THE EVALUATION OF IgM ANTI-DENGUE IMMUNE RESPONSE IN SEQUENTIAL INFECTION

ABSTRACT

The appearance, persistence and the levels of dengue IgM antibodies were studied by MAC-ELISA in 310 confirmed cases of dengue secondary infection. Assays performed on acute and convalescent patient sera showed that IgM antibodies usually present titers lower than 1/3,200. The lack of IgM antibodies in few dengue cases requires special attention concerning the interpretation of MAC-ELISA results since this method is widely used for serodiagnosis of dengue.

INTRODUCTION

Dengue (DEN) viruses belong to the family Flaviviridae, genus Flavivirus, and occur as four antigenically related but distinct serotypes designated DEN-1, 2, 3 and 4 (Westaway et al., 1985). Primary infection with one serotype confers lasting immunity to that serotype and partial and temporary protection against the others. Then secondary infection is possible after a relatively short period of time and has been associated with an increased risk of severe forms of disease (WHO 1997).

DEN viruses are transmitted from infected to susceptible human beings mainly by Aedes aegypti and the infection is prevalent in over 100 tropical and sub-tropical countries, with 2.5 thousand million people in areas of risk infection (Knudsen 1996).

Dengue infection causes a spectrum of illness ranging from a mild nonspecific febrile disease to severe and fatal forms. Dengue fever is a self-limited disease and represents most cases of dengue infection. However, in some situations,
patients infected with dengue virus develop life-threatening complications such as leaking plasma, hemorrhagic manifestations and shock, which are called dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) (WHO 1997). The pathological mechanisms of DHF/DSS have been one of the most important issues on dengue research, being more commonly observed during secondary dengue infections than in primary ones (Halstead 1988, Thein et al., 1997).

Virus isolation, serology and detection of viral RNA are the approaches for dengue laboratory diagnosis. The use of Aedes albopictus clone C6/36 cell line associated with monoclonal antibodies and the reverse-transcriptase polymerase chain reaction (RT-PCR) have been the routine methods used for detection and identification dengue virus (Igarashi 1978, Gubler et al., 1984, Lanciotti et al., 1992).

The haemagglutination inhibition (HI) assay (Clarke & Casals 1958) has been the standard serological method used traditionally for the diagnosis and classification of dengue virus infection. A relatively new test, the IgM capture enzyme-linked immunosorbent assay (MAC-ELISA) has been the serological method of choice during the last decade, since anti-dengue IgM detection in any single serum specimen indicates an active or recent infection (Kuno et al., 1987, Lam et al., 2000).

MAC-ELISA is a simple and rapid method, no sophisticated equipment is required and allows the screening of a large number of serum specimens (Nogueira et al., 1992).

During the DEN-1 epidemic virus in Rio de Janeiro - Brazil (1986-1987), MAC-ELISA (Kuno et al., 1987) was employed for the first time on a large scale and made it possible to determine IgM antibody levels in the population without prior exposure to dengue virus ("virgin soil" epidemic). At that time, assays performed on acute and convalescent sera revealed that IgM antibodies appeared during the early phase of disease (day 2) and persisted for three months after onset (Nogueira et al., 1992).

After the introduction of the DEN-2 virus in Rio de Janeiro in 1990 (Nogueira et al., 1990), MAC-ELISA also proved to be useful for the IgM detection in the secondary infection cases (Nogueira et al., 1993). In this report, we present the appearance, persistence and levels of dengue IgM antibodies detected by MAC-ELISA in secondary/sequential in the state of Rio de Janeiro between 1995 to 2000, when DEN-1 and DEN-2 viruses co-circulated.

**MATERIAL AND METHODS**

**Samples**

Sera from 310 patients with secondary dengue infection previously classified by G-ELISA (Miagostovich et al., 1999) and HI (Clarke & Casals 1958) were evaluated for IgM response to dengue infection. All cases occurred during epidemic periods in the state of Rio de Janeiro between 1995 to 2000. DEN-1 and DEN-2 viruses were the serotypes circulating in the State at that time.

**Serological method and antigens**

IgM capture enzyme-linked immunosorbent assay (MAC-ELISA) – Centers for Disease Control
and Prevention (CDC) protocol - was performed for routine serodiagnosis using serotype-specific DEN-1 and DEN-2 antigens as previously described (Kuno et al., 1987). For titration sera were diluted to 1/10, 1/32, 1/100, 1/320 to 1/10,000.

DEN-1 (Mochizuki strain) and DEN-2 (New Guinea C prototype) antigens used in serological tests were prepared from infected suckling mouse brains by the sucrose acetone extraction method (Clarke & Casals 1958).

Results
The appearance and persistence of dengue IgM antibodies in 310 cases characterized as dengue secondary infections was determined by the analysis of IgM detection according to the onset of disease. Table 1 shows that MAC-ELISA did not detected IgM response in 100% of cases despite of laboratorial confirmation of disease. Titration of IgM antibodies from 92 serum samples showed that IgM response in secondary cases has not reached titers higher than 1/3,200 (Figure 1).

Discussion
In Brazil almost two millions dengue cases were reported in the last fifteen years, resulting from DEN-1 and/or DEN-2 epidemics throughout the country, except for the States of Rio Grande do Sul and Santa Catarina (FUNASA). The co-circulation of the two serotypes in all Brazilian States where dengue epidemics have been reported and the recent introduction of DEN-3 virus in the State of Rio de Janeiro (Nogueira et al., 2001), pointed out the need for good serological tests for epidemiological and clinical surveillance.

The introduction of DEN virus in Brazil during the 80s, lead to the establishment of Public Health Laboratory Network for Dengue Diagnosis with the implementation of the diagnosis methods that included virus isolation in cell cultures and MAC-ELISA as a current serological method (Schatzmayr et al., 1996).

MAC-ELISA has been described as useful in both endemic and non endemic areas for serological surveillance since dengue IgM antibodies have early appearance, are transitory and are produced by primary or secondary dengue infections (Gubler, 1998).

Anti-dengue IgM antibody appears a little faster than IgG antibody and the rapidity with which it develops varies considerably among patients (Gubler, 1998). The appearance and persistence of anti-dengue IgM detected by MAC-ELISA did not reached 100% in any time after the onset of disease in all confirmed secondary dengue. Our findings corroborate previous data showing that IgM antibodies could not be detected during sequential dengue infection at least in a small number of cases (Gubler & Sather 1988). The appearance of IgM response in secondary infection was earlier however decreased faster when compared with data previously reported for primary infection (Nogueira et al., 1992). The lack of IgM antibodies in convalescent sera requires special attention concerning the interpretation of MAC-ELISA results since this method is widely used for serodiagnosis of dengue (Table 1).
Table 1. Anti-dengue IgM confirmed cases of dengue secondary infections in relation to onset of disease.

<table>
<thead>
<tr>
<th>Days after onset of disease</th>
<th>No of positive/no. of tested</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>1-2</td>
<td>8/54</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>11/64</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>14/60</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>17/32</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>14/18</td>
<td>77.8</td>
</tr>
<tr>
<td>7-10</td>
<td>20/22</td>
<td>91</td>
</tr>
<tr>
<td>11-15</td>
<td>15/17</td>
<td>88.2</td>
</tr>
<tr>
<td>16-20</td>
<td>28/35</td>
<td>80</td>
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<tr>
<td>21-25</td>
<td>12/17</td>
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<td>31-60</td>
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<td>66.7</td>
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<tr>
<td>61-90</td>
<td>1/1</td>
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Figure 1. Anti-dengue IgM antibody titers from 92 sera of secondary dengue cases by MAC-ELISA according to days after onset of disease.
A great individual variability in IgM response independent of the type of infection is also observed. In this study with secondary dengue infections in only one case we could observe an IgM titer of 1/3,200. Titers of 1/320 have been considered high for secondary infections (Gubler, 1998). Usually IgM antibodies titers in primary infection are higher than in secondary ones and can reach titers of 1/10,000 (data not shown). Previously, titers as high as 1/5,000 explained the high rate of IgM detection three months after the onset of disease (Nogueira et al., 1988).

The recent introduction of DEN-3 virus in the state of Rio de Janeiro in 2001 resulted in a concomitant circulation of three serotypes (DEN-1, 2, 3) in the state (Nogueira et al., 2001). Taking in account this epidemiological situation, the first recommendation is the inclusion of the new dengue antigen in the “in house” ELISA, since in some cases homotypic response can occur (Nogueira et al., 1988). Another important approach is to combine the use of IGM and IgG tests. This procedure has shown to increase the diagnostic confirmation, since the elevation of IgG levels is an excellent marker of secondary infection (Sang et al., 1988).

Recently, a number of commercial tests have been reported (Wu et al., 1997, Kuno et al., 1998, Lam & Devine 1998, Sang et al., 1998, Vaughn et al., 1999, Lam et al., 2000) and have been showed to be useful at unit cares, where the volume of testing is low or sporadic.

In endemic areas as Brazil the advantage of using commercial kits is that private laboratories could participate on the serological surveillance, since dengue is a disease of compulsory notification. Serological tests are still the most useful methods to confirm dengue infection and are cheaper and faster when compared to virus isolation. In our experience, MAC-ELISA increased the percentage of confirmed cases in 20% during epidemic periods, when combined to virus isolation (Miagostovich et al., 1993).

The commercial tests were also useful to combine the IgM and IgG antibodies detection, distinguishing between primary and secondary infections (Sang et al., 1998). The importance in classifying dengue infections has clinical implications since severe cases are more common in secondary dengue cases (Halstead 1988, Thein et al., 1997). In fact, 55 out of 56 DHF cases occurred in Niteróí city during the epidemic of 1990-1991, were confirmed as secondary infection (Zagno et al., 1994). Previously, the classification of dengue immune response has been done by using HI criteria, although nowadays an G-ELISA is being employed for routine diagnosis presenting several advantages over the conventional HI method (Miagostovich et al., 1999).

In our experience with commercial tests Dengue Duo IgM and IgG Elisa (PanBio, Brisbane, Australia) showed an agreement of 84% (37/44) for primary infections and 90% (26/29) for secondary ones, when compared to HI results. Comparing a Rapid Immunochromatographic test (RIT) (PanBLo, Binax, Inc., Portland, ME, USA) with MAC-ELISA we observed 79% and 93% of sensibility and specificity, respectively (data not published). The false negative results obtained by RIT were described before for primary infection cases showing low HI titers (Sang et al., 1998). RIT was useful to detect cases...
of secondary infection and may be important for severe dengue cases as a presumptive diagnosis. As a rule for all suspected dengue cases presenting negative results in an acute serum by any method, a second serum sample is required as suggested for any viral infection. By other hand, acute sera could be used for viral isolation or nucleic acid detection.

We would like to emphasize the valuable use of paired sera for serological methods and the combined use of both anti-dengue IgM and IgG detection methods, considering that individual fluctuations may occur. We also would like to stress that on an endemic region, serological results should be taken in conjunction with clinical, epidemiological and other laboratorial findings.

**REFERENCES**


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