

# Maternal Antibody and Viral Factors in the Pathogenesis of Dengue Virus in Infants

Cameron P. Simmons,<sup>1</sup> Tran Nguyen Bich Chau,<sup>1</sup> Tran Thi Thuy,<sup>3</sup> Nguyen Minh Tuan,<sup>2</sup> Dang Minh Hoang,<sup>1</sup> Nguyen Thanh Thien,<sup>2</sup> Le Bich Lien,<sup>2</sup> Nguyen Thien Quy,<sup>1</sup> Nguyen Trong Hieu,<sup>4</sup> Tran Tinh Hien,<sup>5</sup> Catriona McElnea,<sup>7</sup> Paul Young,<sup>6</sup> Steve Whitehead,<sup>8</sup> Nguyen Thanh Hung,<sup>2</sup> and Jeremy Farrar<sup>1</sup>

<sup>1</sup>Oxford University Clinical Research Unit, Hospital for Tropical Diseases, <sup>2</sup>Department of Dengue Haemorrhagic Fever, Children's Hospital No. 1, <sup>3</sup>Department of Infectious Diseases, Children's Hospital No. 2, <sup>4</sup>Department of Neonatology, Hung Vuong Hospital, and <sup>5</sup>Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam; <sup>6</sup>School of Molecular and Microbial Sciences, University of Queensland, St. Lucia, and <sup>7</sup>Panbio Ltd., Sinnamon Park, Brisbane, Australia; <sup>8</sup>Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland

**The pathogenesis of dengue in infants is poorly understood. We postulated that dengue severity in infants would be positively associated with markers of viral burden and that maternally derived, neutralizing anti-dengue antibody would have decayed before the age at which infants with dengue presented to the hospital. In 75 Vietnamese infants with primary dengue, we found significant heterogeneity in viremia and NS1 antigenemia at hospital presentation, and these factors were independent of disease grade or continuous measures of disease severity. Neutralizing antibody titers, predicted in each infant at the time of their illness, suggested that the majority of infants (65%) experienced dengue hemorrhagic fever when the maternally derived neutralizing antibody titer had declined to <1:20. Collectively, these data have important implications for dengue vaccine research because they suggest that viral burden may not solely explain severe dengue in infants and that neutralizing antibody is a reasonable but not absolute marker of protective immunity in infants.**

Dengue virus (DENV), of which there are 4 serotypes (DENV1–4), is an important cause of morbidity in many developing countries [1, 2]. Although most DENV infections are unremarkable, occasionally, infection leads to a syndrome called dengue hemorrhagic fever (DHF). DHF is a serious illness characterized by systemic vascular leakage, thrombocytopenia, and, in severe cases, hypovolemic shock.

The epidemiology of DHF in Southeast Asia suggests a bimodal distribution with regard to age at presentation [3]. Infants <12 months of age and, to a greater

extent, children >3 years of age and young adults represent most of the DHF disease burden [3]. Epidemiological studies indicate that DHF in children and adults is associated with secondary DENV infection, typically by a DENV serotype distinct from the individual's first dengue infection [4–7]. In contrast, most cases of DHF in infants represent primary DENV infections [8]. Infants with DHF can be difficult to manage because of their inherently poor capacity to compensate for vascular leakage and because of other systemic organ dysfunction [8].

Antibody-dependent enhancement (ADE) of DENV infectivity is suggested to be central to the pathogenesis of DHF in infants. In infants born to dengue-immune mothers, the decay of maternally derived IgG is suggested to reveal a window period of time in which the infant possesses subneutralizing levels of antibody but levels of antibody that are still capable of enhancing DENV infection in Fc receptor-bearing host cells. Increased viral loads resulting from ADE might then drive the production of inflammatory, vasodilatory molecules that promote vascular permeability [9, 10]. The evi-

Received 10 January 2007; accepted 22 February 2007; electronically published 19 June 2007.

Potential conflicts of interest: none reported.

Financial support: Wellcome Trust; Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health. The NS1-based assays were in part supported by the Australian Research Council.

Reprints or correspondence: Cameron Simmons, Oxford University Clinical Research Unit, Hospital for Tropical Diseases, 190 Ben Ham Tu, Dist. 5, Ho Chi Minh City, Vietnam (camsimmons1@gmail.com).

**The Journal of Infectious Diseases** 2007;196:416–24

© 2007 by the Infectious Diseases Society of America. All rights reserved.

0022-1899/2007/19603-0012\$15.00

DOI: 10.1086/519170

dence to support a role for maternal IgG in the pathogenesis of dengue in infants are inferred from epidemiological data and more directly from a small study of Thai infants [3, 10]. The need for further insights into dengue pathogenesis in infants led to the present study. The main findings—that viral burden was not associated with clinical severity in infants with DHF and that maternally derived neutralizing antibody was a moderate but not definitive marker of protective immunity—have implications for models of dengue pathogenesis and immunity.

## PATIENTS, MATERIALS, AND METHODS

### Patient Recruitment

**Healthy cohort.** A cohort of 55 full-term infants were recruited at birth from Hung Vuong Hospital, Ho Chi Minh City (HCMC). Cord plasma samples were collected at birth, and infant plasma was collected at 6, 9, and 12 months of age.

**Infants with dengue.** Infants <18 months of age with suspected dengue were recruited into the study at Paediatric Hospital Numbers 1 and 2, HCMC, between November 2004 and March 2006. Recruitment also took place in the outpatient department of Paediatric Hospital Number 1 between July 2005 and December 2005. Daily venous or capillary blood samples were collected from infants for 4 days beginning on entry to the study (study day 1) and again 10–14 days after discharge from the hospital. The length of illness for each patient was reported by the mother. The day of illness onset was used as a reference point (rather than the day of defervescence), because several infants were afebrile at study entry. Venous blood samples were collected from the mother at hospital presentation and again 4–8 days later. The extent of hemoconcentration during symptomatic illness was determined by comparing the maximum hematocrit recorded during hospitalization with either the value recorded at follow-up (74% of patients) or against an age- and sex-matched population value (26% of patients). World Health Organization (WHO) classification criteria [11] were applied to each case after review of study notes. Written informed consent was obtained from a parent or guardian of each patient. The study protocols were approved by the scientific and ethical committees at Hung Vuong Hospital, Paediatric Hospital Number 1, Paediatric Hospital Number 2, and the Oxford University Tropical Research Ethical Committee.

### DENV Polymerase Chain Reaction (PCR)

DENV loads in plasma were measured using an internally controlled, serotype-specific, real-time reverse-transcriptase PCR assay that has been described elsewhere [12]. Results were expressed as cDNA equivalents per milliliter of plasma. All reactions were performed in duplicate, and sample measurements were only valid when there was a detectable signal from the internal control amplicon.

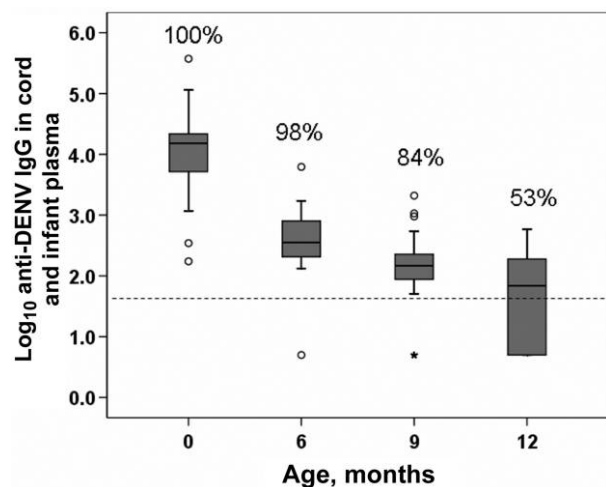
### Serologic Testing

**Capture ELISA.** A capture IgM and IgG ELISA, using both inactivated dengue and Japanese encephalitis (JE) antigens, was performed as described elsewhere [13]. This assay allowed a level of discrimination between dengue- or JE-specific IgM or IgG.

**Indirect ELISA.** A commercial, indirect ELISA (Panbio) was used to measure maternally derived anti-dengue antibody responses in cord serum samples and healthy infants. This assay measures IgG antibodies to purified DENV1–4 virions coated on the plate. The titer was defined as the reciprocal of the dilution giving an optical density of 0.3. A second indirect ELISA was developed to measure IgG antibodies to the DENV E protein. Briefly, dilutions of plasma were added to ELISA plates (Nunc) previously coated with soluble, recombinant E proteins from each of DENV1–4 (80% of the N terminus) (Hawaii Biotech). After washing, bound IgG was detected using a horseradish peroxidase–conjugate antiserum (Sigma). Antibody titer was defined as reciprocal of the last dilution of plasma that gave an optical density value above the cutoff value (defined as the optical density of blank control wells plus  $3 \times \text{SD}$ ). Measles virus antibody concentrations were quantitated using a commercial ELISA (IBL).

### Quantification of sNS1 Levels

Free (non-immune-complexed) dengue viral sNS1 levels were determined in consecutive daily plasma samples by capture



**Figure 1.** Decay of maternally derived, dengue-reactive antibody in healthy Vietnamese infants. Shown are the median and interquartile ranges of anti-dengue virus (DENV) IgG in 55 infants at birth (cord blood) and again at 6, 9, and 12 months. The mean half-life of dengue-reactive IgG in each infant was 42 days (range, 21–77 days). All infants were IgM negative at all time points. The dashed line represents the limit of detection in the indirect ELISA, and the values above the symbols represent the percentage of infants with detectable dengue-reactive IgG at each time point.

**Table 1. Summary of presenting clinical features and outcome in infants with primary dengue and in infants with other febrile illness (OFI).**

| Variable  | Outpatient dengue<br>(n = 9) | Inpatient              |                                   | P <sup>a</sup> |
|---|------------------------------|------------------------|-----------------------------------|----------------|
|   |                              | Dengue<br>(n = 66)     | Other febrile illness<br>(n = 29) |                |
| Male sex  | 50 (4)                       | 59 (39)                | 62 (18)                           | .8             |
| Age, mean (range), months                               | 8 (2–18)                     | 7 (2–13)               | 9 (2–18)                          | .03            |
| Duration of illness, <sup>b</sup> mean<br>(range), days | 5 (3–6)                      | 4 (1–6)                | 3 (1–5)                           | .0001          |
| Platelet nadir, median (range)                          | 101,000 (30,000–367,000)     | 29,150 (20,000–37,600) | 100,000 (78,000–146,000)          | .0001          |
| Petechia  | 0                            | 100 (66)               | 68 (19)                           | .0001          |
| Hepatomegaly  | 0                            | 100 (66)               | 52 (15)                           | .0001          |
| Cardiovascular shock                                    | 0                            | 7 (5)                  | 3 (1)                             | .663           |
| Maximum hemoconcentration,<br>median (range)            | 1.8 (–5.8 to 22.7)           | 25 (0–63)              | 9 (0–28)                          | .0001          |
| Clinical leak <sup>c</sup>                              | ND                           | 47 (31)                | 0 (0)                             | .0001          |
| WHO grade   |                              |                        |                                   |                |
| DF  | 2                            | 0                      |                                   |                |
| DHF I   | 0                            | 0                      |                                   |                |
| DHF II  | 3                            | 60                     |                                   |                |
| DSS   | 0                            | 6                      |                                   |                |
| Indeterminate   | 4                            | 0                      |                                   |                |
| Death   | 0                            | 3 (2)                  | 3 (1)                             | 1.000          |

**NOTE.** Data are % (no.), unless otherwise indicated. DF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome; ND, not done; WHO, World Health Organization.

<sup>a</sup> Equality of populations (Mann-Whitney *U* test) between inpatients with dengue and patients with OFI.

<sup>b</sup> On study day 1.

<sup>c</sup> Clinical evidence of vascular leak revealed by ultrasound or chest x-ray (pleural effusions, ascites, or gall bladder).

ELISA using a dengue group-reactive monoclonal antibody as the detection probe [14]. Samples were initially screened in a blinded fashion at a 1:10 dilution, with positive samples subsequently serially diluted and assayed in duplicate. Levels of sNS1 in patient plasma were quantified by comparison with standard curves generated by analysis of serial dilutions of affinity purified recombinant DENV NS1.

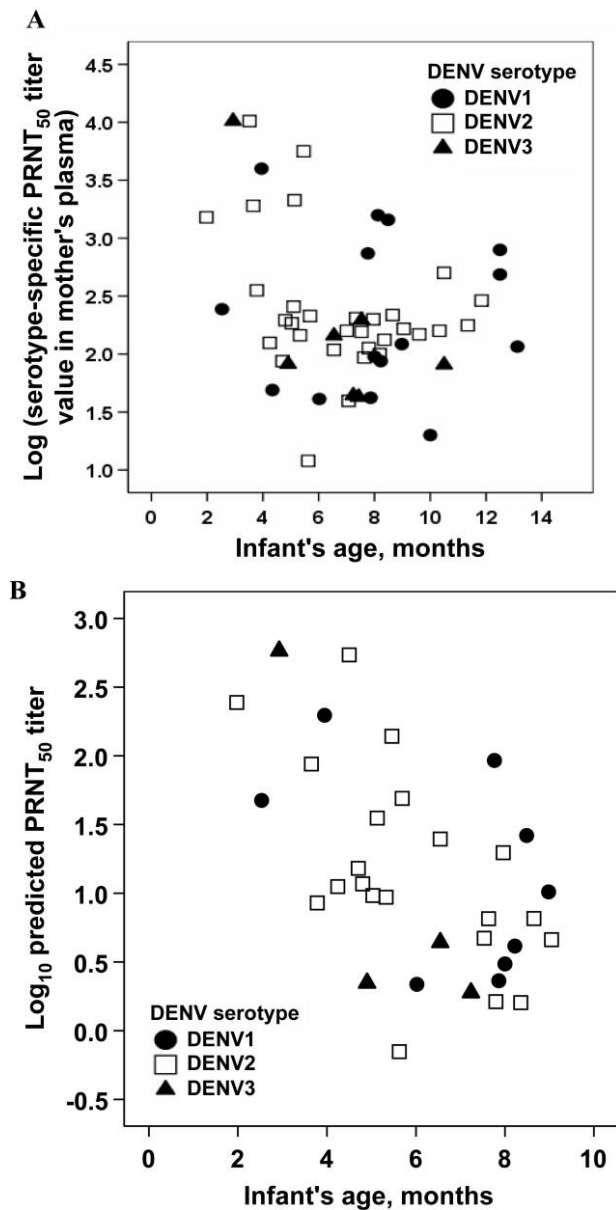
#### Plaque-Reduction Neutralization Test (PRNT)

Neutralizing antibody titers were determined by a complement-enhanced PRNT. Briefly, test plasma were heat inactivated (30 min at 56°C) and serial 2-fold dilutions beginning at 1:10 were prepared in serum-free medium containing 0.25% human serum albumin. Test virus, diluted to 500 pfu/mL in medium containing guinea pig complement (Cambrex) at a complement fixation titer of 1:10, was added to equal volumes of diluted test serum and incubated at 37°C for 30 min. Medium was removed from monolayer cultures of Vero cells on 24-well plates, and 0.1 mL of virus/serum mixture was transferred into duplicate wells. After incubation for 60 min at 37°C, the wells were overlaid with medium containing 1% methylcellulose and 2% fetal bovine serum. Samples were incubated at 37°C for 4–5 days, plaques were visualized by immunoperoxidase staining,

and the PRNT<sub>50</sub> was calculated. We used the following viruses: DENV1 Puerto Rico/94, DENV2 NGC prototype, DENV3 Sleman/78, and DENV4 814669.

#### Statistics

The degree of association between 2 variables was measured by Pearson's correlation analysis. The decay rate of maternally derived DENV- and measles-specific IgG in infants was assumed to follow a monophasic exponential model. The individual slope, (i.e., the individual elimination constant in the exponential model) was calculated using the ordinary least-squares regression method in which the model regresses the log<sub>10</sub> of the specific antibody titers at points in time since birth. For measles antibody decay, the individual slope was estimated from 2 time points. The eliminating half-life of IgG was derived from the eliminating slope through the formula half-life = log<sub>10</sub>(2) / |eliminating slope|. A random-effects model was used to identify factors associated with viremia or NS1 concentration. Parameters included in the model were days of illness, sex, thrombocytopenia, and hemoconcentration. All analyses were done in Stata (version 8.2; StataCorp).



**Figure 2.** Maternal neutralizing antibodies and dengue hemorrhagic fever. *A*, Age of individual infants with primary dengue virus (DENV) at hospital presentation vs. the corresponding maternal plasma 50% plaque-reduction neutralization test (PRNT<sub>50</sub>) titer against the serotype of DENV that infected each infant. Only full-term infants in whom the serotype of the infecting virus was known and whose mothers who did not have evidence of recent DENV infection were included in this analysis ( $n = 64$ ). *B*, Predicted PRNT<sub>50</sub> titer of maternally derived antibody in the infant at the time of infection vs. the age of individual infants at hospital presentation. The predicted PRNT<sub>50</sub> at the time of infection was calculated using the maternal PRNT<sub>50</sub> titer and the half-life of maternal antibody as suggested by the change in measles virus-specific IgG in each mother-infant pair. Only full-term infants <9 months of age in whom the serotype of the infecting virus was known and whose mothers who did not have evidence of recent DENV infection were included in this analysis ( $n = 37$ ). Infants older than 9 months of age were excluded because of the confounding effect of routine measles vaccination at 9 months of age in Vietnam.

## RESULTS

### Decay of Maternally Derived Anti-DENV Antibodies in Infants

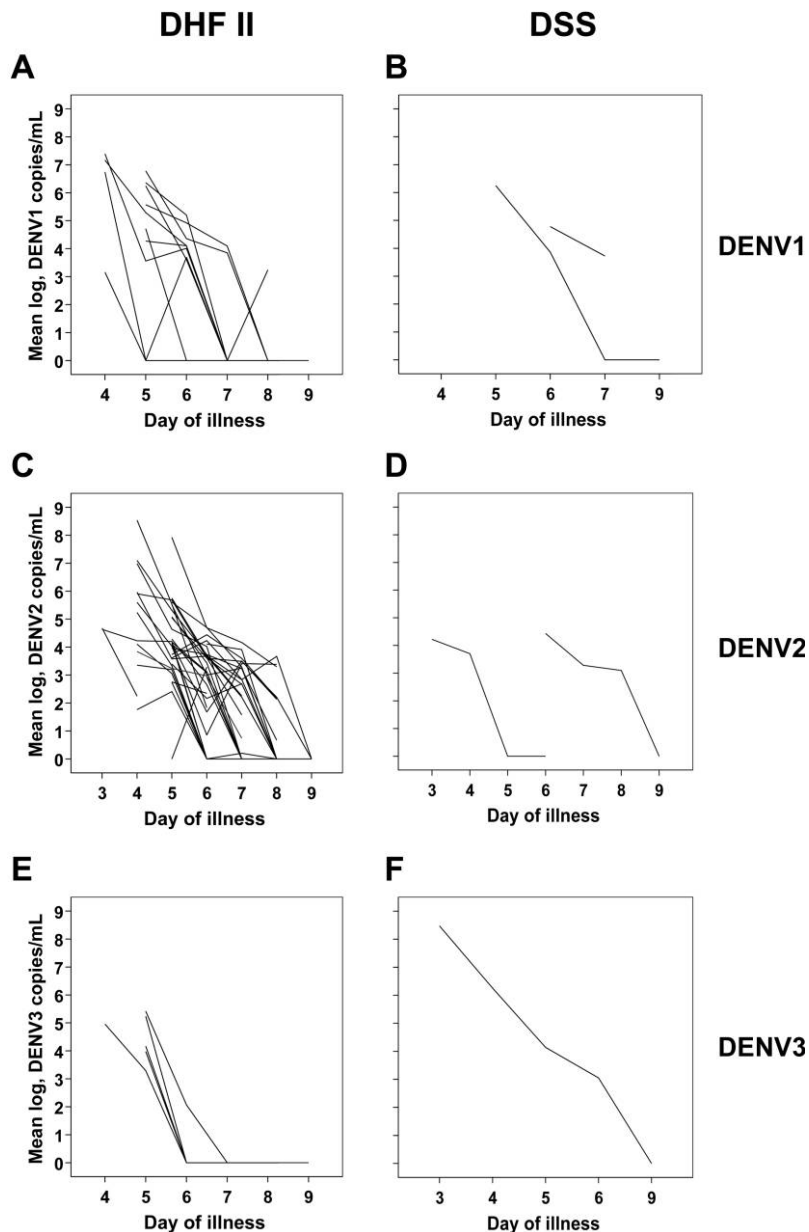
The decay of maternally derived anti-DENV antibody, suggested to be a key determinant of dengue severity during infancy [10], was measured in a cohort of 55 Vietnamese infants followed from birth to 1 year of age. At birth, all cord serum samples contained detectable anti-DENV IgG (median titer, 15,315; range, 174–373,835). Anti-DENV IgG but not IgM was detected in 98% of infants at 6 months, in 84% at 9 months, and in 53% at 12 months (figure 1). Consistent with previous studies, the mean half-life of anti-DENV IgG in these infants was 42 days (range, 21–77 days) [10, 15]. Collectively, these data suggest a 100% dengue seroprevalence rate for Vietnamese women residents of HCMC and reveal the persistence of dengue-reactive IgG for at least 12 months in some infants.

### Clinical Findings in Infants with Dengue

Vietnamese infants ( $n = 158$ ) <18 months of age with suspected dengue at Paediatric Hospital Numbers 1 and 2 in HCMC were recruited into this study. Ninety-seven study subjects were treated as inpatients; 66 (68%) of these were serologically diagnosed with primary dengue and 2 (2%) with secondary dengue, and 29 patients (30%) were designated other febrile illness (OFI). Sixty-one infants were treated as outpatients, of whom 9 (15%) were serologically diagnosed with primary dengue. The demographic and clinical features of these patients are summarized in table 1. Of note, all hospitalized infants with primary dengue were <13 months of age. Hospitalized infants with primary dengue had significantly lower platelet nadirs and greater hemoconcentration and were more frequently observed with petechia, hepatomegaly, and systemic vascular leak than infants with OFI (table 1). Two infants with primary dengue and prolonged dengue shock syndrome (DSS) died. Most inpatient infants with primary dengue were classified as DHF grade II (table 1).

### Maternal Exposure to DENV Infection

The shared environment of a mother and her infant suggested that mothers may themselves have had a recent DENV exposure. Accordingly, serological changes suggestive of recent DENV infection were proportionally more common in mothers of infants with dengue (10/73 [14%]) than without dengue (1/29 [3%]). Serological changes manifested as either 4-fold rises in Env-reactive IgG ( $n = 8$ ; average fold increase, 20; range, 4–64), 4-fold declines in Env-reactive IgG ( $n = 2$ ; average decrease, 10; range, 4–16), and/or the presence of anti-dengue IgM at hospital presentation (1 subject) or discharge (2 subjects). NS1 was not detected in any maternal plasma samples. Collectively, these data suggest recent DENV infection in some



**Figure 3.** Viremia in infants with dengue virus (DENV). Shown are the kinetics of viremia by day of illness in individual infants with dengue hemorrhagic fever (DHF) grade II or dengue shock syndrome (DSS) when caused by primary DENV1 (A and B), primary DENV2 (C and D), or primary DENV3 (E and F). The limit of detection was 650 cDNA equivalents/mL. Patients diagnosed with dengue fever ( $n = 2$ ) had undetectable viremia.

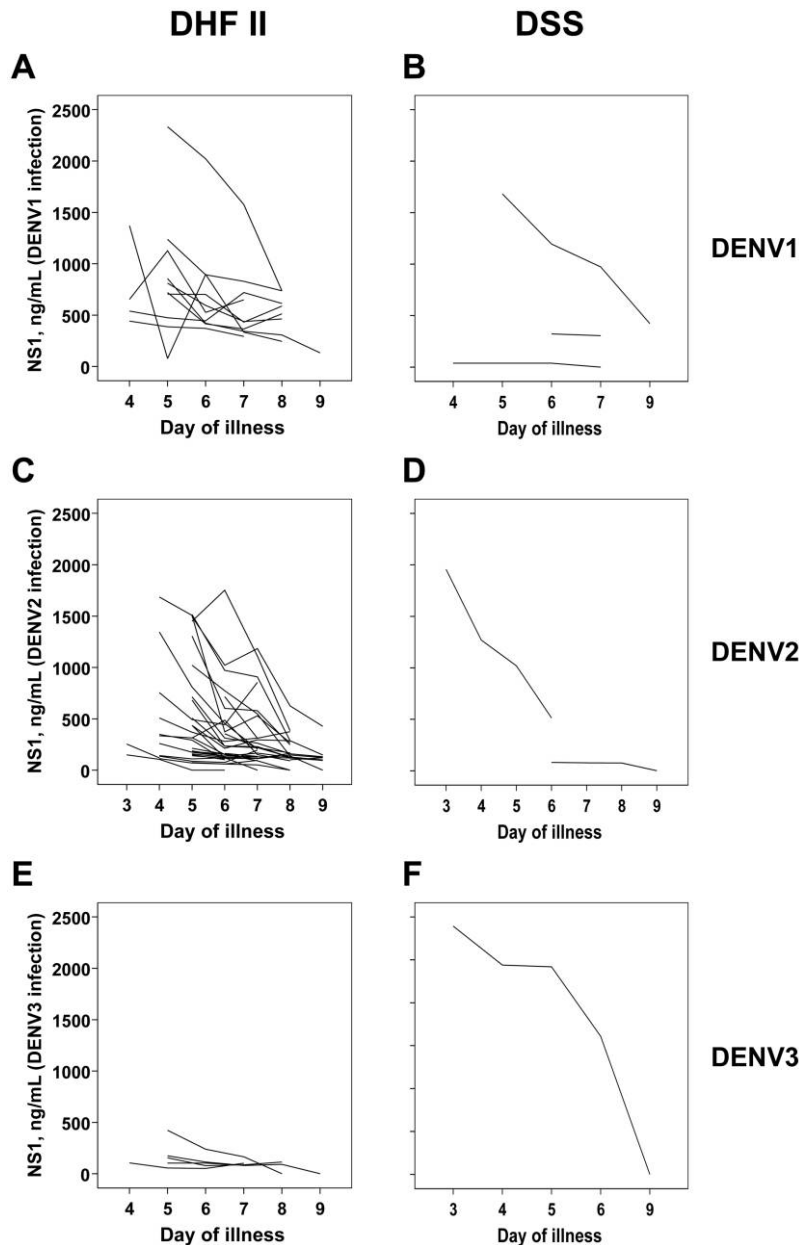
mothers, consistent with a shared exposure, such as in the home, for these mother-infant pairs.

#### Neutralizing Antibody and Immunity

Maternally derived, neutralizing anti-DENV antibodies are postulated to confer immunity to dengue during the first months of life. In the present study, neutralizing antibodies to at least 1 DENV serotype were present in all maternal plasma samples, with 96% of mothers possessing neutralizing antibodies to  $\geq 3$  serotypes (data not shown). Maternal PRNT<sub>50</sub> titers against the serotype of virus that infected each infant were then compared

with the infant's age at illness onset. Only mothers of infants with primary dengue, who delivered their baby after 37 weeks gestation and who did not have serological evidence of recent infection were included in this analysis ( $n = 64$ ). Surprisingly, there was no correlation between serotype-specific neutralizing antibody titers in the mother and the age at which their infant experienced dengue (figure 2A). There was also no correlation between infant age and neutralizing antibody titers to serotypes that did not correspond to the infecting virus (data not shown).

Measurement of the neutralizing maternal antibody levels at the time of DENV exposure in the infant were estimated in-



**Figure 4.** NS1 antigenemia in infants with dengue virus (DENV). Shown are the kinetics of NS1 antigenemia by day of illness in individual infants with dengue hemorrhagic fever (DHF) grade II or dengue shock syndrome (DSS) when caused by primary DENV1 (A and B), primary DENV2 (C and D), or primary DENV3 (E and F). The limit of detection was 45 ng/mL. Patients diagnosed with dengue fever ( $n = 2$ ) had undetectable antigenemias.

directly because of the confounding effect of the infant's own immune response by the time of hospital presentation. To predict the titer of neutralizing anti-DENV antibody in each infant at the time of DENV infection, measles virus-specific IgG was used as a surrogate marker of maternal antibody. In this approach, measles virus-specific IgG titers were measured in each mother-infant pair, and then these data were used to calculate the half-life of maternal antibody in each infant. This method suggested that the mean half-life of measles-specific IgG was 45 days (range, 21–81 days), almost identical to the 42-day

half-life of dengue-reactive antibody measured in healthy infants (figure 1). The maternal DENV PRNT<sub>50</sub> titer, together with the calculated half-life of maternal antibody in each infant, were then used to infer the serotype-specific PRNT<sub>50</sub> titer in each infant at the time of their infection. This approach revealed 3 groups of patients. In the first, the majority (65%) of infants presented with dengue when the passively acquired PRNT titer had declined to <1:20 against their infecting serotype. A second group of patients (21%) had predicted PRNT titers between 1:20 and 1:100 at the time of presentation. A third group of

young infants with DHF (14%) were predicted to possess substantial neutralizing antibody (PRNT<sub>50</sub> range, 138–580) against their infecting DENV serotype (figure 2B) at hospital presentation. These data suggested that maternal neutralizing antibody, as measured by PRNT<sub>50</sub> assays, was a generally good, although not absolute, predictive marker of immunity to DHF in infants.

### Viral Burden in Infants with Primary Dengue

We postulated that markers of viral burden would be positively associated with disease severity in infants. To explore this hypothesis, plasma viral loads were measured in infants for 4 consecutive days after hospital presentation and again at hospital discharge (figure 3A–F). DENV1 (14/75 [19%]), DENV2 (36/75 [48%]), and DENV3 (8/75 [11%]) were detected in admission plasma samples of patients with primary dengue, with a further 17 (22%) of 75 patients aviremic at study entry. A feature of these data was the heterogeneity in viremia in patients with highly similar clinical presentations. Thus, some patients with DHF had substantial viremia in excess of 10<sup>6</sup> cDNA equivalent copies/mL, whereas others, including several patients with DSS, had 1000-fold lower viral burdens on the same day of illness (figure 3A–F).

Plasma NS1 concentrations were also highly variable in magnitude and duration in clinically similar patients; for example, patients with DHF grade II had plasma NS1 concentrations ranging from 105 to 1686 ng/mL on the fourth day of illness (figure 4A–F). Interestingly, plasma NS1 concentrations did not correlate with plasma viral load in the same sample (figure 5), suggesting that these markers of viral burden are not interdependent.

A random-effects model was used to identify associations between day of illness, viral serotype, viral load, NS1 antigenemia, hemoconcentration, and thrombocytopenia. NS1 concentrations were significantly higher in patients with DENV1 infection ( $P = .01$ ). However, no other associations were observed between markers of viral burden and categorical or continuous measures of disease severity.

### Kinetics of Viral and Antibody Events in Primary Dengue

The relationship between day of illness onset, NS1 antigenemia, viral load (PCR), and IgM and IgG antibody responses are summarized in figure 6. Overall, against IgM as a gold standard, diagnosis of dengue by detection of NS1 at hospital presentation had a sensitivity of 95% and a specificity of 100%. PCR was less sensitive at 77% (PCR was not performed on IgM-negative patients). Moreover, NS1 remained detectable in plasma samples for up to 11 days after illness onset, several days after the PCR results had become negative. DENV-specific IgM was detected in most infants by the fourth day of illness and in 100% of infants after the seventh day of illness, around the same time at which DENV-specific IgG was first detected. Collectively,

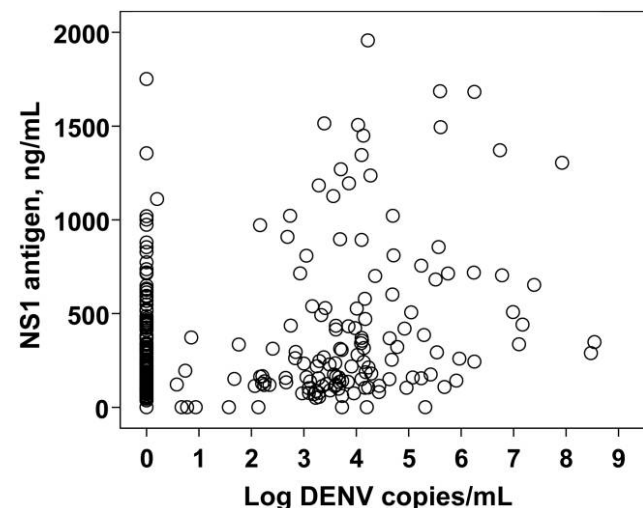
these data identify NS1 as a sensitive and long-lived marker of DENV infection in infants.

## DISCUSSION

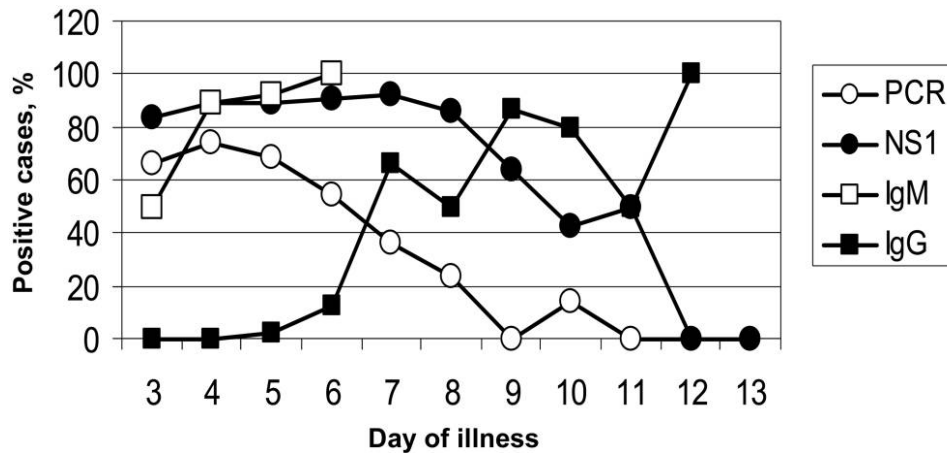
In this study, we postulated that dengue severity in infants would be positively associated with markers of viral burden. Instead, we found significant heterogeneity in viremia and NS1 antigenemia in infants with DHF and DSS, and these factors were not related to markers of clinical severity. These data imply that there is a more complex pathogenesis in infants and that other factors besides viral burden, such as host genetic or innate immune responses, should be considered in the infant pathogenesis model. A second hypothesis was that maternally derived, neutralizing anti-dengue antibody would have decayed before the age at which infants with DHF presented to hospital. Accordingly, most, but not all, cases of DHF occurred in infants with estimated maternally derived neutralizing-antibody levels <1:20.

DHF/DSS in infants is associated with a higher mortality than that in older children because of their inherently poor capacity to compensate for vascular hypovolemia and by occasional severe complications such as gastrointestinal bleeding, hepatic dysfunction, or encephalopathy [8, 16]. Early diagnosis is likely to benefit infants with dengue, and, therefore, our observation that NS1 was a sensitive and early diagnostic marker of infection should assist prompt and appropriate management in this group of patients.

Neutralizing antibody is suggested to provide protective immunity against dengue and is widely used as a primary measure



**Figure 5.** Plasma viremia vs. NS1 antigenemia in infants with dengue virus (DENV). Shown is the relationship between plasma viremia and NS1 antigenemia in individual infants with primary DENV and at least 1 measurable parameter at all time points assessed. There was no significant correlation between viral load and NS1 concentration.



**Figure 6.** Relationship between illness history and serological and virological markers of infection. Shown is the proportion of patients with primary dengue virus (DENV) with plasma that tested positive by either polymerase chain reaction (PCR), NS1 antigen detection assay, or IgM or IgG capture ELISA, according to the day of blood sampling.

of immunogenicity in dengue vaccine trials. The ability of DENV PRNT<sub>50</sub> assays to accurately predict protective immunity is contentious, however [17, 18]. Studies in infants are the only setting in which the protective efficacy of anti-dengue antibody can be investigated without the confounding influence of pre-existing cellular immunity. This study predicted PRNT<sub>50</sub> titers in infants at the time of their DENV infection by using anti-measles virus antibody concentrations to define the half-life of maternal antibody in each infant. This approach relies on several assumptions, most of which are supported by evidence. First, it assumes that maternal anti-dengue antibody concentrations are equivalent to cord blood concentrations at birth—an assumption supported by our own unpublished data and recent evidence [19]. Second, it assumes that anti-dengue IgG catabolizes at the same rate as anti-measles IgG—this is supported by the observation that the mean half-life of anti-dengue IgG in a healthy baby cohort (figure 1) was almost identical to the calculated mean half-life of measles IgG (42 vs. 45 days, respectively). Third, it assumes no change in the anti-dengue IgG concentration in the mother between parturition and the time the infant presented with dengue—our analysis excluded mothers with serological evidence of recent infection, but we cannot exclude the remote possibility that some mothers had DENV exposures shortly after birth that would result in an inaccurate prediction of the PRNT<sub>50</sub> titer in the infant at the time of infection. Fourth, these data assume that PRNT<sub>50</sub> titers obtained using prototype DENVs from each serotype will mimic those obtained using viruses recovered from individual patients. Although we have no evidence to support this assumption, it was not feasible to perform PRNT<sub>50</sub> assays using maternal plasma samples versus individual viruses isolated from every patient. Under these assumptions, we found most cases of DHF (65%) occurred in infants with estimated PRNTs<sub>50</sub> of

<1:20 at the time of infection. However, the finding that a small number of infants (all <6 months of age) developed DHF at an age when they were predicted to possess substantial neutralizing antibody suggests that either our estimates of maternally derived antibody titers were wrong in these infants or that other factors can influence infection outcome. These factors might include the immunological immaturity of young infants, the size of the infectious challenge, or genetic predisposition. If factors such as these are indeed critical determinants of infection outcome, then the results have implications for dengue vaccine development by underlining the potential limitations of PRNT<sub>50</sub> data as correlates of immunity, as has been suggested previously in children with secondary dengue [18].

During secondary DENV infections, viremia and NS1 antigenemia is higher in children with DHF than in those with dengue fever [20, 21]. In this study, neither viremia or antigenemia correlated with WHO disease grade or continuous measures of disease severity. This might reflect the measurement of viral parameters only in the days after hospital presentation, which in most subjects may be after the time of peak viremia and NS1 antigenemia. Nonetheless, it seems reasonable to expect most infants with high initial viral burdens will also have relatively high viral burdens at hospital presentation. Interestingly, in this patient population, NS1 antigenemia did not correlate with viremia in the same sample, leaving open the question as to which marker of DENV infection is the best correlate of in vivo viral burden. Collectively, these data do not suggest that dengue severity in infants is solely related to viral burden, albeit the present study was mostly limited to patients with DHF and DSS. Other factors besides viral burden may contribute to dengue severity in infants. For example, the nutritional status of Vietnamese infants has been previously linked with DHF; infants of low weight and height for age are un-



derrepresented among those with DHF/DSS [22]. Innate immune responses, represented by elevated plasma concentrations of tumor necrosis factor- $\alpha$ , interferon- $\gamma$ , interleukin (IL)-10, and IL-6, have been described in infants with DHF [8]. Innate immune responses and other antiviral mechanisms are influenced by polymorphic host genetic factors and may account for some aspect of disease susceptibility and pathogenesis.

The role of maternally derived antibody in the pathogenesis of DHF in infants requires further investigation. In this study, we did not directly evaluate whether DHF in infants occurred because of ADE. Indeed, measuring ADE and its association with clinical outcomes remains controversial [17]. To the best of our knowledge, DHF has not yet been described in an infant born to a dengue-naive mother. This observation represents an indirect but compelling argument that maternal anti-DENV antibody contributes to DHF pathogenesis in infants. However, further studies of infants with DHF, particularly in regions with low or moderate dengue endemicity or where outbreaks occur among previously naive populations, are needed to unequivocally confirm that maternally derived anti-DENV antibody is a critical risk factor for DHF in infants. A greater understanding of dengue in infants and in particular the role of ADE can be derived from large, prospectively followed birth cohorts; these are currently under way in Vietnam.

## Acknowledgments

We thank the nurses at Paediatric Hospital Numbers 1 and 2 and Hung Vuong Hospital for expert assistance and also the families of infants who participated in these studies. We also thank Dr. Nguyen Minh Tuan for assistance with statistical analyses.

## References

- Guzman MG, Kouri G. Dengue: an update. *Lancet Infect Dis* **2002**; *2*: 33–42.
- Monath TP. Dengue: the risk to developed and developing countries. *Proc Natl Acad Sci USA* **1994**; *91*:2395–400.
- Halstead SB, Lan NT, Myint TT, et al. Dengue hemorrhagic fever in infants: research opportunities ignored. *Emerg Infect Dis* **2002**; *8*:1474–9.
- Graham RR, Juffrie M, Tan R, et al. A prospective seroepidemiologic study on dengue in children four to nine years of age in Yogyakarta, Indonesia. I. Studies in 1995–1996. *Am J Trop Med Hyg* **1999**; *61*: 412–9.
- Thein S, Aung MM, Shwe TN, et al. Risk factors in dengue shock syndrome. *Am J Trop Med Hyg* **1997**; *56*:566–72.
- Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. *Am J Trop Med Hyg* **1988**; *38*:172–80.
- Sangkawibha N, Rojanasuphot S, Ahandrik S, et al. Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. *Am J Epidemiol* **1984**; *120*:653–69.
- Nguyen TH, Lei HY, Nguyen TL, et al. Dengue hemorrhagic fever in infants: a study of clinical and cytokine profiles. *J Infect Dis* **2004**; *189*: 221–32.
- Halstead SB. Antibody, macrophages, dengue virus infection, shock, and hemorrhage: a pathogenetic cascade. *Rev Infect Dis* **1989**; *11*(Suppl 4):S830–9.
- Kliks SC, Nimmanitya S, Nisalak A, Burke DS. Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. *Am J Trop Med Hyg* **1988**; *38*:411–9.
- World Health Organization (WHO). Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. Geneva: WHO, **1997**.
- Cameron P, Simmons SP, Dolocek C, et al. Patterns of host genome-wide gene transcript abundance in the peripheral blood of patients with acute dengue hemorrhagic fever. *J Infect Dis* **2007**; *195*: 1097–1107.
- Cardosa MJ, Wang SM, Sum MS, Tio PH. Antibodies against prM protein distinguish between previous infection with dengue and Japanese encephalitis viruses. *BMC Microbiol* **2002**; *2*:9.
- Young PR, Hilditch PA, Bletchly C, Halloran W. An antigen capture enzyme-linked immunosorbent assay reveals high levels of the dengue virus protein NS1 in the sera of infected patients. *J Clin Microbiol* **2000**; *38*:1053–7.
- Watanaveeradej V, Endy TP, Samakoses R, et al. Transplacentally transferred maternal-infant antibodies to dengue virus. *Am J Trop Med Hyg* **2003**; *69*:123–8.
- Nguyen TH, Nguyen TL, Lei HY, et al. Volume replacement in infants with dengue hemorrhagic fever/dengue shock syndrome. *Am J Trop Med Hyg* **2006**; *74*:684–91.
- Laoprasopwattana K, Libraty DH, Endy TP, et al. Dengue virus (DV) enhancing antibody activity in preillness plasma does not predict subsequent disease severity or viremia in secondary DV infection. *J Infect Dis* **2005**; *192*:510–9.
- Endy TP, Nisalak A, Chunsuttitwat S, et al. Relationship of preexisting dengue virus (DV) neutralizing antibody levels to viremia and severity of disease in a prospective cohort study of DV infection in Thailand. *J Infect Dis* **2004**; *189*:990–1000.
- Pengsaa K, Luxemburger C, Sabchareon A, et al. Dengue virus infections in the first 2 years of life and the kinetics of transplacentally transferred dengue neutralizing antibodies in Thai children. *J Infect Dis* **2006**; *194*:1570–6.
- Libraty DH, Young PR, Pickering D, et al. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. *J Infect Dis* **2002**; *186*:1165–8.
- Vaughn DW, Green S, Kalayanarooj S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* **2000**; *181*:2–9.
- Nguyen TH, Nguyen TL, Lei HY, et al. Association between sex, nutritional status, severity of dengue hemorrhagic fever, and immune status in infants with dengue hemorrhagic fever. *Am J Trop Med Hyg* **2005**; *72*:370–4.