Journal of Clinical Virology 69 (2015) 63–67

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Antibody avidity following secondary dengue virus type 2 infection across a range of disease severity

Louis Lau[a], Angela M. Green[a], Angel Balmaseda[b], Eva Harris[a,*]

[a] Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, USA
[b] Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua

ARTICLE INFO

Article history:
Received 20 March 2015
Received in revised form 20 May 2015
Accepted 2 June 2015

Keywords:
Dengue virus
Antibody avidity
Dengue hemorrhagic fever/dengue shock syndrome
Disease severity

ABSTRACT

Background: The four dengue virus serotypes (DENV1-4) are responsible for the most prevalent mosquito-borne viral illness in humans. DENV causes a spectrum of disease from self-limiting dengue fever (DF) to severe, life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Antibodies from one infection can contribute to either protection or increased disease severity in a subsequent infection with a distinct DENV serotype. The effectiveness of the antibody response is modulated by both the affinity and avidity of the antibody/antigen interaction.
Objectives: We investigated how antibody avidity developed over time following secondary DENV2 infection across different disease severities.
Study design: We analyzed sera from 42 secondary DENV2-infected subjects (DF, $n = 15$; DHF, $n = 16$; DSS, $n = 11$) from a pediatric hospital-based dengue study in Nicaragua. IgG avidity against DENV2 virions was measured in samples collected during acute and convalescent phases as well as 3, 6, and 18 months post-illness using a urea enzyme-linked immunosorbent assay.
Results: The data show a significant increase in avidity from acute to convalescent phase followed by a decrease from convalescent phase to 3 months post-symptom onset, then a plateau. Linear regression analysis comparing antibody avidity between disease severity groups over time indicate that individuals with more severe disease (DHF/DSS) experienced greater decay in antibody avidity over time compared to less severe disease (DF), and ROC curve analysis showed that at 18 months post-illness, lower avidity was associated with previously having experienced more severe disease.
Conclusions: These data suggest that increased dengue disease severity is associated with lower antibody avidity at later time-points post-illness.

1. Background

The four dengue virus serotypes (DENV1-4) are responsible for the most prevalent mosquito-borne viral illness in humans, with up to 96 million symptomatic dengue cases annually and 3.6 billion people at risk for infection in tropical and subtropical regions worldwide [1]. DENV causes a spectrum of disease ranging from self-limiting dengue fever (DF) to the severe, life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2]. People exposed to primary DENV infections develop long-term serotype-specific neutralizing antibody responses, whereas secondary DENV infections generally result in neutralizing serotype cross-reactive responses [3]. Although the majority of secondary DENV infections with a heterotypic serotype result in subclincal or less severe disease (DF), secondary infection is a risk factor for more severe disease (DHF/DSS) [3].

In Nicaragua, the epidemiology of dengue is generally characterized by waves of one dominant serotype followed by another [4,5]. DENV infections in Nicaragua in 2006–2007 were predominantly DENV2 and marked by heightened clinical severity [5]. DENV2 has been reported to result in more severe disease than infection with other serotypes [6–9]. In addition, a change in the circulating DENV2 clade, together with the host's serotype-specific immunity, contributed to increased disease severity upon DENV2 infection during this period in Nicaragua [5]. Here, we examined the anti-DENV2 serum IgG avidity in samples collected in 2006–2007 as part of a pediatric hospital-based study in Managua, Nicaragua. In this study, suspected dengue cases are enrolled at presentation, treated and studied during the acute phase of illness, and followed

* Corresponding author at: Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, 185 Li Ka Shing Center, 1951 Oxford Street, Berkeley, CA 94720-3370, USA. Fax: +1 510 642 6350.
E-mail address: eharris@berkeley.edu (E. Harris).

http://dx.doi.org/10.1016/j.jcv.2015.06.003
1386-6532/

longitudinally for 18 months, with healthy blood samples collected at 14–21 days ("convalescent"), 3, 6, and 18 months post-illness [10].

The mechanism(s) underlying the potential for increased disease severity in secondary DENV infection are not fully understood but are thought to involve both serotype cross-reactive T cell responses and cross-reactive, poorly-neutralizing antibodies that can participate in antibody-dependent enhancement (ADE), whereby the Fc portion of non-neutralizing antibodies complexes with infecting virions to facilitate virus entry into Fc receptor-bearing target cells [3,11–14]. However, antibodies and T cells also contribute to protection in secondary infections [3]. The effectiveness of the antibody response is modulated by both the affinity and avidity of the antibody/antigen interaction, where avidity is the overall strength of antibody binding to its antigen and is influenced by antibody affinity, antibody valency, epitope accessibility, and epitope density [15]. What role antibody avidity contributes to protection or pathogenesis during DENV infection has yet to be elucidated. A previous study from our laboratory reported an association between anti-DENV3 serum IgG avidity and neutralization during DENV3 secondary infections [16]. Utilizing the same modified urea avidity enzyme-linked immunosorbent assay (ELISA), we assessed the association between disease severity and antibody avidity.

2. Objectives

The focus of this study was to evaluate anti-DENV2 IgG avidity over time after secondary infection and to examine the association of avidity with disease severity.

3. Study design

3.1. Ethics statement

The protocol for this study was reviewed and approved by the Institutional Review Boards of the University of California, Berkeley, and the Nicaraguan Ministry of Health. Parents or legal guardians of all subjects provided written informed consent, and subjects 6 years of age and older provided assent [10,16].

3.2. Study population

Study design and execution, subject enrollment and inclusion/exclusion criteria, and classification of disease severity according to the 1997 WHO Guidelines [2] were as previously described [10,17]. Study enrollment occurred in the Nicaraguan National Pediatric Reference Hospital, Hospital Infantil Manual de Jesús Rivera (HIIMJR), from August 1, 2006 to February 28, 2008. Subjects were between 6 months and 14 years of age. Serum samples were collected during the acute phase (3 consecutive days after enrollment), convalescence (14–21 days post-symptom onset), and at 3, 6 and 18 months post-onset of illness. DENV infection was confirmed by RT-PCR detection of viral RNA; isolation of DENV on C6/36 cells; and/or seroconversion by IgM ELISA or a ≥4-fold increase in total antibody titer as measured by Inhibition ELISA in paired acute- and convalescent-phase samples [18–20]. Secondary infection was defined by an antibody titer by Inhibition ELISA of ≥10 in acute samples or ≥2560 in convalescent samples [10]. Sample sizes were as follows: 15 DF, 16 DHF, and 11 DSS ($n = 42$) samples at acute phase; 15 DF, 15 DHF, and 11 DSS ($n = 41$) at 18 months; and 14 DF, 15 DHF, and 10 DSS ($n = 39$) samples at all 5 time-points (Table 1).

Table 1
Demographic data about study participants.

Parameter	*N*	%
General		
Female	19	45.2
Male	23	54.8
Age (mean, S.D.)		
Participant age (years)	9.5	3.8
Disease classification		
Dengue fever (DF)	15	35.7
Dengue hemorrhagic fever (DHF)	16	38.1
Dengue shock syndrome (DSS)	11	26.2
Immune status		
Secondary infection	42	100

3.3. Viruses and cell line

DENV2 virions were harvested from infected *Aedes albopictus* C6/36 cells (gift from Paul Young, University of Queensland, Australia) as previously described [16]. Cell supernatants were concentrated by Amicon filters (100 kDa, 3750 rpm for 30 min at 4 °C), then virus was pelleted by ultracentrifugation (26,000 rpm for 4 h at 4 °C, no brakes). The virus pellet was then resuspended in PBS and divided into aliquots for storage at −80 °C. DENV2 (strain N172, passage 5) was isolated in 2006 and was obtained from the National Virology Laboratory in Managua, Nicaragua.

3.4. Avidity assay

Serum avidity was measured using a modified ELISA protocol with urea washes [16]. Virions purified from Nicaraguan DENV2 N172 clinical isolate were used as antigen. To determine the amount of antigen to coat the plate, an indirect ELISA with pan-DENV mouse monoclonal antibody 4G2 (2 μg/mL) was used. Briefly, serial dilutions of viral antigen were plated, and the dilution of DENV2 antigen that yielded an optical density (OD) of 1 was selected. Ninety-six-well ELISA plates were coated with viral antigen overnight at 4 °C and then blocked in 5% non-fat dry milk in PBS for at least 1 h. Plates were incubated with heat-inactivated patient serum (1:100) for 1 h, and then treated with either 9 M urea or PBS for 10 min [16]. Next, biotinylated anti-human IgG antibody (1:1000, donkey anti-human IgG, Jackson ImmunoResearch) was added, followed by a streptavidin–alkaline phosphatase conjugate (1 μg/mL, Invitrogen) and PnPP substrate (1 mg/mL, Invitrogen), and OD was read at 405 nm on an ELx808 ELISA reader [16]. Background levels were determined with normal human serum consisting of pooled samples from Oakland Red Cross blood donors (1:100). Serum IgG avidity was calculated as the ratio of the OD of background-adjusted IgG bound to urea-treated wells compared to PBS-treated wells, as follows:

$$\left(\frac{\text{mean}_{\text{urea}}}{\text{mean}_{\text{PBS}}}\right) \pm \left(\frac{\text{mean}_{\text{urea}}}{\text{mean}_{\text{PBS}}}\right) \times \sqrt{\left[\left(\frac{\text{SD}_{\text{urea}}}{\text{mean}_{\text{urea}}}\right)^2 + \left(\frac{\text{SD}_{\text{PBS}}}{\text{mean}_{\text{PBS}}}\right)^2\right]}.$$

A positive control consisting of pooled DENV-immune Nicaraguan sera from donors to the Nicaraguan National Blood Bank was included on each plate. Acceptable ranges for the positive control were determined with a positive quality control plate, treating half the plate with 9 M urea and the other half with PBS. Experimental plates were included only if the background absorbance was <0.2 OD_{405nm}, positive control absorbance >5X background OD, and experimental positive control within one standard deviation of the positive quality control plate.