Immunoglobulin A Antibody Responses in Dengue Patients: a Useful Marker for Serodiagnosis of Dengue Virus Infection

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We determined the usefulness of an immunoglobulin A (IgA) antibody-capture enzyme-linked immunosorbent assay for serodiagnosis of dengue virus infections. The results indicate that the presence of IgA and IgM in serum samples assures recent primary dengue virus infection even with a single serum sample.

Dengue viruses cause dengue fever and dengue hemorrhagic fever. Dengue fever/dengue hemorrhagic fever is one of the most serious public health problems in tropical and subtropical areas in the world. Monitoring of dengue virus infections is an important component in assessing the risk to humans. Reverse transcriptase PCR and immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (IgM-capture ELISA) have been carried out in laboratories (3, 7). In the present study, we assess the usefulness of the anti-dengue virus IgA antibody response in serological diagnosis of dengue virus infection.

In the present study, 94 serum samples from 62 dengue patients in Japan were tested. Sera were also collected from 32 healthy Japanese adults who had never been to areas of a dengue virus epidemic or areas to which dengue virus is endemic. Four prototype dengue virus strains and a Japanese encephalitis virus (JEV) strain, JaGAr01, were used for antigens. Preparation of viral antigens was carried out according to previously described methods (4). IgA-capture ELISA was carried out according to the previously described IgM-capture ELISA technique (2, 3, 4). Antibody titrations were carried out to determine the assay dilution in IgA-capture ELISA, using one (each) serum sample from a confirmed dengue patient and a healthy Japanese donor (data not shown). The result was quantified as a positive-to-negative (P/N) ratio: P/N ratio = A492 reading with the viral antigen/A492 reading with the uninfected control antigen. Specific absorbance stayed at the plateau level at the dilution ranges from 1:50 to 1:400 and decreased gradually at serum dilutions higher than 1:400. Low levels of nonspecific reaction were detected with dengue virus-negative control serum at low dilutions. P/N ratios clearly differentiated the dengue virus-positive serum from the negative

<table>
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<th>Table 1. Comparison of the results obtained with IgA ELISA and IgM ELISA</th>
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<td><strong>IgA ELISA result</strong></td>
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<td>Positive</td>
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<td>Negative</td>
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FIG. 1. Detection of dengue virus-specific IgA (A) and IgM (B) on specific disease days. Closed and open columns indicate positive and negative, respectively.
one at the dilution ranges in the assay. Based on these results, we decided to dilute the serum samples at 1:400 for IgA-capture ELISA in the present study. In order to determine the cutoff value in IgA-capture ELISA, 32 negative sera were assayed. Mean P/N ratios plus two standard deviations obtained at 1:400 dilutions of the sera were 1.64 $\pm$ 0.254 and 1.73 $\pm$ 0.258 for dengue virus-negative, anti-JEV HI antibody-negative (lower than 1:10) and dengue virus-negative, anti-JEV HI antibody-positive sera (higher than 1:10), respectively. Therefore, the cutoff value was defined as follows: mean P/N ratio $\pm$ 2 standard deviations $\pm$ 2.00 in IgA-capture ELISA.

Ninety-four serum samples from 62 dengue patients collected on various days were tested by IgA- and IgM-capture ELISA (Table 1). Twenty-three (from 19 cases) of the 94 samples were IgA positive, and the remaining 71 were IgA negative. Seventy-three (from 52 cases) of the 94 samples were IgM positive, and the remaining 21 were IgM negative. There was no sample which was IgA positive and IgM negative. The agreement of results between the IgA and IgM tests was 32% (23/73). To investigate the differences between IgA and IgM tests, we analyzed the kinetics of IgA and IgM responses.

Seventy-three serum samples with defined disease days were tested by IgA- and IgM-capture ELISA (Fig. 1A and B). Disease days were defined according to the report by Vaughn et al. (6). Disease day 1 is the day of onset, which is usually characterized by fever. IgA was positive as early as disease day 6 and as late as disease day 23, and IgA responses were mostly positive on disease days 9 to 15 (Fig. 1A). On the other hand, IgM responses were mostly positive on disease days 5 to 50 (Fig. 1B). The results suggest that serum IgA antibody responses reflect dengue virus infection; however, IgA antibody remains positive for a short period of time compared to IgM antibody.

We next examined virus specificities of antibodies among patients. We previously reported that most of the dengue cases were primary dengue virus infection in Japan but demonstrated immune responses as secondary flavivirus infection because of immunity to JEV (2). We compared titers of IgA and IgM for dengue virus and JEV antigens in 31 serum samples from 26 dengue patients (Fig. 2A and B). Twenty-two serum samples from 22 Japanese encephalitis (JE) patients were also tested (Fig. 2C and D). Ten of the serum samples were kept at the Department of Virology, Institute of Tropical Medicine, Nagasaki University, and 12 were kept at National Institute of Infectious Diseases, Tokyo, Japan. Serum IgA antibody in dengue patients was cross-reactive to dengue virus and JEV antigens, whereas IgM antibody was specific for dengue virus antigen (Fig. 2A and B). Titers of IgA and IgM for dengue virus
and JEV antigens were also compared in serum samples from JE patients. IgA and IgM for JEV antigen were detected but not IgA and IgM for dengue virus antigen (Fig. 2C and D).

There is no domestic dengue virus infection in Japan. Therefore, all the dengue patients are imported cases. On the other hand, most of the Japanese population possesses antibodies to JEV by vaccination or natural infection. Therefore, most dengue cases are also considered secondary flavivirus infection. The present data are different from the reports by the groups of Talarmin et al. (5) and Balmaseda et al. (1). The specificity and positive predictive value of their data are over 90% for dengue patients’ sera, which were collected in French Guiana and Nicaragua, respectively. This may have been due to the difference in epidemiological status. French Guiana and Nicaragua are countries to which dengue virus is endemic, whereas Japan is not. Therefore, serum samples were probably from secondary dengue virus infections in those studies but from primary infections in our study. The results in the present study indicate that dengue virus infection elicits serum IgA antibodies as well as IgM in patients, but IgA persists for a shorter period of time. Thus, the presence of both IgA and IgM suggests an early phase of dengue virus infection, and assures dengue virus infection even with a single serum sample, when dengue patients are primary infections. However, it should be noted that the absence of IgA does not necessarily rule out dengue virus infection. The combination of IgM-capture ELISA and IgA-capture ELISA increases the assurance of the serological diagnosis of dengue virus infection, especially when only a single serum sample is available.

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