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MAJOR ARTICLE

Dengue Virus (DENV) Neutralizing Antibody Kinetics in Children After Symptomatic Primary and Postprimary DENV Infection

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The immune response to dengue virus (DENV) infection is complex and not fully understood. Using longitudinal data from 181 children with dengue in Thailand who were followed for up to 3 years, we describe neutralizing antibody kinetics following symptomatic DENV infection. We observed that antibody titers varied by serotype, homotypic vs heterotypic responses, and primary versus postprimary infections. The rates of change in antibody titers over time varied between primary and postprimary responses. For primary infections, titers increased from convalescence to 6 months. By comparing homotypic and heterotypic antibody titers, we saw an increase in type specificity from convalescence to 6 months for primary DENV3 infections but not primary DENV1 infections. In postprimary cases, there was a decrease in titers from convalescence up until 6 months after infection. Beginning 1 year after both primary and postprimary infections, there was evidence of increasing antibody titers, with greater increases in children with lower titers, suggesting that antibody titers were boosted due to infection and that higher levels of neutralizing antibody may be more likely to confer a sterilizing immune response. These findings may help to model virus transmission dynamics and provide baseline data to support the development of vaccines and therapeutics.

Keywords. dengue; neutralizing antibody; longitudinal antibody kinetics; vaccine.

Dengue is a vector-borne disease found across much of the world, with an expanding geographical range [1]. There are 4 dengue virus (DENV) serotypes, DENV1–4, consisting of multiple genetically distinct viruses that are classified into genotypes. Thailand has a large burden of dengue, with all 4 DENV serotypes circulating [2].

Antibody responses to DENV infection are thought to provide long-lasting protection against subsequent disease due to the same serotype. For a short time (months) following primary DENV infection, the immune response has been found to prevent symptomatic disease following subsequent secondary DENV experimental challenge [3, 4], with this finding supported by observational studies [5–7]. Following this short period of protection, there is a time when, through a process called antibody-dependent enhancement, antibodies bind but do not neutralize virus, facilitating entry into host cells and resulting in an increased likelihood of severe disease [8]. Tertiary and quaternary infections have also been detected [9], but they appear less likely to cause severe disease [10]. One consistent finding across studies is the observation that antibody responses vary over time, as does their ability to protect individuals or place them at risk for a severe outcome from subsequent DENV infection.

Careful characterization of antibody responses after infection is an important step in understanding how these immunological phenomena are important to dengue pathogenesis and epidemiology. To date, no careful analysis of longitudinal antibody responses has been conducted.

MATERIALS AND METHODS

Study and Assay Description

Children were enrolled during acute DENV infection from 2 hospitals in Thailand: the Queen Sirikit National Institute of Child Health, a large children's hospital in the center of Bangkok, and the Kamphaeng Phet provincial hospital, a regional hospital located in rural northern Thailand. The study has been detailed elsewhere [11]. Between 1994 and 1997, children between the ages of 6 months and 15 years who presented to the outpatient department or hospital ward, had had fever (temperature, ≥38°C) for <72 hours, and had no localizing signs of infection were identified and invited to participate in the study

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with parental consent. Exclusion criteria included signs of shock or an alternate, nondengue diagnosis. Blood samples were obtained from study participants daily during acute infection until the day following defervescence; at convalescence (around study day 10); and, for children with confirmed dengue, at 6 months, 1 year, 2 years, and 3 years after DENV infection. Primary or postprimary DENV infection was confirmed by an immunoglobulin G/immunoglobulin M enzyme-linked immunosorbent assay and a hemagglutination-inhibition assay as described previously [12]. Infecting virus serotype was determined by virus isolation in mosquitoes and/or reverse transcription–polymerase chain reaction analysis as described previously [13, 14]. Antibody titers were measured using a plaque reduction neutralization test as described in detail elsewhere [15, 16]. In brief [15], a monolayer of continuous LLC-MK2 was infected with DENV in the presence of 4-fold serial dilutions of heat-inactivated serum (range, 1:10–1:2560). For each dilution, the number of viral plaques was counted and compared to the number of plaques in control plates, where no serum was added. The titer required to reduce the number of DENV plaque by 50% ($PRNT_{50}$) was determined by means of log probit regression analysis, using SPSS software (IBM [previously SPSS]), and was reported as a reciprocal titer.

Analysis

The antibody titers and changes between measurements at early convalescence, 6 months, 1 year, 2 years, and 3 years after infection were assessed. The homotypic response refers to the response to the infecting serotype (eg, the homotypic DENV1 response is the DENV1 antibody titer in those infected with DENV1), and the heterotypic response refers to the response to a serotype in individuals infected with a different serotype (eg, the heterotypic DENV1 response is the DENV1 antibody titer in those infected with DENV2, 3, or 4). Grouping in this way enabled comparisons between responses to each serotype in individuals infected with one of the serotypes and those infected with the other serotypes, taking into account the different magnitudes of titers to each serotype. Titers of <1:10 were below the assay limit of detection and were treated as 5. All analyses were performed in R [17].

We calculated geometric mean titers (GMTs) of the neutralizing antibody titers at each time point after infection (convalescence, 6 months, 1 year, 2 years, and 3 years) for primary and postprimary infections, for the homotypic and heterotypic responses. We also calculated the linear rate of change of $\log_{10}$ titers between measurements and compared these findings between study subjects with primary and postprimary infections, between subjects with infections of each serotype, between responses to each serotype, and between the homotypic and heterotypic response. Distributions of titers and rates of change were compared between groups, using the Mann–Whitney test.

At each time point, we assessed the correlations between titers and the rate of change in titers to the next time point, as well as the correlations between the rates of change in titers to each serotype for an individual (eg, we analyzed whether, in a given individual, an increase in DENV1 antibody titer was associated with an increase in DENV2 antibody titer). From 1 year on, we assessed the proportion of cases that had a >4-fold increase to any DENV serotype as an indication of intervening DENV infection, similar to the approach by Montoya et al [7].

RESULTS

This study enrolled 189 children between 1994 and 1997. One hundred eighty-one children who had a serotyped DENV infection with a primary or postprimary classification were included in the analysis. Population characteristics are shown in Table 1. There were more postprimary cases than primary cases. This was particularly noticeable for DENV2 and DENV4, as previously observed [18, 19], with no children with primary DENV4 infection and only 1 with primary DENV2 infection. All plaque reduction neutralization tests used in this analysis were performed over a single period. Individuals who were enrolled in 1995 had titers up until 2 years after infection, and those enrolled in 1996 and 1997 had titers up until 1 year after infection, leading to less power in the 2-year and 3-year analyses.

Magnitude of Titers

At all time points after infection, DENV3 titers were significantly lower than the titers against the other serotypes, and DENV2 titers were the highest (Tables 2 and 3 and Figure 1).

Convalescence Until 6 Months

From convalescence to 6 months, for titers to DENV1 and 3 an increase occurred for 5%–9% of children with postprimary infections and for 64%–71% of children with primary infections (Figure 2), despite there being no differences in the mean time (±SD) after infection at which the convalescent titer was observed (11.25 ± 1.4 days for primary cases and 11.0 ± 1.6 for postprimary cases). Thirty-five percent of children had a ≥4-fold increase in $PRNT_{50}$ values to at least 1 serotype in this period. An increase in the antibody titer of 1 log at day 10 led to a mean decrease in the rate of change of 0.1 log (95% confidence interval [CI], 0.01–0.13 logs); that is, titers in subjects with lower titers at the time of the convalescent blood specimens collection were more likely to increase, or to decrease at a lower rate, than titers in subjects with higher titers.

The changes in titers to DENV3 from convalescence to 6 months after primary infection were greater (more positive; $P < .05$) for the homotypic response (mean increase per month, 0.18; 95% CI, −.10 to .45), compared with the heterotypic response (mean increase per month, 0.05; 95% CI, −.3 to .4; Figure 2), suggesting an increase in type specificity for DENV3 over this period during primary infections. There were no differences between the rates of change of homotypic and heterotypic

Table 1. Summary Characteristics of the Population, Overall and by Dengue Virus (DENV) Serotype

Characteristic	All	DENV1	DENV2	DENV3	DENV4
Underwent measurements during infection					
Overall	181	53	52	57	20
Primary infection	36	17	1	18	0
Postprimary infection	145	36	51	29	20
Enrollment year					
1994	54	12	17	10	15
1995	39	14	11	13	2
1996	54	15	15	21	3
1997	34	12	9	13	0
Enrolled in Bangkok, subjects, %	77	81	71	81	70
Age, y, mean ± SD	7.5 ± 2.9	7.4 ± 3.2	7.9 ± 2.8	7.3 ± 2.9	7.9 ± 2.1
Illness duration at enrollment, d, mean (95% CI)	3 (2–4)	2.9 (2–4)	2. 9 (2–4)	3.2 (2–4)	3.5 (2–4.5)
Had measureable titer, by time point(s)					
Convalescence					
Overall	160	47	42	53	18
Primary infection	33	14	1	18	0
Postprimary infection	127	33	41	35	18
6 mo					
Overall	150	45	42	45	18
Primary infection	31	15	1	15	0
Postprimary infection	119	30	41	30	18
1 y					
Overall	140	35	41	47	17
Primary infection	29	12	1	16	0
Postprimary infection	111	23	40	29	17
2 y					
Overall	77	21	22	19	15
Primary infection	13	6	1	6	0
Postprimary infection	64	15	21	13	15
3 y					
Overall	49	11	16	8	1
Primary infection	4	3	1	0	0
Postprimary infection	45	8	15	8	1
Both convalescence and 6 mo					
Overall	131	40	33	42	16
Primary infection	28	12	1	15	0
Postprimary infection	103	28	32	27	16
Both 6 mo and 1 year					
Overall	98	24	25	35	14
Primary infection	22	8	1	13	0
Postprimary infection	76	16	24	22	14
Both 1 y and 2 y					
Overall	54	13	13	17	11
Primary infection	11	4	1	6	0
Postprimary infection	43	9	12	11	11
Both 2 y and 3 y					
Overall	32	7	7	6	12
Primary infection	3	2	1	0	0
Postprimary infection	29	5	6	6	12
All time points					
Overall	30	7	7	6	10
Primary infection	3	2	1	0	0
Postprimary infection	27	5	6	6	10

Data are no. of children, unless otherwise indicated.

Abbreviation: CI, confidence interval.

Table 2. Geometric Mean Neutralizing Titers (GMTs) to Each Dengue Virus (DENV) Serotype at 6 Months, 1 Year, 2 Years, and 3 Years After Infection for Postprimary Cases in Those Infected With That Serotype and in Those Infected With a Different Serotype

Infection Type, DENV Serotype	Convalescence	6 mo	1 y	2 y	3 y
Primary infection					
GMT to infecting serotype (homotypic response)					
DENV1	146 (108–185)	317 (303–331)	409 (397–422)	654 (649–659)	1555 (1551–1559)
DENV2	590	216	636	1295	3060
DENV3	24 (14–34)	148 (142–153)	146 (141–151)	190 (187–194)	. . .
DENV4	. . .	. . .	. . .	. . .	. . .
GMT to this serotype in those infected by any of the other serotypes (heterotypic response)					
DENV1	44 (9–78)	60 (51–69)	148 (138–159)	672 (664–679)	653
DENV2	140 (122–158)	212 (205–219)	72 (55–90)	354 (348–360)	641 (638–643)
DENV3	17 (9–25)	22 (14–31)	7 (0–19)	47 (41–52)	51 (47–55)
DENV4	35 (2–69)	29 (21–37)	24 (13–35)	117 (113–122)	78 (73–83)
Postprimary infection					
GMT to infecting serotype (homotypic response)					
DENV1	2734 (2714–2754)	624 (614–634)	642 (635–648)	532 (548–540)	280 (274–286)
DENV2	7370 (7361–7380)	935 (929–942)	337 (329–345)	915 (907–924)	1417 (1411–1423)
DENV3	444 (341–547)	71 (53–90)	52 (44–60)	62 (56–67)	189 (185–193)
DENV4	12223 (12 208–12 237)	781 (773–788)	374 (368–380)	280 (272–288)	550 (545–555)
GMT to this serotype in those infected by any of the other serotypes (heterotypic response)					
DENV1	1549 (1482–1616)	290 (275–260)	204 (188–219)	382 (317–339)	482 (472–491)
DENV2	9474 (9715–9778)	640 (629–652)	368 (361–376)	446 (433–459)	1505 (1498–1512)
DENV3	899 (848–950)	63 (49–78)	39 (27–52)	60 (49–71)	209 (202–215)
DENV4	3078 (3061–3096)	154 (144–164)	120 (107–132)	147 (138–155)	285 (280–290)

Values in parentheses denote 95% confidence intervals.

responses for primary DENV1 infection (Figure 2). There was a lower rate of decay in the homotypic response, compared with the heterotypic response, for postprimary infections for DENV2 (mean change, −0.15 per month [95% CI, −.37 to .069] vs −0.21 [95% CIs, −.46 to .037]; $P<.05$) and DENV3 (mean change, −0.14 [95% CI, −.39 to .12] vs −0.19 [95% CI, −.45 to .058]; $P<.05$) but not for DENV1 and DENV4 (Figure 2). In children with a decrease in titers, the mean rate of decrease of the homotypic response was not different between primary and postprimary cases of DENV1 infection (−0.16 $\log_{10}$ per month for primary infection and −0.14 $\log_{10}$ per month for postprimary infection), but it was different for cases of DENV3 infection (−0.02 $\log_{10}$ per month for primary infection vs −0.16 $\log_{10}$ per month for postprimary infection).

Table 3. From 6 Months Onward, Geometric Mean Titers (GMTs) at the Start of a Period in Children Who Had Increasing Titers in the Next Period and Those Whose Titers Stayed the Same or Decreased

	GMT (95% CI)		
Serotype	Children With Increase	Children With No Increase	*P* Value[a]
DENV1	94 (80–107)	662 (653–671)	<.001
DENV2	309 (296–322)	635 (627–645)	.03
DENV3	21 (7–34)	132 (126–1347)	<.001
DENV4	58 (436–70)	290 (282–297)	<.001

Abbreviations: CI, confidence interval; DENV, dengue virus.

[a] By the Mann–Whitney Wilcoxon test.

6 Months to 1 Year

Titers were the lowest 6 months after infection in children with primary infections, compared with those at other time points and with titers in children with postprimary infection (Table 2 and Figure 1). At 6 months, homotypic titers were higher after postprimary DENV1 infections than after primary DENV1 infections but were higher after primary DENV3 infections than after postprimary DENV3 infections (Table 2 and Figure 1). From 6 months to 1 year, the decrease in antibody titers continued for the majority (60%–70%, depending on the serotype) of subjects. Among titers that decreased between 6 months and 1 year, the rate of decrease was, on average, about half of the rate of decrease up to 6 months, with an average rate for all serotypes of −0.08 $\log_{10}$ per month (95% CIs, −.1 to −.06; $\log_{10}$ per month; Figure 3). From 6 months to a year, children with primary infections were once again more likely to have increasing titers than those with postprimary infections ($P < .05$ for comparison among serotypes; Figure 1). For all serotypes, the mean rate of change was 0.021 for primary infection and −0.03 for postprimary infection.

1 Year to 2 Years and 2 Years to 3 Years

From 1 to 2 years 50% of individuals had a ≥4-fold increase in $PRNT_{50}$ values to at least one serotype (37% had ≥8-fold). From 2 to 3 years, 43% of individuals had a ≥4-fold increase in $PRNT_{50}$ values to at least 1 serotype (25% had an ≥8-fold increase). This is also reflected in the average titers over time (Figure 1 and Table 2) and the average rates of change (Figure 3).

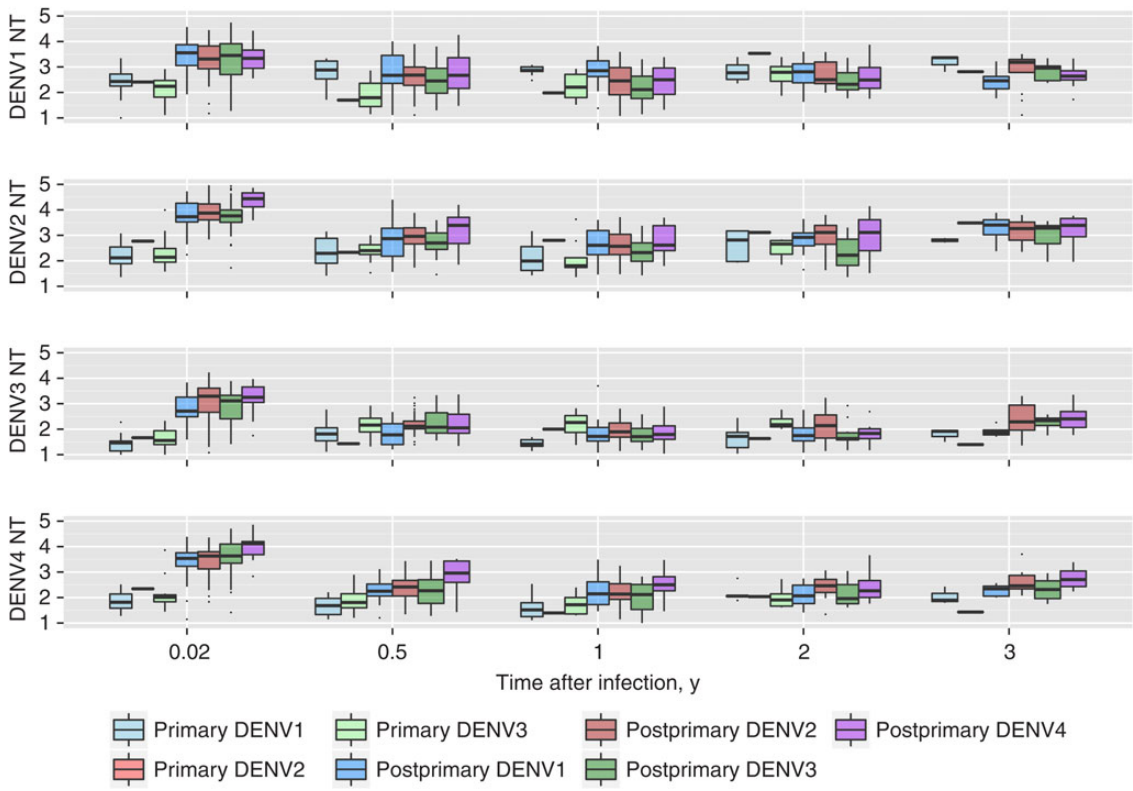

Figure 1 Log_{10} neutralizing titers (NTs) to dengue virus serotypes 1–4 (DENV1–4) at different time points after infection; 0.02 is the convalescence time point. Data are median values, interquartile ranges, and 95% confidence intervals.

From 1 to 2 years, between 25% and 35% of titers against each serotype increased ≥4-fold (and between 6% and 20% increased ≥8-fold). In this time frame, there was a significant relationship between the rate of change of each serotype to the others within an individual ($P < .01$ for all correlations except DENV2 and DENV3, and DENV3 and DENV4). From 2 to 3 years, there was a greater variation across serotypes: 16% of DENV1 and 2, 32% of DENV3, and 9% of DENV4 titers had a ≥4-fold increase in $PRNT_{50}$ values (6% of DENV1, 10% of DENV2, 11% of DENV3, and 0% of DENV4 titers had a ≥8-fold increase), and there was no relationship between the rate of change of each serotype to the others within an individual.

There was no difference in the rate of change between primary and postprimary cases in the 1–2 year period (although the numbers were small for primary cases in this time frame). The numbers of children with primary infections were too small for comparison in the 2–3 year period.

In those that decreased, from 1 year to 2 years the decrease rate was −0.03 log_{10} per month (95% CI, −.06 to −.01 log_{10} per month) and from 2 years to 3 years it was −0.04 log_{10} per month (95% CIs, −.07 to −.01 log_{10} per month).

Consistent Observations From 6 Months Onward

At all time points from 6 months onward, the rate of change of titers from a time point onward was negatively correlated with the magnitude of titers at the starting time point; that is, individuals with lower titers to a serotype were more likely to see an increase in titers to this serotype and to have these titers increase at a higher rate, compared with individuals with higher titers. The correlations varied between −0.5 and −0.7 depending on the serotype and the time point ($P<.01$ for all comparisons). For DENV1–4, the geometric mean titers in individuals who had decreasing titers in the next time frame were 662, 635, 132, and 290, respectively, and 94, 309, 21, and 58, respectively for those who had increasing titers (Table 3).

From 6 months on, there were no observed differences in rates of change in homotypic versus heterotypic titers (Figure 3). Although there were not differences in the rates of change, at all time points, for all serotypes, homotypic titers were on average higher than heterotypic titers for both primary and postprimary infections (Figure 1 and Table 2).

DISCUSSION

We present a detailed description of DENV antibody dynamics as measured by $PRNT_{50}$ values following natural infection. This analysis details the antibody dynamics for children after infection for primary and postprimary cases due to different serotypes and is informative about how immunity to DENV changes over time.

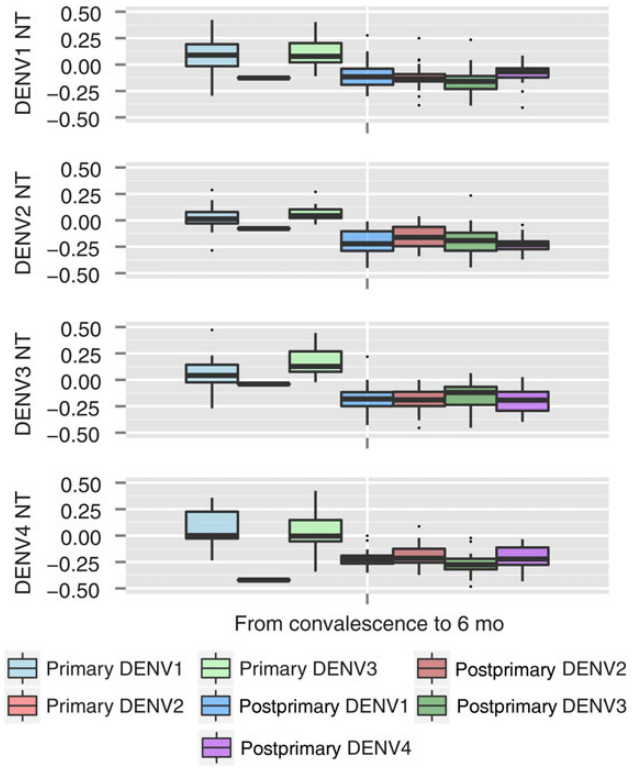

Figure 2. The rate of change per month of the $\log_{10}$ of the titers to dengue virus serotypes 1–4 (DENV1–4) from convalescence to 6 months after infection. Data are mean values, interquartile ranges, and 95% confidence intervals for the rate of change per month. Abbreviation: NT, neutralizing titer.

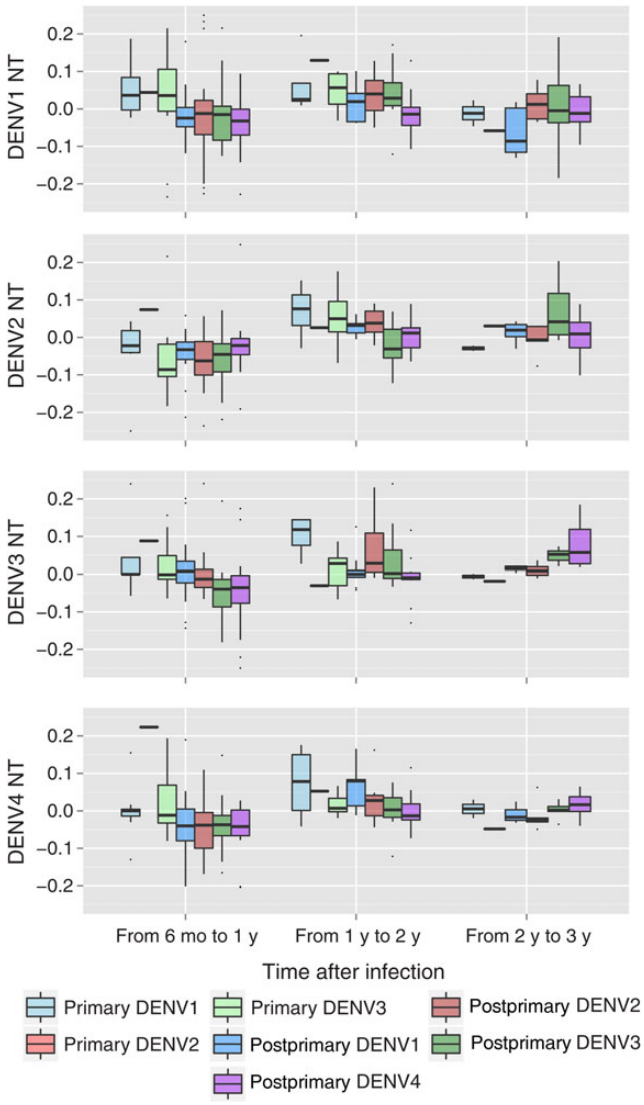

Figure 3. The rate of change per month of the $\log_{10}$ of the titers to dengue virus serotypes 1–4 (DENV1–4) between time points, beginning 6 months after infection. Data are mean values, interquartile ranges, and 95% confidence intervals for the rate of change per month. Abbreviation: NT, neutralizing titer.

From convalescence to 6 months, DENV antibody titers decreased rapidly following postprimary infections. In primary cases, titers were still increasing in this period, consistent with the more slowly developing titers following primary infection. By comparing the homotypic response to the heterotypic response, it is possible to consider changes in type specificity over time. There was some evidence for an increase in type specificity (for DENV3 but not DENV1) between 10 days and 6 months after primary infection, as DENV3 homotypic titers increased more in this period than the heterotypic titers, but there was less evidence for this in the postprimary cases, with only a small proportion (5%–9%) of children with titers increasing in this period. There were suggestions of differences in the rates of decrease for postprimary DENV2 and DENV3 cases, so it could be that the heterotypic response decays faster than the homotypic response, although both are decaying. From 6 months on, there were no observed differences in rates of change of homotypic or heterotypic titers, so there is no evidence for an increase in type specificity in this time frame, but the inference becomes difficult due to infections occurring in this time frame. Over this time frame, we have evidence that the homotypic titers may start higher and remain higher, but there are not differential boosts to the previously infecting serotype. Previous evidence for increases in type specificity came from Cuba, where titers to the infecting serotype were shown to be higher and titers to other serotypes lower at 22 years after infection than (in different individuals) at 4–8 years after infection [20]. Further work investigating kinetics of the immune response after vaccination and natural and challenge infection will be of great use in understanding whether type specificity increases after DENV infection.

Experimental infections and cohort studies suggest that individuals are in a period of cross-protection at 6 months and possibly at 1 year after a primary infection [3, 4, 7]. However, the lowest titers were observed for heterotypic responses at 6 months and 1 year following primary infection. Recent work in Kamphaeng Phet (with plaque-reduction neutralization tests

undertaken in the same laboratories used here, although at a different time) [21] suggested that titers of 11, 323, and 16 are protective against DENV1, 2, and 4, respectively. Although these titers were from a different population, it is interesting to consider these findings in the context of our data and what they mean for population-level immunity. If we apply these cutoffs to our data, we estimate that 90% of individuals with a primary infection caused by DENV3 would be protected against DENV1 after 6 months and 1 year. Similarly, of those with primary DENV1 or 3 infection, 56% would be protected against DENV2 at 6 months, and 48% would be protected after 1 year. Finally, primary infections by DENV1 or 3 result in protection against DENV4 infections in 91% of individuals after 6 months and in 86% after 1 year. In a recent vaccine trial, protection was not conferred against DENV2 [22], although postvaccination $PRNT_{50}$ values were comparable to those for the other serotypes. Our detection of higher DENV2 titers, as well as the suggestion of inferred lower protection against DENV2 compared to the other serotypes after DENV1 and DENV3 primary infection, accord with this. The observed differences in titers to the serotypes could be due to assay differences and so must be considered in multiple epidemiological and laboratory settings.

The observed increase in average antibody titers up until 2 and 3 years after infection is consistent with boosting of titers due to subsequent infection. These observations will be a function not only of the immune response, but also of the transmission setting. With the $\geq$ 4-fold increase as a cutoff, the serotype distributions of increases are consistent with the observed cases in 1996 and 1997, with similar values for DENV1-3 in 1996 (spread across both 1–2-year and 2–3-year periods) and greater values for DENV3 than the other serotypes in 1997 (2–3 years only) [2]. This adds strength to the conclusion that these increases are due to exposure. However, there are some inconsistencies. First, there were more DENV4 increases than would be expected due to the case numbers (perhaps due to larger proportions of nonclinical cases for DENV4). In addition, the proportion with increases was high (higher than one would expect from force of infection calculations (as shown by Rodriguez-Barraquer et al [23], for example). However, because the children in this study have already experienced at least one infection, they may have a higher exposure to dengue than average. Finally, this result is not robust to differences in the infection cutoff used. Therefore, more work is needed to establish the cutoff for determining whether an infection has occurred and whether it varies across serotypes. Boosting did not occur in all individuals, and there were lower titers in those with boosting, compared with those who did not, perhaps indicating that, at higher levels, the neutralizing antibody becomes sterilizing, clearing virus before a measureable immune boost occurs. Further work is needed in cohorts to determine these levels. Titer boosting over these longer time frames after postprimary infections is suggestive of tertiary or quaternary exposures, but we do not have information on whether such exposures were clinically apparent or sufficiently infectious to support transmission. Although the serotype specificity in titer increases suggest that the titer increases were due to infection, maturation of the immune response cannot definitively be ruled out. If occurring, these antibody boosts could be important in maintaining protective immunity in individuals in dengue-endemic countries.

Our results show the importance of analyzing data at an individual level, but there were several limitations to our analysis. We did not have information on previous infecting serotypes for the postprimary cases: we only had enough cases of primary DENV1 and DENV3 for comparison, and the numbers of subjects followed were small in the later time points. Thus, we can only speculate on antibody kinetics after a first infection with DENV2 and DENV4. Also, all subjects had symptomatic DENV infection that led them to seek medical attention; antibody kinetics might differ after milder or subclinical DENV infection. We did not have any information on cell-mediated immunity or the capacity of antibody to enhance. Further studies that consider antibody dynamics in different settings, as well as those that include other antibody measurements or more-detailed characterization of the immune response, as in the study by de Alwis et al [24], will be useful in furthering our understanding of the antibody response after infection.

This study characterized the antibody titer trajectories after natural primary and postprimary DENV infection in children in a setting of high dengue transmission. A thorough understanding of these dynamics will aid in understanding the interactions between serotypes in dengue vaccine studies and in identifying natural correlates of protection.

Notes

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