



Immunoglobulin G antibody response in children and adults with acute dengue 3 infection

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ABSTRACT

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Using a serological test, different criteria have been established for classifying a case as primary or secondary dengue virus infection. Considering the dengue epidemiological situation in Cuba, IgG antibody response to dengue virus infection in serum samples from children and adults with a dengue 3 infection, in Havana city during the 2001–2002 epidemic was evaluated. Samples were collected on days 5–7 of fever onset and tested by an ELISA inhibition. A total of 713 serum samples positive for IgM antibody, 93 from children and 620 from adult patients were studied. Serum samples collected from healthy blood donors and patients not infected with dengue were included as controls. An IgG primary infection pattern was observed in sera collected from children, with titers of ≤ 20 in the 89.3% of the patients, while both, a primary and secondary patterns were observed in sera collected from adult patients with titers of ≤ 20 (13.4%) and ≥ 1280 (83.9%), respectively. These results permitted the definition of a primary or secondary case of dengue virus infection in serum samples collected during the acute phase of dengue virus infection.

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1. Introduction

Dengue is one of the most important human arthropod-borne viral diseases in terms of morbidity and mortality with a wide clinical spectrum, from fever, classical dengue fever (DF) to the severe dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). The infection is caused by any of the four dengue virus (Den-1 to Den-4) (Guzman and Kouri, 2002). Two main types of serological responses are observed: primary and secondary. The primary response is observed in those individuals not immune to dengue viruses. The secondary response is observed in individuals with a previous dengue virus infection caused by a different serotype (Guzman and Kouri, 2004; Guzman and Vazquez, 2002; Shu and Huang, 2004).

Different criteria have been established for classifying a case as a primary or a secondary dengue infection by enzyme-linked immunosorbent assay (ELISA) applied to a single serum, which is

comparable with the results obtained by the hemagglutination inhibition (HI) test considered as "the gold standard" test (Cuzzubbo et al., 2000; Kuno et al., 1991; Miagostovich et al., 1999). For the detection of IgM and IgG dengue antibodies, two ELISA tests: MAC-ELISA (Vazquez et al., 1998) and ELISA inhibition (EIM) (Fernandez and Vazquez, 1990; Vazquez et al., 1997), were developed by the Arbovirus Laboratory of the "Pedro Kourí" Institute, including a comparison with HI as "the gold standard" test, considering the established criteria for defining a primary or secondary case. MAC-ELISA and EIM systems are employed as part of the Cuban laboratory surveillance and have been of considerable use for the serological diagnosis of epidemics, both in Cuba and abroad (Guzman, 1995; Guzman et al., 2000, 1997; PAHO, 1994; Vazquez et al., 2007, 2005; WHO, 1997).

The serological classification of a dengue case as primary or secondary is of importance for evaluating the risk of DHF/DSS in individuals and populations since most of the severe cases are associated with secondary dengue infection. To obtain the necessary paired sera for serological confirmation of dengue infection is difficult as is the identification of the type of infection. For this reason and considering the epidemiological situation of dengue in Cuba (Kouri et al., 1986; Pelaez et al., 2004; Valdes et al., 1999) the IgG antibody response to dengue virus infection was evaluated in a single serum sample collected during the acute phase of illness from children and adult patients by EIM with the aim of identifying a primary or secondary infections. Serum samples collected from

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healthy blood donors and non-dengue patients without dengue as controls.

2. Materials and methods

2.1. Serum samples

A total of 713 serum samples (collected from 5 to 7 days of fever onset) from patients with a clinical diagnosis during a dengue 3 epidemic in Havana, 2001–2002 (Pelaez et al., 2004) were studied. Ninety-three (13%) were collected from children (younger than 15-year old) and 620 (87%) from adults. A positive anti-dengue IgM determined by MAC-ELISA was employed as a criterion of infection (Vazquez et al., 1998). EIM was used to determine the IgG titer in each serum (Vazquez et al., 1997, 2003b).

A total of 419 serum samples as negative controls (153 collected from healthy blood donors and 266 from patients without dengue) were included. All the samples were taken during a period of absence of dengue infection (January 2004 to March 2005) and tested by MAC-ELISA and EIM.

2.2. Virus and antigens

Dengue antigens used were prepared from the brain of infected suckling mice extracted by the sucrose–acetone method (Clark and Casals, 1958). Prototype strains were used: dengue 1 (Hawaii strain), dengue 2 (New Guinea C strain), dengue 3 (H-87 strain) and dengue 4 (H-241 strain).

2.3. Capture IgM specific dengue antibodies by MAC-ELISA

A MAC-ELISA “in-house” test was used (Vazquez et al., 2005, 1998). Briefly, NUNC MaxiSorp plates were coated with goat IgG anti-human IgM (Sigma). After blocking, 50 μ L of 1/20 serum dilution in PBS plus 0.5% bovine serum albumin (BSA) (BDH) were added. Positive (duplicated) and negative (quadruplicated) controls were included in each test. Samples were incubated for two hrs at room temperature. An antigen mix of the four dengue serotypes was added. After overnight incubation, 50 μ L of human conjugate IgG anti-dengue (IPK, Havana, Cuba) diluted 1/5000 was added. Orthophenildiamine (OPD) (Merck, Darmstadt, Germany) and hydrogen peroxide (Sigma–Aldrich, Steinhen, Germany) in phosphate citrate buffer (pH 5) was used as substrate. The reaction was stopped and the plates were read at 492 nm. Optical density ratio (OD ratio) = P/N was calculated for all samples. P represents the OD of each serum sample and N represents the ODs mean of the negative control. All samples with an OD ratio ≥ 2 were considered as positive.

2.4. ELISA inhibition method (EIM) for detection of IgG anti-dengue

EIM was carried out as described previously (Vazquez et al., 1997, 2007, 2003a). Polystyrene plates (Costar) were adsorbed with human anti-dengue IgG at 10 μ g/mL concentration, in coating buffer pH 9.6 overnight at 4 °C. The following day, the plates were washed three times and the surface was blocked with 150 μ L/well of 1% BSA solution in coating buffer pH 9.6 and incubated 1 h at 37 °C. After blocking, a volume of 100 μ L of Den-3 antigen diluted 1/40 in PBS plus 0.05% Tween 20 was added in each well and incubated 1 h at 37 °C. Volumes of 100 μ L of serum samples diluted from 1/20 to 1/40,960, including a negative control serum were added and incubated 1 h at 37 °C. Volumes of 100 μ L human IgG anti-dengue peroxidase conjugate diluted 1/3000 in PBS plus a 0.05% Tween 20 and 2% fetal bovine serum was added and incubated 1 h at 37 °C. Substrate containing OPD was added. The reaction was stopped after 30 min incubation at room temperature. The test was read at 492 nm. The inhibition percentage was calculated as

$$\text{Inhibition \%} = \left[1 - \left(\frac{\text{OD sample}}{\text{OD negative control}} \right) \right] \times 100$$

The antibody titer of each serum was considered as the highest dilution with a percentage of inhibition ≥ 50 . A serum with a percentage of inhibition < 50 was considered negative for IgG dengue antibodies (< 20).

2.5. Statistical analysis

A descriptive analysis of the IgG antibody titers in the serum samples was performed. The GraphPad Prism 5 program, 2007 was employed for to determine the geometric mean titer (GMT) with confidence intervals (CI) of 95%.

3. Results

3.1. IgG response in sera from children and adults infected by dengue 3 virus by EIM

Fig. 1A shows the IgG antibody titers in sera collected from children. The 81.7% (76/93) had an IgG dengue antibody titer < 20 followed by 7.5% (7/93) with a titer of 20 and 5.4% (5/93) with a titer of 40. Only 5 sera presented values of 8–320 (5.4%). IgG titers equal or higher than 640 were not observed. The GMT was 40 (CI 95%: 20–80).

The distribution of the IgG antibody titers in adult serum samples is shown in Fig. 1B. From the total sera, 11.3% (70/620) had titers < 20 , while 2.6% (16/620) presented values of 20 and only 1

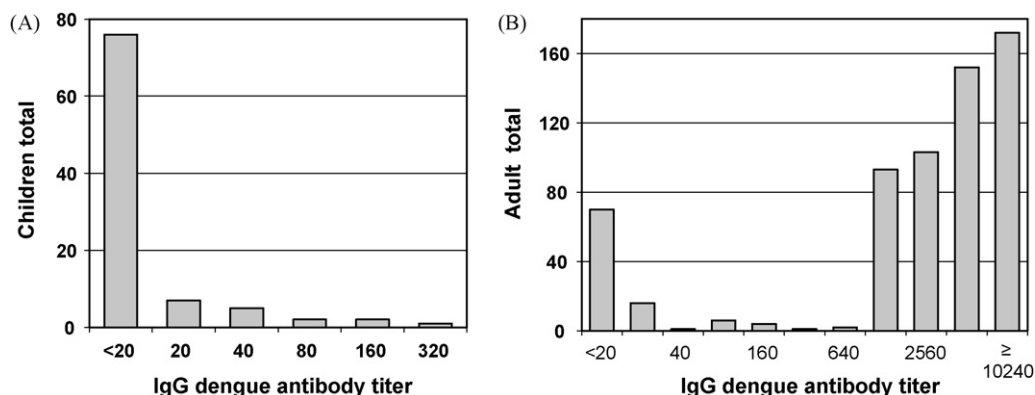


Fig. 1. Distribution of dengue IgG titer in sera from children and adult patients with a dengue 3 infection.

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