Use of an Immunoglobulin G Avidity Test To Discriminate between Primary and Secondary Dengue Virus Infections

Vanda Akico Ueda Fick de Souza,1 Silvana Fernandes,1 Evaldo Stanislau Araújo,2 Adriana Fumie Tateno,1 Olimpia M. N. P. F. Oliveira,2 Renato dos Reis Oliveira,1 and Cláudio Sérgio Pannuti1*

Laboratório de Virologia (LIMHC-52), Instituto de Medicina Tropical de São Paulo, Departamento de Moléstias Infecciosas e Parasitárias da Faculdade de Medicina, Universidade de São Paulo,1 and Hospital Ana Costa, Santos, São Paulo, Brazil

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An enzyme-linked immunosorbent assay-based immunoglobulin G (IgG) antibody avidity test was evaluated by using sera from 57 patients with acute dengue infection. Overall, 55 of 57 patients were correctly classified (27 of 27 with primary dengue and 28 of 30 with secondary dengue). We conclude that the IgG avidity test can be useful for differentiating between acute, primary, and secondary dengue infections.

An estimated 2.5 billion people in over 100 countries are at risk of acquiring dengue infection. Up to 50 million infections, resulting in 500,000 cases of dengue hemorrhagic fever (DHF) and 22,000 deaths, occur annually (World Health Organization website [http://www.who.int/ctd/dengue/burdens.htm]). The pathogenesis of DHF is still unclear (6, 8), although there is good evidence that secondary infection by a different serotype in patients harboring preexisting heterologous dengue antibodies increases the risk of DHF (3, 7, 9, 15, 20). The mechanism postulated to explain this epidemiological correlation is known as immune enhancement (9, 10, 16, 25, 29).

The immunoglobulin M (IgM) capture enzyme immunoassay is the most widely used serologic test for dengue diagnosis (6). In addition, the hemagglutination inhibition test has traditionally been used to classify dengue infections as primary or secondary (29). However, when the interval between the acute- and convalescent-phase samples is less than 7 days, in the absence of a change in antibody titers in acute- and convalescent-phase samples or in single specimens, interpretation of the dengue hemagglutination inhibition antibody response becomes difficult (6, 29). The hemagglutination inhibition test also requires serum pretreatment with acetone or kaolin to remove nonspecific inhibitors of hemagglutination and further absorption with gander or human type O red blood cells (29) and is not commercially available. Methods based on IgM/IgG ratios have also been used (13, 28). The assessment of IgG antibody avidity is a very useful tool for differentiating between primary and secondary immune responses (12). The IgG antibody avidity test makes use of the fact that in the primary infection, the specific IgG antibody response begins with low-avidity IgG antibodies which gradually evolve to high-avidity antibodies. In the secondary infection, the rapid antibody response is characterized by the production of high-avidity antibodies (23). The IgG antibody avidity test has been used to diagnose acute viral infections (5, 12, 17, 21, 27), to discriminate recent primary infection from reinfection or reactivation (1, 2, 4), to estimate the efficacy of vaccines (22), and to identify vaccine failures (18, 19). The aim of the present study was to evaluate the IgG avidity test for differentiating primary from secondary dengue infections.

Sera were obtained between March and July 2002 during a dengue outbreak in Santos, São Paulo, Brazil. During the study period, the Health Department of the State of São Paulo reported the occurrence of three dengue virus serotypes (Den 1, Den 2, and Den 3) in the city of Santos (Centro de Vigilância Epidemiológica website [http://www.cve.saude.gov.br/htm/z00/if_sem_den02.htm]). Blood samples were collected during the acute phase of infection, and a convalescent-phase sample was obtained at least 7 days later. The study was approved by the Ethics and Research Committee of the University of São Paulo Medical School. Confirmation of acute dengue was obtained by the detection of IgM antibodies. IgG and IgM antibodies were detected by using Dengue ELISA (enzyme-linked immunosorbent assay) IgG and Dengue ELISA IgM (Focus Technologies, Cypress, Calif.). To standardize the IgG avidity test, primary and secondary dengue infections were defined by using strict diagnostic criteria. Primary dengue infection was defined by a negative IgG test for a serum sample from the acute phase collected at least 4 days after disease onset, followed by seroconversion in the convalescent-phase serum sample. Secondary dengue infection was defined by a positive IgG test for an acute-phase sample obtained within 4 days of disease onset and a laboratory-confirmed diagnosis of acute dengue infection. The IgG avidity test was performed by using a modified Dengue ELISA IgG kit (Focus Technologies) to which a urea incubation step was introduced. Briefly, serum samples diluted 1:100 were dispensed in duplicate into wells coated with dengue antigen. After 1 h of incubation at room temperature, the plates were rinsed once with washing buffer; half of the wells were then rinsed with phosphate-buffered saline (pH 7.2) containing urea, and the other half were rinsed with phosphate-buffered saline without urea. After two further rinses, the test was performed according to the manufacturer’s instructions. The avidity index, expressed as a percentage, was calculated as

* Corresponding author. Mailing address: Laboratório de Virologia, Instituto de Medicina Tropical de São Paulo, Av. Eneas de Carvalho Aguiar, 470, CEP 05043-000 São Paulo (SP), Brazil.
the ratio of the optical density with urea to the optical density without urea times 100. Samples whose absorbances were above the limit of the ELISA reader were retested after a 1:1,000 or 1:10,000 dilution to allow better calculation of the avidity index.

Variability in results when ELISA is used to test avidity has been reported when different commercial plates and different sources of antigen or coating techniques have been used (14, 26). Different urea concentrations and elution times also have been employed for different antigens (2, 11). For this reason, we tested different schedules of urea washing to standardize the procedure for the commercial dengue ELISA used here. Sera from 12 convalescent patients with confirmed primary infections and sera from 12 patients with confirmed secondary dengue infections were tested by using washing schedules of 7 M urea for 10 min, 7 M urea for 10 min, and 8 M urea for 5 min. Similar results were obtained when schedules of 7 M urea for 10 min and 8 M urea for 5 min were used to discriminate a primary from a secondary immune response. The mean avidity index for primary infection was 11 with urea at 7 M for 10 min and as well as urea at 8 M for 5 min. The mean avidity indices for secondary infection were 57 ± 17 and 57 ± 17 for urea at 7 M for 10 min and for urea at 8 M for 5 min, respectively (Fig. 1). The schedule of 7 M urea for 10 min was chosen, since it has been used to evaluate IgG avidity in measles virus infections (22).

The performance of the IgG avidity test with serum samples selected from convalescent patients with laboratory-confirmed acute dengue infections was evaluated. Serum samples from 57 patients were collected within 30 days of disease onset. Of these samples, 27 were obtained from patients with confirmed primary infection and 30 were obtained from patients with confirmed secondary infection according to the definition criteria adopted. A receiver operating characteristic (ROC) curve analysis employing Analyze-it software was used to evaluate the ability of the avidity test to distinguish between primary and secondary dengue infections. The cutoff point was defined by the highest sum of the sensitivity and specificity estimates. The area under the ROC curve was used as a measure of test accuracy (24). Quantitative data (avidity indices and times of sample collection) were compared by one-way analysis of variance (data are presented here as means ± standard deviations).

The highest sum of the sensitivity and specificity estimates used to define secondary infection was obtained at a cutoff point of ≥24% for the IgG avidity index. At this cutoff point, the IgG avidity test provided correct classifications of 55 of 57 patients (27 of 27 patients with primary dengue [100%] and 28 of 30 patients with secondary dengue [93%]). The test was highly accurate, as the area under the ROC curve was almost perfect (0.995). The mean avidity index for secondary infection was 50 ± 18.0, significantly higher than that for the primary-infection group (12 ± 4.6) (Table 1). It is known that the avidity of IgG antibodies increases according to the time elapsed after infection (23). However, the higher avidity indices found for secondary infection in the present study were not related to the duration of infection, since all samples were collected within 30 days of disease onset and the mean times elapsed for sample collection were similar for the primary (20 ± 3.4 days) and secondary (19 ± 2.7 days) infection groups (P = 0.71).

We conclude that the IgG avidity test, which uses a single blood sample and a commercially available kit, has the potential to be a useful tool for differentiating between acute primary and secondary dengue infections.

REFERENCES


