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Short communication

Anti-dengue virus envelope protein domain III IgG ELISA among infants with primary dengue virus infections

Daniel H. Libraty[a,*], Lei Zhang[a], AnaMae Obcena[b], Job D. Brion[c], Rosario Z. Capeding[b,d]

[a] Division of Infectious Diseases and Immunology, Department of Medicine, University of Massachusetts Medical School, Worcester, MA, USA
[b] Department of Medicine, Research Institute for Tropical Medicine, Manila, Philippines
[c] San Pablo City Health Office, San Pablo, Philippines
[d] Department of Microbiology, Research Institute for Tropical Medicine, Manila, Philippines

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ABSTRACT

Dengue is the most prevalent arthropod-borne viral illness in humans. The current gold standard serologic test for dengue virus (DENV) infection is a neutralizing antibody assay. We examined a DENV recombinant (r)E protein domain III IgG ELISA among infants with primary DENV infections. Infants experience a primary DENV infection in the presence of maternally derived anti-DENV IgG. The estimated DENV rE protein domain III IgG levels to the infecting serotype at the time of infant primary symptomatic DENV2 and DENV3 infections correlated with the 50% plaque reduction neutralization reciprocal antibody titers ($PRNT_{50}$). Anti-DENVs 1–4 rE protein domain III IgG levels all correlated with each other, and the estimated rE protein domain III IgG level to the infecting serotype at the time of infection inversely correlated with dengue disease severity. The anti-DENV rE protein domain III IgG ELISA may be a useful and potentially high-throughput alternative to traditional DENV neutralizing antibody assays.

1. Introduction

Dengue is the most prevalent arthropod-borne viral illness in humans with half of the world's population at risk. The global burden of symptomatic dengue is on the order of 100 million cases/year (Bhatt et al., 2013). The dengue viruses (DENVs) are single-stranded, positive-sense, RNA-containing enveloped viruses belonging to the *Flavivirus* genus within the *Flaviviridae* family (Henchal and Putnak, 1990). There are four serotypes of DENVs (DENV1–4). DENV infections produce a wide spectrum of clinical illness. It ranges from asymptomatic or mild illness to a severe and potentially life threatening disease, dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS). The global spread of dengue, and the incidence of epidemic DHF, have increased dramatically over the past 50 years and continue on an upward trajectory (Halstead, 2007; Kyle and Harris, 2008).

The current gold standard serologic test for DENV infection is a neutralizing antibody assay. Most neutralizing antibodies against DENVs are directed against the major surface viral protein, the envelope (E) glycoprotein. The DENV E glycoprotein has been divided into three domains (domains I–III), and domain III has been found to be highly antigenic (Chavez et al., 2010).

Among infants with primary DENV infections, the DENV infection occurs in the presence of maternally derived anti-DENV IgG. We have been conducting a prospective clinical study of DENV infections during infancy in the Philippines (Libraty et al., 2009). We therefore examined a DENV recombinant (r)E protein domain III ELISA of IgG among infants with primary DENV infections. We found that estimated DENV rE protein domain III IgG levels to DENV2 and DENV3 at the time of infant primary symptomatic DENV infections correlated with the 50% plaque reduction neutralization reciprocal antibody titers ($PRNT_{50}$). Anti-DENVs 1–4 rE protein domain III IgG levels all correlated with each other, and the estimated rE protein domain III IgG level to the infecting serotype at the time of infection inversely correlated with dengue disease severity.

2. Methods

2.1. Ethics statement

The study protocol was approved by the institutional review boards of the Research Institute for Tropical Medicine, Philippines, and the University of Massachusetts Medical School. Mothers and their healthy infants were recruited and enrolled after providing written informed consent.

* Corresponding author at: Rm S6-862, Division of ID, UMMS, 55 Lake Ave N, Worcester, MA 01655, USA. Tel.: +1 508 856 4182; fax: +1 508 856 4890.
E-mail address: daniel.libraty@umassmed.edu (D.H. Libraty).

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2.2. Clinical study

The study began in January 2007 in San Pablo, Laguna, Philippines, and has been previously described (Libraty et al., 2009). Blood samples were collected from the infant and mother at the first study visit when the infant was between approximately 6 and 18 weeks old. Clinical and epidemiological information were collected at the study visit. We conducted surveillance year-round for hospitalized acute febrile illnesses in study infants across the seven hospitals serving San Pablo, Philippines. During the rainy season (June–November), mothers were encouraged to bring their infants to the San Pablo City Health Office for evaluation of outpatient febrile illnesses. Acute- and convalescent-phase (day 14) blood samples were obtained from study infants with febrile illnesses that did not have an obvious source at time of presentation (*e.g.* lobar pneumonia, bacterial meningitis, pyelonephritis). Routine clinical information was abstracted daily during any hospitalization and at the acute and convalescent time points for all febrile study infants.

A DENV infection was identified in febrile infants by serotype-specific RT-PCR in acute-phase sera (Lanciotti et al., 1992) and DENV IgM/IgG ELISA in paired acute and convalescent phase sera. Primary or secondary DENV infections were identified by previously established serologic criteria for the paired IgM/IgG ELISA results (Innis et al., 1989). The infecting DENV serotype was identified by RT-PCR for all the symptomatic infants.

Serial blood samples at three study visits over the first year of life from a subset of 250 infants in 2007 and 150 infants in 2009 without reported febrile illnesses were screened for evidence of clinically inapparent DENV infection using a hemagglutination-inhibition (HAI) assay to DENVs 1–4 (Clarke and Casals, 1958) or a single dilution flow cytometry neutralizing antibody assay (Kraus et al., 2007). A primary DENV infection was then identified by a >4-fold rise in DENV neutralizing antibody titer between two time points with a monotypic pattern (Endy et al., 2004). The DENV serotype with the highest neutralizing antibody titer in a monotypic pattern was assumed to be the serotype that produced the clinically inapparent infection.

2.3. DENV rE protein domain III ELISA

Briefly, 0.05 μg/ml of purified rE protein domain III for DENVs 1–4 produced in *Escherichia coli* (Table 1) (GenWay Biotech) were used as antigens on 96-well flat-bottomed microtiter plates (Thermo Scientific). Plates were blocked with Protein-Free T20 Blocking Buffer (Pierce Protein Biology). A 1:10 dilution of maternal sera was added to the plates and incubated for 2 h at room temperature. Then, horseradish peroxidase (HRP)-conjugated anti-human IgG was used as a secondary antibody (Santa Cruz Biotechnology). The ELISA plates were developed using TMB Substrate Solution (Pierce Technologies), and the reactions were stopped with 2 N sulfuric acid. Relative IgG concentrations were determined using optical spectrophotometer readings at 450 nm (OD values) using a MultiSkan FC microplate reader and analyzed with the MultiSkan software.

The estimated levels for maternally derived anti-DENV rE protein domain III IgG at the time of infant primary DENV infection were calculated using the following assumptions: (i) the anti-DENV IgG levels measured in the maternal sera obtained at the first study visit were assumed to be the levels present in the infant at birth (full-term birth for all study infants), (ii) maternally derived anti-DENV rE protein domain III IgG was assumed to decay in the infant with first order kinetics and a $t_{1/2} = 40$ days (Libraty et al., 2009), (iii) for infants with symptomatic DENV infections, the date of fever onset was assumed to be the date of DENV infection onset, (iv) for infants with asymptomatic/inapparent DENV infections, the date of the DENV infection could not be ascertained. In these cases, the date of the first blood collection in the paired sample seroconversion was used as the onset of the DENV infection.

Table 1
Amino acid sequences of the recombinant (r)E protein domain III peptides used in the IgG ELISAs.[a]

DENV1 rE protein domain III	VMCTGSFKLE KEVAETQHGT
	VLVQVKYEGT DAPCKIPFST
	QDEKGVTQNG RLITANPIVT
	DKEKPVNIEA EPPFGESYIV
	VGAGEKALKL SWFKKGSV
DENV2 rE protein domain III	SYSMCTGKFK IVKEIAETQH
	GTIVIRVQYE GDGSPCKIPF
	EIMDLEKRHV LGRLITVNPI
	VTEKDSPVNI EAEPPFGDSY IIIGVEPGQL
	KLDWFKKGSS IGQMFETTMR GAKRMA
DENV3 rE protein domain III	GSGMSYAMCL NTFVLKKEVS
	ETQHGTILIK VEYKGEDAPC
	KIPFSTEDGQ GKAHNGRLII
	ANPVVTKKNE PVNIEAEPPF
	GESNIVIGIG KALWINWYRK
	GSSIGKTTMR GAKRMA
DENV4 rE protein domain III	SYTMCYGMFS IDKEMAETQH
	GTTVGTVKYE GEGAPCKVPI
	EIRDVNKEKV VGRIISSTPF AEYTNSVTNI
	PLEPFGDSYI GIGVGDSALT
	LHWFRKGSSI VSSIGKTTMR GAKRMA

[a] Recombinant E protein domain III for DENVs 1–4 produced in *E. coli* were purchased from GenWay Biotech.

2.4. Statistical analysis

The SPSS software package (version 20.0) was used for statistical analyses. Correlations were determined using the non-parametric Spearman rank-order test. p-Values <0.05 were considered significant.

3. Results

3.1. DENV rE protein domain III sequences

The amino acid sequences of the DENV rE protein domain III sequences used in the ELISAs are shown in Table 1.

3.2. DENV rE protein domain III ELISA IgG levels correlate with plaque reduction neutralizing antibody titers

We estimated the DENV $PRNT_{50}$ at the time of infection among infants with symptomatic primary DENV infections using an infant serum sample obtained pre-infection. The methods have been previously described (Libraty et al., 2009). We estimated the DENV rE protein domain III IgG levels at the time of infection by ELISA, as described in Section 2. Among infants with primary symptomatic DENV2 infections, the DENV2 $PRNT_{50}$ correlated with the DENVs 1–4 rE protein domain III IgG OD values (Spearman $r = 0.98$, 0.98, 0.90, 0.88, DENVs 1–4 rE protein domain III IgG ODs, respectively, $p \leq 0.001$, $n = 10$). Among infants with primary symptomatic DENV3 infections, the DENV3 $PRNT_{50}$ correlated with the DENVs 1–4 rE protein domain III IgG OD values (Spearman $r = 0.44$, 0.42, 0.48, 0.43 DENVs 1–4 rE protein domain III IgG ODs, respectively, $p \leq 0.002$, $n = 49$) (Table 2). There was only one DENV1 and no DENV4 cases that had both $PRNT_{50}$ and rE domain III IgG OD values.