outside dominant antibody epitopes may have little antigenic consequence. Consequently, genetic surveillance alone may not adequately identify emerging DENV-1 lineages with altered susceptibility to existing humoral immunity, emphasizing the need to integrate functional neutralization analyses into assessments of DENV-1 diversity.

Using antigenic cartography, we identified amino acid substitutions present in DENV-1 lineages with large antigenic distances from the exposure strain. In analyses of vaccinated participants, two lineages (1I_E.1, containing substitutions 52D and 312L, and 1I_B, containing substitutions 225T and 351V) showed high antigenic distances from the exposure strain. These four positions therefore represent candidate determinants of altered antibody recognition. In addition to individual candidate amino acid sites, the impact on neutralization could depend on interactions with other residues, changes in envelope protein quaternary structure, or substitutions in prM. Further mutagenesis and epitope mapping studies will be needed to establish causality and determine the structural mechanisms underlying the observed phenotypes. Nevertheless, these results provide a focused set of candidate residues and lineages for future investigation using reciprocal mutagenesis and epitope mapping to help identify specific regions of the envelope protein that contribute to DENV-1 antigenic variation.

Our analyses indicated that the antigenic composition and context of the initial DENV exposure influenced both the magnitude and breadth of DENV-1 neutralizing antibody responses. Before challenge, sera from TDENV-PIV/LAV vaccine recipients exhibited a range of pre-existing NAbs to DENV-1 variants. Following challenge, average NAb levels were higher compared to the unvaccinated group; however, there was more variation, with the range of NAb titers across variants being much greater in the vaccinated group relative to the unvaccinated/challenged group. These patterns suggest that neutralization following DENV-1 challenge of previously vaccinated individuals contains a substantial contribution from serotype cross-reactive NAb, whereas the response following primary DENV-1 challenge appears to be dominated by DENV-1 serotype-specific NAbs. This is supported by the data from the DENV-2 and WNV RVP controls and is consistent with an element of antigenic imprinting. In the unvaccinated/challenge cohort, DENV-2 and WNV were consistently below the lower limit of quantification or minimally neutralized. However, DENV-2 NAb titers were substantially increased

following DENV-1 challenge of previously vaccinated participants – NAb titers for DENV-2 were even greater than titers for the DENV-1 challenge strain across most time points for most participants, and WNV was modestly neutralized in most samples. These findings are consistent with recall of memory B-cell responses that are cross-reactive to multiple DENV serotypes. Our data indicate that the epitopes involved in cross-reactivity at the DENV serotype level may be distinct from those mediating cross-reactivity at the lineage level within a serotype, which is consistent with previous studies of DENV-2 showing that monovalent and tetravalent vaccination produced distinct neutralization profiles against genetically diverse DENV-2 strains (28).

Our data also suggest that the dynamics of cross-reactive immune responses may change over time. Even with as little as a five-month interval from Day 28 to Day 180, the antigenic map of the vaccinated group indicated ongoing shifts in which specificities are driving neutralization, as DENV-2 relocated from a central to peripheral position on the antigenic map. In contrast to the Day 28 map, the day 180 map of the vaccinated group more closely resembled the maps following DENV-1 monotypic infection or challenge of flavivirus-naïve individuals. These findings suggest that the most recent flavivirus exposure can disproportionately shape the circulating NAb landscape, and that this landscape continues to evolve after exposure. This may be particularly relevant in endemic regions, where sequential infections repeatedly reshape pre-existing immunity. For vaccination, there are likely relevant differences in protective immunity mediated by cross-reactive versus serotype-specific NAb. Vaccines that are dominated by one serotype component that elicits abundant cross-reactive NAb may exhibit limited breadth in protective immunity against diverse lineages of heterologous serotypes.

Several important observations and caveats emerged during the development of our DENV-1 RVP panel. Our initial PARNAS analysis identified 20 representative DENV-1 lineages; however, RVPs corresponding to three isolates (MN933667, KJ755855, and MT397277) could not be produced at viable titers. These isolates were among the most divergent sequences in our panel, forming long branches in the phylogenetic tree, which could reflect the presence of deleterious mutations, sequencing errors, or sequence-specific incompatibilities with the RVP production system. Although our panel was designed primarily to capture diversity in the envelope protein, each RVP also contained the corresponding prM sequence from the same isolate to support proper protein folding and virion assembly. Antibodies recognizing prM also influence neutralization activity (70, 71). Although our analyses did not identify individual prM substitutions associated with altered neutralization, combinations of substitutions in prM and envelope could contribute to some of the phenotypes observed in our panel. Although our serum cohorts are generally well characterized, the timing and infecting DENV-1 lineage are unknown for individuals in the natural-infection cohort. Finally, the PARNAS-based approach for determining representative lineages is based on publicly available sequences; however, the true diversity and distribution of DENV-1 lineages globally may not be well reported due to regional differences in genomic surveillance.

DENV epidemiology and vaccine design have traditionally been guided by the principle that infection with one serotype provides durable protection against subsequent infection by the same serotype. Although

our study does not directly measure protection from reinfection, we found substantial lineage-dependent variation in neutralization following natural infection, experimental challenge, and tetravalent vaccination, indicating that measurements against a single reference strain may not fully capture the breadth of immunity within a serotype. These findings support evaluating vaccine responses against genetically and antigenically diverse lineages. The comparatively resistant variants and candidate amino acid substitutions identified here may also provide useful benchmarks for assessing the breadth of future DENV vaccines and guiding immunogen design.

Growing epidemiologic evidence further indicates that homotypic DENV reinfection occurs and may contribute to viral transmission and evolution. A recent study in Southeast Asia estimated that, in highly endemic settings, approximately 60% of individuals may experience homotypic reinfection by 40 years of age (29), challenging a strict model of lifelong homotypic immunity. Consistent with concerns about durability, we observed significant declines in neutralization between days 28 and 180 in both vaccinated and unvaccinated challenge cohorts. Continued waning could disproportionately reduce neutralization against less susceptible DENV variants, emphasizing the importance of longitudinally evaluating both the magnitude and lineage-level breadth of vaccine-induced immunity. Future studies will be needed to determine whether the antigenic differences identified here are associated with breakthrough infection or homotypic reinfection and whether similar patterns extend to other DENV serotypes.

Declarations

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Data Availability

The code and data underlying antigenic cartography analyses are publicly available on https://github.com/Piantadosi-Lab/Variation-among-dengue-virus-1-lineages-affects-the-neutralization-breadth-.git

References

1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504–7.
2. Yang X, Quam MB, Zhang T, Sang S. Global burden for dengue and the evolving pattern in the past 30 years. Journal of travel medicine. 2021;28(8):taab146.
3. Nakase T, Giovanetti M, Obolski U, Lourenço J. Global transmission suitability maps for dengue virus transmitted by Aedes aegypti from 1981 to 2019. Scientific Data. 2023;10(1):275.
4. Lambrechts L, Scott TW, Gubler DJ. Consequences of the expanding global distribution of Aedes albopictus for dengue virus transmission. PLoS neglected tropical diseases. 2010;4(5):e646.

5. Nishiura H, Halstead SB. Natural history of dengue virus (DENV)—1 and DENV—4 infections: reanalysis of classic studies. The Journal of infectious diseases. 2007;195(7):1007–13.
6. Schilling S, Ludolfs D, Schmitz H. Laboratory diagnosis of primary and secondary dengue infection. Journal of Clinical Virology. 2004;31(3):179–84.
7. Soo K-M, Khalid B, Ching S-M, Chee H-Y. Meta-analysis of dengue severity during infection by different dengue virus serotypes in primary and secondary infections. PloS one. 2016;11(5):e0154760.
8. Aggarwal C, Ahmed H, Sharma P, Reddy ES, Nayak K, Singla M, et al. Severe disease during both primary and secondary dengue virus infections in pediatric populations. Nat Med. 2024;30(3):670–4.
9. Valdés K, Alvarez M, Pupo M, Vázquez S, Rodríguez R, Guzmán MaG. Human dengue antibodies against structural and nonstructural proteins. Clinical Diagnostic Laboratory Immunology. 2000;7(5):856–7.
10. Churdboonchart V, Bhamarapravati N, Peampramprecha S, Sirinavin S. Antibodies against dengue viral proteins in primary and secondary dengue hemorrhagic fever. The American journal of tropical medicine and hygiene. 1991;44(5):481–93.
11. AbuBakar S, Azmi A, Mohd-Saad N, Shafee N, Chee H. Antibody responses of dengue fever patients to dengue 2 (New Guinea C strain) viral proteins. Malaysian Journal of Pathology. 1997;19:41–52.
12. Abubakar S, Azila A, Suzana M, Chang L-Y. Antigenic cell associated dengue 2 virus proteins detected in vitro using dengue fever patients sera. The Malaysian journal of pathology. 2002;24(1):29–36.
13. Lazaro-Olan L, Mellado-Sanchez G, Garcia-Cordero J, Escobar-Gutierrez A, Santos-Argumedo L, Gutierrez-Castaneda B, et al. Analysis of antibody response in human dengue patients from the Mexican coast using recombinant antigens. Vector-Borne and Zoonotic Diseases. 2008;8(1):69–80.
14. Zhang Y, Zhang W, Ogata S, Clements D, Strauss JH, Baker TS, et al. Conformational changes of the flavivirus E glycoprotein. Structure. 2004;12(9):1607–18.
15. Pitcher TJ, Sarathy VV, Matsui K, Gromowski GD, Huang CY-H, Barrett AD. Functional analysis of dengue virus (DENV) type 2 envelope protein domain 3 type-specific and DENV complex-reactive critical epitope residues. Journal of General Virology. 2015;96(2):288–93.
16. Christian EA, Kahle KM, Mattia K, Puffer BA, Pfaff JM, Miller A, et al. Atomic-level functional model of dengue virus Envelope protein infectivity. Proceedings of the National Academy of Sciences. 2013;110(46):18662-7.
17. Lin S-R, Zou G, Hsieh S-C, Qing M, Tsai W-Y, Shi P-Y, et al. The helical domains of the stem region of dengue virus envelope protein are involved in both virus assembly and entry. Journal of virology. 2011;85(10):5159–71.
18. Phadungsombat J, Nakayama EE, Shioda T. Unraveling dengue virus diversity in Asia: an epidemiological study through genetic sequences and phylogenetic analysis. Viruses. 2024;16(7):1046.

19. Bell SM, Katzelnick L, Bedford T. Dengue genetic divergence generates within-serotype antigenic variation, but serotypes dominate evolutionary dynamics. Elife. 2019;8:e42496.
20. Katzelnick LC, Fonville JM, Gromowski GD, Arriaga JB, Green A, James SL, et al. Dengue viruses cluster antigenically but not as discrete serotypes. Science. 2015;349(6254):1338–43.
21. Hill V, Cleemput S, Pereira JS, Gifford RJ, Fonseca V, Tegally H, et al. A new lineage nomenclature to aid genomic surveillance of dengue virus. PLoS biology. 2024;22(9):e3002834.
22. Teoh B-T, Sam S-S, Tan K-K, Johari J, Shu M-H, Danlami MB, et al. Dengue virus type 1 clade replacement in recurring homotypic outbreaks. BMC evolutionary biology. 2013;13(1):213.
23. Barde P, Kori B, Shukla M, Bharti P, Chand G, Kumar G, et al. Maiden outbreaks of dengue virus 1 genotype III in rural central India. Epidemiology & Infection. 2015;143(2):412–8.
24. Pratt D, Larbi YA, Ofori M, Agbodzi B, Wiley J, Mante AO, et al. Outbreak of dengue fever in Ghana: The emergence of DENV-1 serotype. PLOS Neglected Tropical Diseases. 2026;20(5):e0014248.
25. Gularte JS, Sacchetto L, Demoliner M, Girardi V, da Silva MS, Filippi M, et al. DENV-1 genotype V linked to the 2022 dengue epidemic in Southern Brazil. Journal of Clinical Virology. 2023;168:105599.
26. Nyathi S, Rezende IM, Walter KS, Thongsripong P, Mutuku F, Ndenga B, et al. Geographic origin and evolution of dengue virus serotypes 1 and 3 circulating in Africa. Virus Evol. 2025;11(1):veae116.
27. Shrestha B, Brien JD, Sukupolvi-Petty S, Austin SK, Edeling MA, Kim T, et al. The development of therapeutic antibodies that neutralize homologous and heterologous genotypes of dengue virus type 1. PLoS pathogens. 2010;6(4):e1000823.
28. Martinez DR, Yount B, Nivarthi U, Munt JE, Delacruz MJ, Whitehead SS, et al. Antigenic variation of the dengue virus 2 genotypes impacts the neutralization activity of human antibodies in vaccinees. Cell reports. 2020;33(1).
29. Andrade J, de Girardier AM, Huang AT, Buddhari D, Hamins-Puertolas M, Alera M-T, et al. Long-term antibody dynamics challenge the paradigm of lifelong homotypic immunity to dengue virus. Proceedings of the National Academy of Sciences of the United States of America. 2026;123(22):e2606206123.
30. Hadinegoro SR, Arredondo-García JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. New England Journal of Medicine. 2015;373(13):1195–206.
31. Villar L, Dayan GH, Arredondo-García JL, Rivera DM, Cunha R, Deseda C, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. New England Journal of Medicine. 2015;372(2):113–23.
32. Sabchareon A, Wallace D, Sirivichayakul C, Limkittikul K, Chanthavanich P, Suvannadabba S, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. The Lancet. 2012;380(9853):1559–67.
33. Rivera L, Biswal S, Sáez-Llorens X, Reynales H, López-Medina E, Borja-Tabora C, et al. Three-year efficacy and safety of Takeda's dengue vaccine candidate (TAK-003). Clinical Infectious Diseases.

2022;75(1):107–17.

34. Thomas SJ, Eckels KH, Carletti I, De La Barrera R, Dessy F, Fernandez S, et al. A phase II, randomized, safety and immunogenicity study of a re-derived, live-attenuated dengue virus vaccine in healthy adults. The American journal of tropical medicine and hygiene. 2013;88(1):73.
35. Putnak R, Barvir DA, Burrous JM, Dubois DR, D'Andrea VM, Hoke CH, et al. Development of a purified, inactivated, dengue-2 virus vaccine prototype in Vero cells: immunogenicity and protection in mice and rhesus monkeys. Journal of Infectious Diseases. 1996;174(6):1176–84.
36. Danko JR, Kochel T, Teneza-Mora N, Luke TC, Raviprakash K, Sun P, et al. Safety and immunogenicity of a tetravalent dengue DNA vaccine administered with a cationic lipid-based adjuvant in a phase 1 clinical trial. The American journal of tropical medicine and hygiene. 2018;98(3):849.
37. Waggoner JJ, Balmaseda A, Gresh L, Sahoo MK, Montoya M, Wang C, et al. Homotypic dengue virus reinfections in Nicaraguan children. The Journal of infectious diseases. 2016;214(7):986–93.
38. Lin L, Koren MA, Paolino KM, Eckels KH, De La Barrera R, Friberg H, et al. Immunogenicity of a live-attenuated dengue vaccine using a heterologous prime-boost strategy in a phase 1 randomized clinical trial. The Journal of infectious diseases. 2021;223(10):1707–16.
39. Koren M, Pollett S, Akerele K, Lee C, Mills K, Moon JE, et al. Evaluation of inactivated and live attenuated, tetravalent dengue vaccine prime-boost strategies in a randomized, open-label, phase I study: implications for understanding immune correlates of protection. mSphere. 2026;11(9):e00497-26.
40. Lyke KE, Chua JV, Koren M, Friberg H, Gromowski GD, Rapaka RR, et al. Efficacy and immunogenicity following dengue virus-1 human challenge after a tetravalent prime-boost dengue vaccine regimen: an open-label, phase 1 trial. The Lancet infectious diseases. 2024;24(8):896–908.
41. Martinez LJ, Lin L, Blaylock JM, Lyons AG, Bauer KM, De La Barrera R, et al. Safety and immunogenicity of a dengue virus serotype-1 purified-inactivated vaccine: results of a phase 1 clinical trial. The American journal of tropical medicine and hygiene. 2015;93(3):454.
42. Puri B, Nelson WM, Henchal EA, Hoke CH, Eckels KH, Dubois DR, et al. Molecular analysis of dengue virus attenuation after serial passage in primary dog kidney cells. J Gen Virol. 1997;78 (Pt 9):2287–91.
43. Vilsker M, Moosa Y, Nooij S, Fonseca V, Ghysens Y, Dumon K, et al. Genome Detective: an automated system for virus identification from high-throughput sequencing data. Bioinformatics. 2019;35(5):871–3.
44. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Molecular Biology and Evolution. 2020;37(5):1530–4.
45. Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature methods. 2017;14(6):587–9.
46. Letunic I, Bork P. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic acids research. 2024;52(W1):W78-W82.

47. Markin A, Wagle S, Grover S, Vincent Baker AL, Eulenstein O, Anderson TK. PARNAS: objectively selecting the most representative taxa on a phylogeny. Systematic Biology. 2023;72(5):1052–63.
48. Lingemann M, Amaro-Carambot E, Lamirande EW, Pierson TC, Whitehead SS. Simultaneous quantitation of neutralizing antibodies against all four dengue virus serotypes using optimized reporter virus particles. Journal of Virology. 2024;98(7):e00681-24.
49. Pierson TC, Sánchez MD, Puffer BA, Ahmed AA, Geiss BJ, Valentine LE, et al. A rapid and quantitative assay for measuring antibody-mediated neutralization of West Nile virus infection. Virology. 2006;346(1):53–65.
50. Mukherjee S, Pierson TC, Dowd KA. Pseudo-infectious reporter virus particles for measuring antibody-mediated neutralization and enhancement of dengue virus infection. Dengue: Methods and Protocols: Springer; 2014. p. 75–97.
51. Ansarah-Sobrinho C, Nelson S, Jost CA, Whitehead SS, Pierson TC. Temperature-dependent production of pseudoinfectious dengue reporter virus particles by complementation. Virology. 2008;381(1):67–74.
52. Dowd KA, DeMaso CR, Pierson TC. Genotypic differences in dengue virus neutralization are explained by a single amino acid mutation that modulates virus breathing. MBio. 2015;6(6):10.1128/mbio. 01559–15.
53. Reverberi R, Reverberi L. Factors affecting the antigen-antibody reaction. Blood transfusion. 2007;5(4):227.
54. Stirling DR, Swain-Bowden MJ, Lucas AM, Carpenter AE, Cimini BA, Goodman A. CellProfiler 4: improvements in speed, utility and usability. BMC bioinformatics. 2021;22(1):433.
55. Wilks S. Racmacs: R antigenic cartography macros. 2022.
56. Team RC. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. 2021.
57. Rössler A, Netzl A, Lasrado N, Chaudhari J, Mühlemann B, Wilks SH, et al. Nonhuman primate antigenic cartography of SARS-CoV-2. Cell Reports. 2025;44(1).
58. Sharma A, Zhang X, Dejnirattisai W, Dai X, Gong D, Wongwiwat W, et al. The epitope arrangement on flavivirus particles contributes to Mab C10's extraordinary neutralization breadth across Zika and dengue viruses. Cell. 2021;184(25):6052–66. e18.
59. Rothen DA, Dutta SK, Krenger PS, Vogt A-CS, Lieknina I, Sobczak JM, et al. Preclinical evaluation of novel sterically optimized VLP-based vaccines against all four DENV serotypes. Vaccines. 2024;12(8):874.
60. McCracken MK, Kuklis CH, Kannadka CB, Barvir DA, Sanborn MA, Waickman AT, et al. Enhanced dengue vaccine virus replication and neutralizing antibody responses in immune primed rhesus macaques. npj Vaccines. 2021;6(1):77.
61. Gallichotte EN, Henein S, Nivarthi U, Delacruz M, Scobey T, Bonaparte M, et al. Vaccine-induced antibodies to contemporary strains of dengue virus type 4 show a mechanistic correlate of protective immunity. Cell reports. 2022;39(10).

62. Rojas-Gallardo D, Ferrell T, Escobar-Pereira PM, Lopez D, Giraldo B, Restrepo-Chica J, et al. Spatiotemporal dispersion of DENV-1 genotype V in Western Colombia. Virus Evol. 2025;11(1):veaf018.
63. Pratt D, Larbi YA, Ofori M, Agbodzi B, Wiley J, Mante AO, et al. Outbreak of dengue fever in Ghana: The emergence of DENV-1 serotype. PLOS Neglected Tropical Diseases. 2026;20(5):e0014248-e.
64. Jamal Z, Haider SA, Hakim R, Humayun F, Farooq MU, Ammar M, et al. Serotype and genomic diversity of dengue virus during the 2023 outbreak in Pakistan reveals the circulation of genotype III of DENV-1 and cosmopolitan genotype of DENV-2. Journal of Medical Virology. 2024;96(6):e29727.
65. de La-Roque DGL, Santos EV, Oliveira RAM, Slavov SN, Rodrigues ES, Fonseca V, et al. DENV-1 genotype V circulation during the nonepidemic period in the Northeast of São Paulo State endemic area. Journal of Medical Virology. 2024;96(3).
66. Masrinoul P, Sun-Arlee P, Yoksan S, Wanlayaporn D, Juntarapornchai S, Punyahathaikul S, et al. Intra-serotypic antigenic diversity of dengue virus serotype 3 in Thailand during 2004–2015. Epidemiology & Infection. 2024;152:e11.
67. Tamura T, Zhang J, Madan V, Biswas A, Schwoerer MP, Cafiero TR, et al. Generation and characterization of genetically and antigenically diverse infectious clones of dengue virus serotypes 1–4. Emerging Microbes & Infections. 2022;11(1):227–39.
68. Velasco JM, Klungthong C, Chinnawirotpisan P, Diones PC, Valderama MT, Leonardia S, et al. Genetic diversity of dengue virus circulating in the Philippines (2014–2019) and comparison with dengue vaccine strains. PLOS Neglected Tropical Diseases. 2024;18(12):e0012697.
69. Zhang C, Mammen Jr MP, Chinnawirotpisan P, Klungthong C, Rodpradit P, Monkongdee P, et al. Clade replacements in dengue virus serotypes 1 and 3 are associated with changing serotype prevalence. Journal of virology. 2005;79(24):15123–30.
70. Smith SA, Nivarthi UK, de Alwis R, Kose N, Sapparapu G, Bombardi R, et al. Dengue virus prM-specific human monoclonal antibodies with virus replication-enhancing properties recognize a single immunodominant antigenic site. Journal of virology. 2016;90(2):780–9.
71. Wang P-G, Kudelko M, Lo J, Siu LYL, Kwok KTH, Sachse M, et al. Efficient assembly and secretion of recombinant subviral particles of the four dengue serotypes using native prM and E proteins. PLoS One. 2009;4(12):e8325.

Figures

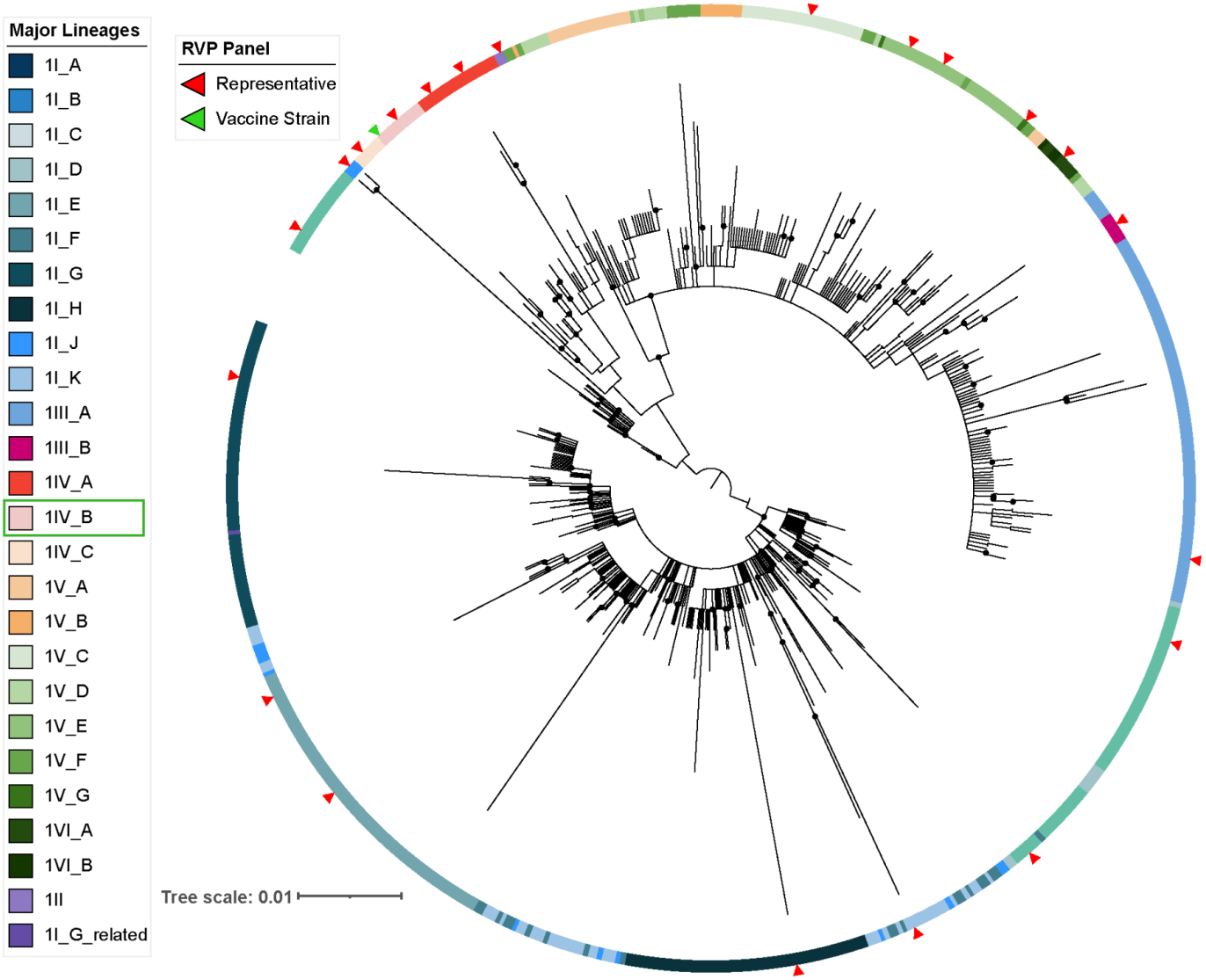


Figure 1

Maximum-likelihood tree of 624 DENV-1 envelope sequences. Twenty representative sequences were selected by PARNAS (red triangles) to represent the global diversity across DENV-1, as shown in the legend. The WHO DENV-1 reference strain used to generate the TDENV-PIV vaccine is highlighted with a neon green triangle, and its corresponding major lineage is highlighted in a green rectangle, as shown in the legend. The black circles indicate nodes with an ultrafast bootstrap value of at least 90.

Envelope	Domain I			Domain II																Domain I									
Strain	8	37	52	55	58	83	84	85	88	89	96	104	106	113	114	115	117	129	145	146	147	155	161	163	169	170	171	180
DVU88535_1IV_B.1	N	D	N	V	K	V	E	E	T	N	F	G	G	L	I	T	A	I	T	G	D	T	T	T	P	T	S	A
KY586544_1I_A	S	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.
MW945976_1I_B	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.
GQ199830_1I_C	S	.	.	.	.	M	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.
MN018332_1I_E1	S	.	D	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	T	.
JQ287663_1I_G	S	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	T	.	.	.	S	.	.	.	S	T	.
KJ755855_1I_H	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MT397277_1I_J	S	.	.	.	.	.	.	.	A	.	.	E	.	L	.	.	.	.	I	A	A	S	.	I	.	.	T	.
MN933667_1I_K	S	.	.	.	.	.	R	G	A	I	.	V	.	F	.	M	D	.	.	.	.	S	.	.	H	.	T	S
MW945844_1II	.	N	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
MN886856_1III_A	.	N	.	.	.	.	.	.	A	.	.	.	.	.	L	.	.	.	.	.	.	.	I	.	.	.	.	.
MK858134_1III_B	.	N	.	.	.	.	.	.	A	.	.	.	.	.	L	.	.	.	.	.	.	.	I	.	.	.	.	.
OK469341_1IV_A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.
MH921566_1IV_A	.	.	.	.	R	.	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	I	.	.	.	.	.
MT832044_1IV_B.1	.	.	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
JN544411_1IV_C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.	.	.
KJ189307_1V_C	.	N	.	I	.	.	.	.	A	.	.	.	.	.	L	.	.	.	.	.	.	.	I	.	.	.	.	.
KC692516_1V_E	.	N	.	.	.	.	.	.	A	.	.	.	.	.	L	.	.	.	.	.	.	.	I	.	.	.	.	.
GU131949_1V_G	.	N	.	.	.	.	.	.	A	.	.	.	.	.	L	.	.	.	.	.	.	.	I	.	.	.	.	.
MZ130534_1VI_A	.	N	.	.	.	.	.	.	A	.	.	.	.	.	L	.	.	.	.	.	.	.	I	.	.	.	.	.

Envelope	Domain II															Domain III																											
Strain	202	203	204	225	234	235	249	253	257	258	264	266	269	270	272	273	283	284	285	293	294	297	305	312	320	324	337	339	343	345	346	347	348	351	352	358	365	369	378	380	386	394
DVU88535_1IV_B.1	E	K	K	S	Q	D	E	L	E	G	L	G	E	I	T	S	L	K	C	T	L	M	S	V	V	V	F	S	K	V	T	Q	N	L	I	V	V	A	I	V	A	K
KY586544_1I_A	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	T	R	.	.	.	.	.	.	.	.	.	.	I	.	.
MW945976_1I_B	K	E	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	V	.	.	.	.	.	.	.	.
GQ199830_1I_C	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	T	.	.	.	.	.	.	.	.	.	.	.	I	.	.
MN018332_1I_E1	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	L	.	I	.	T	.	.	.	.	.	.	.	.	.	.	.	I	.	.
JQ287663_1I_G	K	E	.	.	K	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	T	.	.	.	.	.	.	.	.	.	.	.	I	.	.
KJ755855_1I_H	K	.	.	.	.	G	V	D	L	I	R	.	.	.	.	.	P	.	.	S	.	.	.	.	.	I	.	C	.	.	.	.	.	.	.	.	.	.	.	I	.	.
MT397277_1I_J	K	E	.	.	L	F	.	.	.	.	.	.	A	L	.	F	.	N	I	.	.	.	.	.	I	I	.	T	.	.	.	.	.	.	.	.	.	.	.	I	.	.
MN933667_1I_K	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.	.	.	I	C	T	.	.	A	R	K	.	.	.	.	.	.	I	.	.
MW945844_1II	K	E	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	.	.	.
MN886856_1III_A	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	I	T	.	.	.	.	.	.	.	.	.	T	.	I	.	.
MK858134_1III_B	K	E	.	.	.	.	.	.	.	.	.	.	.	.	M	.	.	.	.	.	.	V	.	.	.	.	.	I	.	.	.	.	.	.	A	.	T	.	I	.	.
OK469341_1IV_A	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.
MH921566_1IV_A	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	P	.	.	.	.	.	.	.	I	.	.	.	.	.	I	.	.	.	.	.
MT832044_1IV_B.1	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	L	.	.	.
JN544411_1IV_C	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.
KJ189307_1V_C	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.
KC692516_1V_E	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	R
GU131949_1V_G	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.
MZ130534_1VI_A	K	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.	I	.	.	.	.	.	.	T	.	.	T	.

Figure 2

DENV-1 envelope protein amino acid variation between selected representatives. Amino acid substitutions are noted relative to the vaccine reference strain (blue text). Each envelope domain (I/II/III) is color-coded and separated by dashed lines. Dots represent conserved positions. Sequence-specific substitutions are highlighted in green, and substitutions shared by multiple sequences are shown in black. Only amino acid positions with variation from the reference strain are shown.

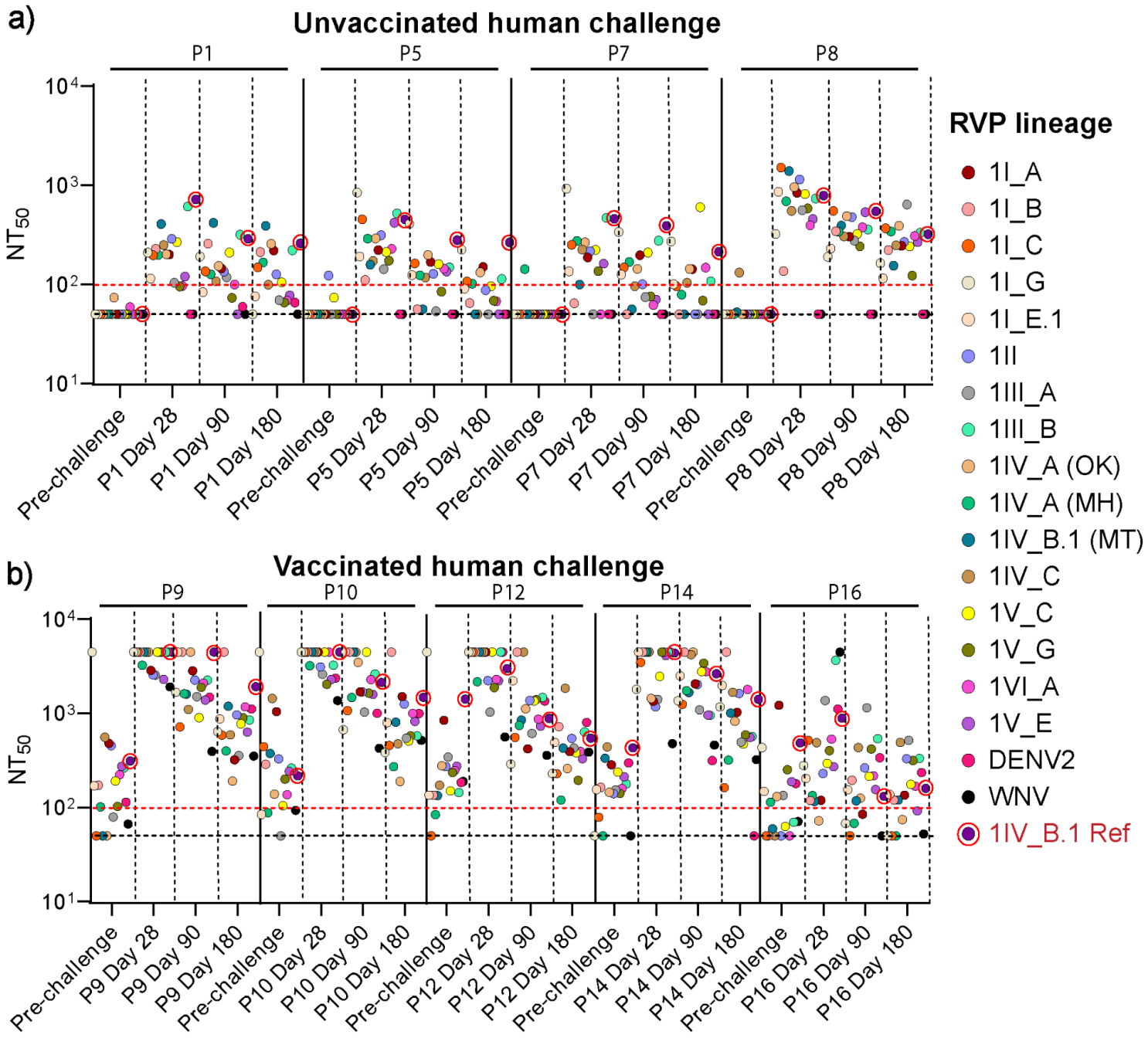
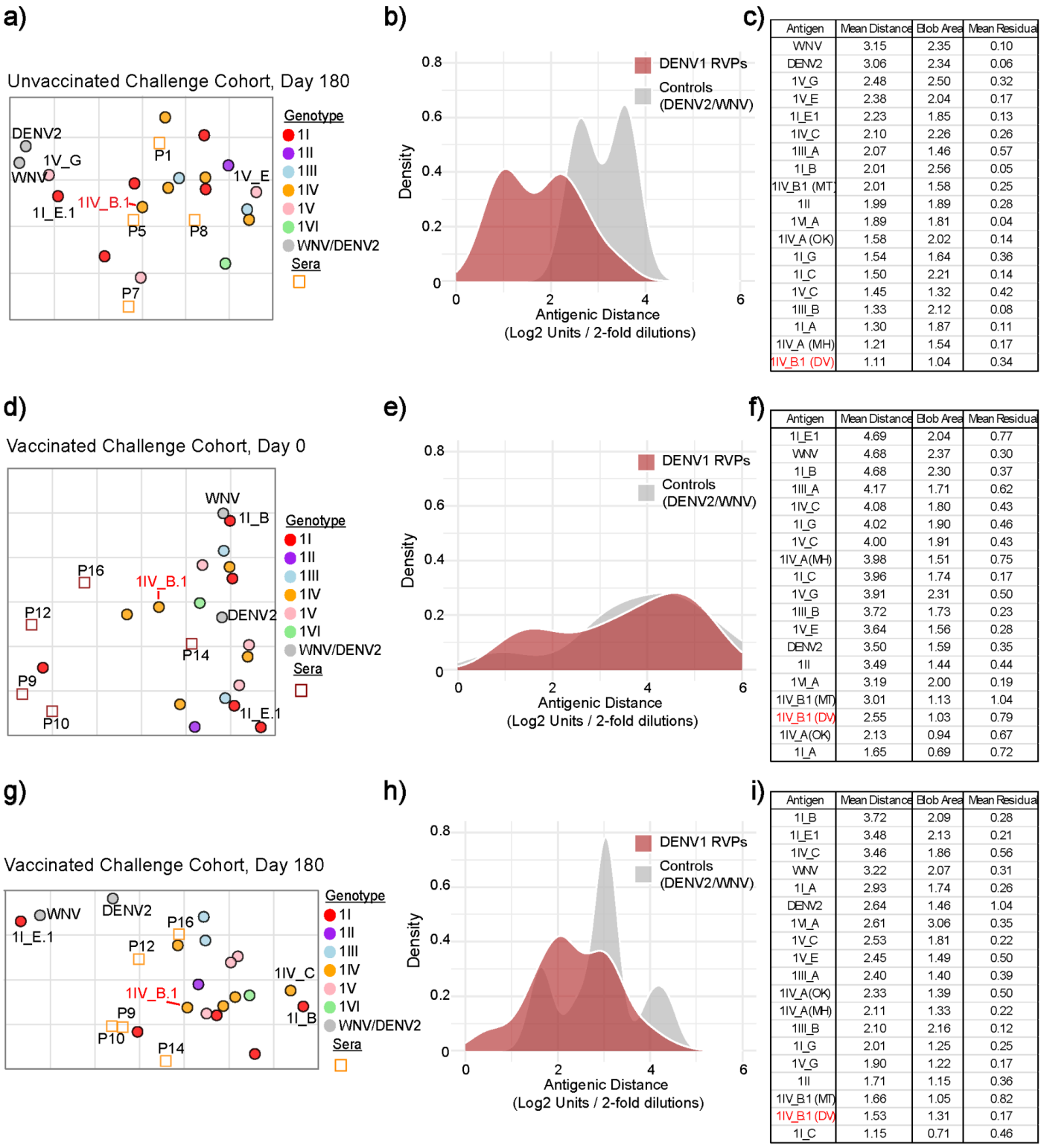


Figure 3

Neutralization efficiency of sera from unvaccinated/challenge cohort and vaccination/challenge cohort against DENV-1 major lineages. ***(A)***. NT50 values for sera from each participant (n=4) from the unvaccinated/challenge cohort tested against a panel of DENV-1 major lineages (n=17), DENV-2 and WNV RVPs. ***(B).*** NT50 values for sera from vaccination/challenge Participants (n=5) tested against the same RVP panel as in A. For both panels, the vertical dashed lines separate the sera tested. The black horizontal dashed line represents the minimum detection level, and the red horizontal dashed line represents the heuristic threshold at NT50 = 100. The maximum level of detection for this assay is a serum dilution of 1:4480. The red circles highlight the challenge and vaccine lineage (45AZ5).



Antigen	Mean Distance	Blob Area	Mean Residual
WNV	3.15	2.35	0.10
DENV2	3.06	2.34	0.06
1V_G	2.48	2.50	0.32
1V_E	2.38	2.04	0.17
1I_E1	2.23	1.85	0.13
1IV_C	2.10	2.26	0.26
1III_A	2.07	1.46	0.57
1I_B	2.01	2.56	0.05
1IV_B1 (MT)	2.01	1.58	0.25
1II	1.99	1.89	0.28
1VI_A	1.89	1.81	0.04
1IV_A (OK)	1.58	2.02	0.14
1I_G	1.54	1.64	0.36
1I_C	1.50	2.21	0.14
1V_C	1.45	1.32	0.42
1III_B	1.33	2.12	0.08
1I_A	1.30	1.87	0.11
1IV_A (MH)	1.21	1.54	0.17
1IV_B1 (DV)	1.11	1.04	0.34

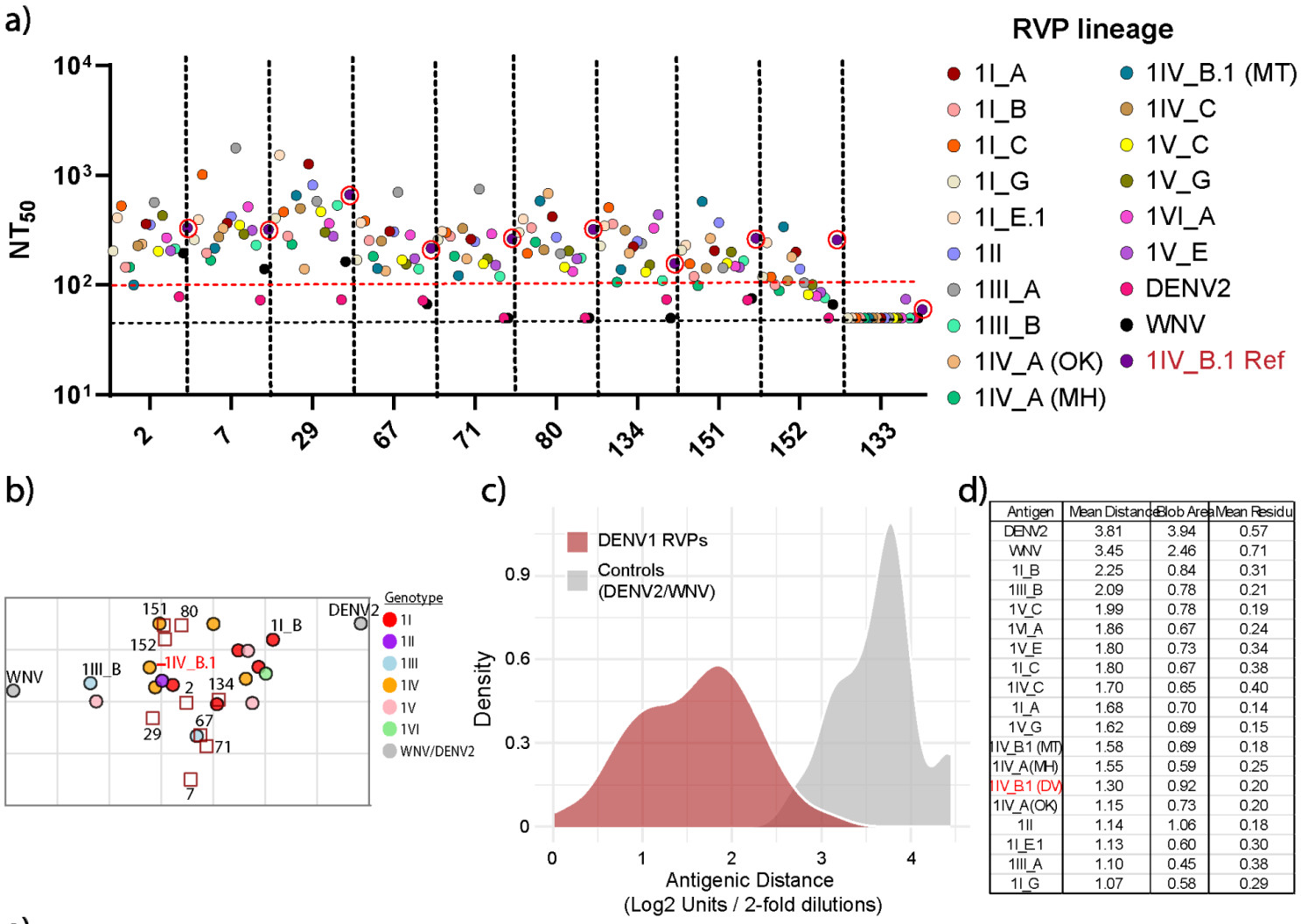
Antigen	Mean Distance	Blob Area	Mean Residual
1I_E1	4.69	2.04	0.77
WNV	4.68	2.37	0.30
1I_B	4.68	2.30	0.37
1III_A	4.17	1.71	0.62
1IV_C	4.08	1.80	0.43
1I_G	4.02	1.90	0.46
1V_C	4.00	1.91	0.43
1IV_A(MH)	3.98	1.51	0.75
1I_C	3.96	1.74	0.17
1V_G	3.91	2.31	0.50
1III_B	3.72	1.73	0.23
1V_E	3.64	1.56	0.28
DENV2	3.50	1.59	0.35
1II	3.49	1.44	0.44
1VI_A	3.19	2.00	0.19
1IV_B1 (MT)	3.01	1.13	1.04
1IV_B1 (DV)	2.55	1.03	0.79
1IV_A(OK)	2.13	0.94	0.67
1I_A	1.65	0.69	0.72

Antigen	Mean Distance	Blob Area	Mean Residual
1I_B	3.72	2.09	0.28
1I_E1	3.48	2.13	0.21
1IV_C	3.46	1.86	0.56
WNV	3.22	2.07	0.31
1I_A	2.93	1.74	0.26
DENV2	2.64	1.46	1.04
1VI_A	2.61	3.06	0.35
1V_C	2.53	1.81	0.22
1V_E	2.45	1.49	0.50
1III_A	2.40	1.40	0.39
1IV_A(OK)	2.33	1.39	0.50
1IV_A(MH)	2.11	1.33	0.22
1III_B	2.10	2.16	0.12
1I_G	2.01	1.25	0.25
1V_G	1.90	1.22	0.17
1II	1.71	1.15	0.36
1IV_B1 (MT)	1.66	1.05	0.82
1IV_B1 (DV)	1.53	1.31	0.17
1I_C	1.15	0.71	0.46

Figure 4

Antigenic cartography analysis of DENV-1 major lineages against serum from unvaccinated/challenge and vaccination/challenge cohorts. (**A-C**) Antigenic profiles for the unvaccinated challenge cohort at day 180. (**A**) Antigenic cartography map showing the relative positions of all RVPs and serum samples based on NT50 values. (**B**) Density distribution of antigenic distances for all RVPs based on antigenic distances from panel A. Red distributions represent all DENV-1 RVPs in this study, and grey distributions

represent antigenic controls (DENV-2 [strain: 16681] and WNV [strain: NY99]). (**C**) Summary table of each RVP's antigenic profile, including the mean antigenic distance across all sera, blob area (reflecting the positional uncertainty of each RVP), and mean residual (indicating the agreement between predicted distances and observed neutralization titers). The vaccine derivative strain is shown in red.**(D-F)** Antigenic profiles for the vaccinated/challenged cohort at day 0, structured as in panels A–C. **(G-I)** Antigenic profiles for the vaccinated/challenged cohort at day 180, structured as in panels A-C.



Antigen	Mean Distance	Blob Area	Mean Residu
DENV2	3.81	3.94	0.57
WNV	3.45	2.46	0.71
1I_B	2.25	0.84	0.31
1III_B	2.09	0.78	0.21
1V_C	1.99	0.78	0.19
1VI_A	1.86	0.67	0.24
1V_E	1.80	0.73	0.34
1I_C	1.80	0.67	0.38
1IV_C	1.70	0.65	0.40
1I_A	1.68	0.70	0.14
1V_G	1.62	0.69	0.15
1IV_B.1 (MT)	1.58	0.69	0.18
1IV_A(MH)	1.55	0.59	0.25
1IV_B.1 (DV)	1.30	0.92	0.20
1IV_A(OK)	1.15	0.73	0.20
1II	1.14	1.06	0.18
1I_E.1	1.13	0.60	0.30
1III_A	1.10	0.45	0.38
1I_G	1.07	0.58	0.29

e)

Sera Cohort	Collection Point	Potency (Average NT50)	Breadth (# RVPs < 100) Across Particpants	Minimum Breadth	Maximum Breadth
Unvaccinated, human challenged	Pre-challenge	55	0	0	0
	Day 28	389	12	13	15
	Day 90	211	10	10	13
	Day 180	168	4	7	10
Vaccinated, human challenged	Pre-challenge	403	4	9	15
	Day 28	3057	16	16	17
	Day 90	1790	13	13	17
	Day 180	760	12	12	17
Natural Infection	Single point	300	12	12	17

Figure 5

RVP neutralization and antigenic cartography profile of DENV-1 lineages against sera from naturally infected individuals. (**A**) Serum from each participant (n=9) from the naturally infected cohort was tested against a panel of DENV-1 variants (n=17), DENV-2, and WNV. The vertical dashed lines separate the sera tested, while the horizontal dashed line represents the minimum detection level. The red circles highlight the vaccine derivative strain (shown in red). (**B**) Antigenic cartography map showing the relative positions of all RVPs based on NT50 values for all sera. (**C**) Density distribution of antigenic distances. Red distributions represent all DENV-1 variants in this study, and grey distributions represent controls (DENV-2 [strain: 16681] and WNV [strain: NY99]). (**D**) Summary table of each RVP's antigenic profile, including the mean antigenic distance across all sera, blob area (reflecting the positional uncertainty of each RVP), and mean residual (indicating the agreement between predicted titers and observed neutralization titers). The vaccine derivative strain is highlighted in red. **(E)** Comparative analysis of potency and breadth across sera from naturally infected, unvaccinated, and vaccinated/challenged individuals. The average potency for each cohort across timepoints was calculated. Breadth is the number of RVPs in the panel with values above the heuristic threshold of 100 NT50 across each set. The minimum and maximum breadth correspond to the lowest and highest number of RVPs above our NT50 threshold for any individual participant serum.

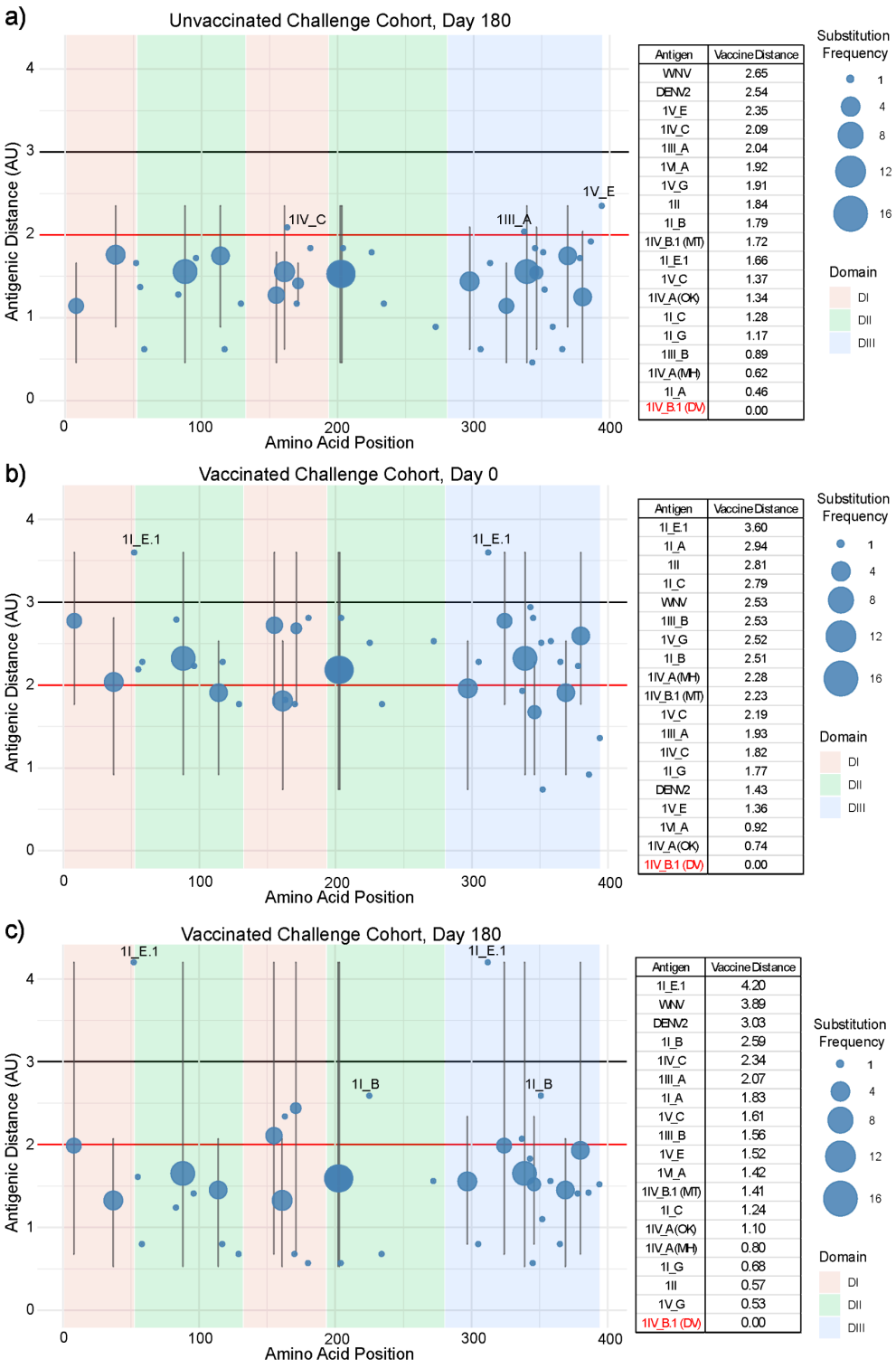


Figure 6

Envelope amino acid substitutions and antigenic variation among DENV-1 RVPs in unvaccinated/challenged and vaccinated/challenged cohorts. The relationship between antigenic distance and amino acid substitutions relative to the reference strain (**WestPac-74 derivative**) was assessed using antigenic profiles generated from sera in the **(A)** unvaccinated/challenged cohort at Day 180, **(B)** vaccinated/challenged cohort at Day 0, and **(C)** vaccinated/challenged cohort at Day 180. Circle

size indicates the frequency of RVPs carrying a substitution at each envelope amino acid position, and Y-axis position represents the average antigenic distance of all RVPs with a substitution at that site. The three structural domains of the envelope protein are color-coded. The red and black horizontal lines are thresholds for potential differences from the most susceptible strain, representing 4-fold and 8-fold distances, respectively.

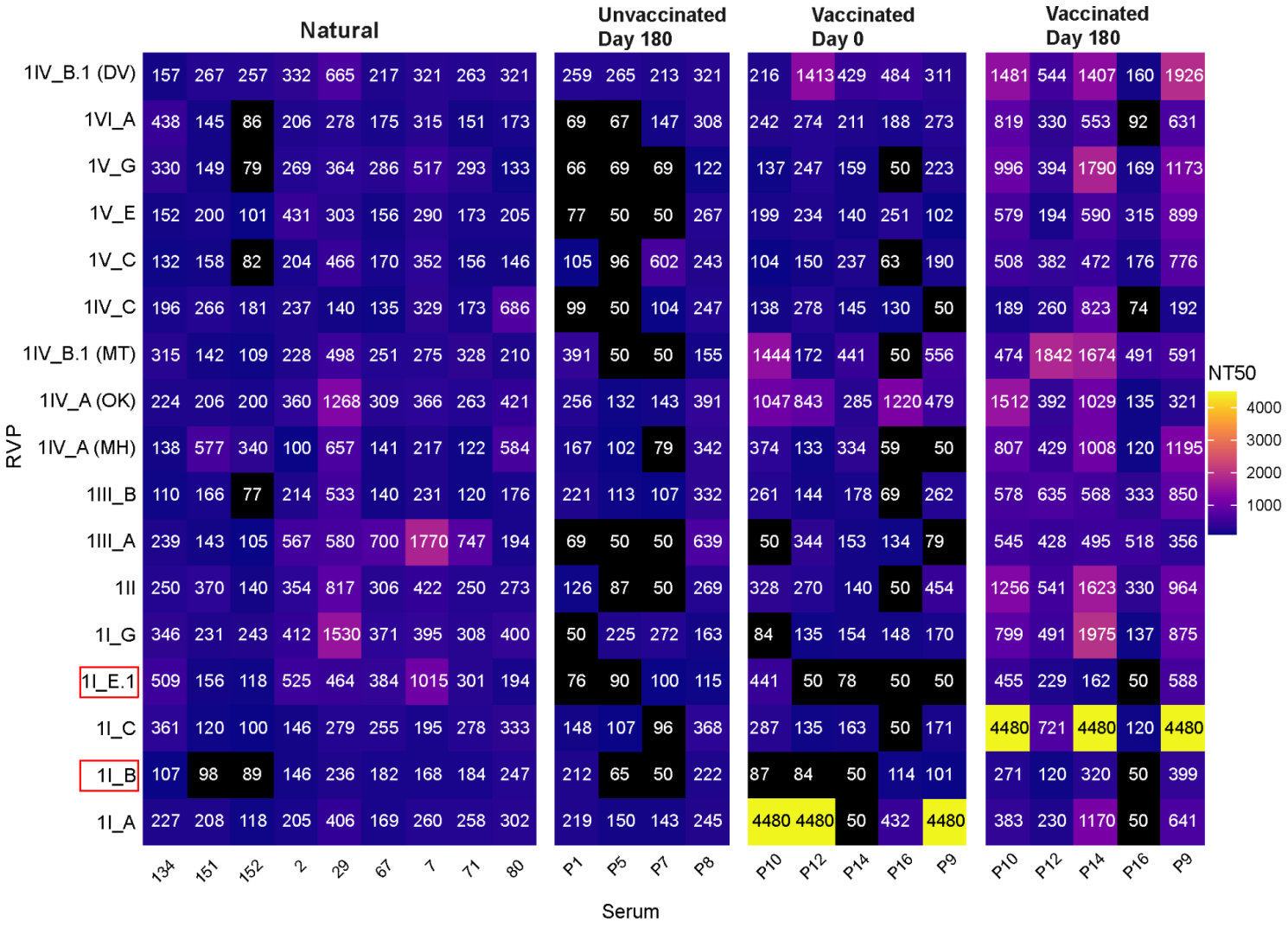


Figure 7

Heatmap of DENV-1 lineage-specific neutralization profiles across the unvaccinated/challenged day 180, vaccinated/challenged day 0/180, and naturally infected cohorts. Heatmap shows neutralization titers (NT_{50}) for each participant in the unvaccinated/challenged (n = 4), vaccinated/challenged (n = 5), and naturally infected (n = 9) cohort tested against a panel of major DENV-1 lineages (n = 17). Day labels indicate the time point after challenge. Each tile represents the NT50 value for a given serum–lineage pair. The lower limit of detection was set at 50, and the maximum antibody dilution tested was 1:4480.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalFigures.pdf