Genetic Characterization of Newly Reintroduced Dengue Virus Type 3 in Martinique (French West Indies)

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Dengue virus (DENV) infection is recognized as a major public health problem. An estimated 100 millions humans are infected each year worldwide (13), and the incidence of the severe, sometimes lethal, forms of the disease is increasing (5). DENV is a mosquito-borne flavivirus with a single-stranded, nonsegmented, positive-sense RNA genome approximately 11 kb long. There are four antigenically distinct DENV serotypes, 1 to 4 (17). Infection with any of these serotypes leads to a large clinical spectrum, ranging from subclinical infection or classical dengue fever (DF) to severe forms (dengue hemorrhagic fever [DHF] and dengue shock syndrome [DSS]). Possible roles of antibody-dependent enhancement of infection (6), increased pathogenicity of viral strains (19), or other possible unidentified factors in promoting DHF and DSS have been proposed.

Like other Caribbean islands, Martinique experiences dengue epidemics. Since the Cuban epidemic in 1981, DHF and DSS have been frequently reported in the region (the Caribbean, northern South America, Central America, and Mexico). However, since the first report of DF and DSS in Martinique in 1995 (12), DHF has been rarely reported, contrary to observations from other countries in the region (24; http://www.carc.org/data/dengue).

However, factors potentially responsible for an increase in severe forms of dengue infections have been identified in Martinique. DENV type 2 (DENV-2) was principally responsible for the 1998 epidemic. Sequence determination established that it belongs to a recently identified genotype of the American region whose presence correlates with the increase in DHF and DSS in South and Central America (18, 22). Between November 1999 and December 2001, DENV-3 reappeared on the island and became prominent. During this period, 97 French soldiers presenting with dengue-like syndromes were received in the Infirmerie du Bataillon d’infanterie de marine. A total of 147 blood samples withdrawn at early and late stages of the infection were received in our laboratory in Marseilles. All of the samples were found to be positive for dengue infection by the presence of immunoglobulin M (IgM), the elevation of specific IgG, or both, using dengue virus-specific enzyme-linked immunosorbent assay (ELISAs) developed in house (MAC ELISA for IgM and sandwich ELISA for IgG). Virus isolation by coinoculation of white blood cells with C6/36 cells (22) was positive in 29 patients. DENV-3 was identified by indirect immunofluorescence assay with serotype-specific monoclonal antibodies (kindly provided by Nick Karabatsos, Centers for Disease Control and Prevention, Fort Collins, Colo.). The 97 patients developed DF and recovered fully.

The population of Martinique also suffered from the disease, and between September 2001 and January 2002, DENV transmission became epidemic, with 24,000 patients suspected of having dengue (3). Of these patients, 217 were hospitalized with at least one symptom of severe infection (elevated temperature, malaise, or thrombocytopenia). Serum samples from hospitalized patients underwent reverse transcription (RT)-PCR at the Fort de France University Hospital in accordance with a previously published protocol (9), as did another 154 serum samples from outpatients. Of the 371 RT-PCR assays performed, 133 indicated DENV-3 infection. A DENV-2-positive RT-PCR result was found only once. Despite the existence of symptoms of severe infection in the 217 hospitalized patients, only three fatal cases were classified as DHF or DSS in accordance with World Health Organization criteria (1). In relation to the total number of suspected cases (clinical evidence of dengue infection without virological confirmation), the incidence of DHF during the epidemic was lower than 0.02%, an unexpected value in view of the presence of potentially more pathogenic DENV-2 and the recent reintroduction of DENV-3.

The virus strain isolated in December 1999 from a military patient was the first DENV-3 strain isolated in Martinique in 20 years (3, 15). The complete sequence of this DENV strain, named D3Mart1243, was determined as previously described (22) (Table 1). Between November 1999 and December 2001, the 97 military cases constituted a homogeneous group with regard to the clinical course and basic virological parameters (serotype, cytopathogenic effect on Vero cells) when virus iso-
TABLE 1. Sequenced DENV-3 strains and corresponding GenBank accession numbers

<table>
<thead>
<tr>
<th>Identification no.</th>
<th>Sequenced region</th>
<th>GenBank accession no.</th>
<th>Isolation date</th>
</tr>
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<tbody>
<tr>
<td>D3Mart1243</td>
<td>Complete</td>
<td>AY099337</td>
<td>December 1999</td>
</tr>
<tr>
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<td>AY099338</td>
<td>April 2000</td>
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<td>AY099342</td>
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<td>AY099347</td>
<td></td>
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<tr>
<td>D3SriLa1266</td>
<td>Complete</td>
<td>AY099336</td>
<td>September 2000</td>
</tr>
</tbody>
</table>

The strains sequenced in this work are indicated by asterisks. The level of identity between the strains from Martinique (99.5 to 99.9% paired identity), which all have an 11-nucleotide-long insertion between positions 10275 and 10276 in the 3′ untranslated region (AGTGAAAAAGA), interestingly, the same insertion was found in D3SriLa1266, with a one-base difference (AGTGAAAAAGA), indicating that it probably has a recent ancestor in common with the Martinique viruses.

A phylogenetic tree was constructed on the basis of partial sequences of the prM/M-E gene region (positions 437 to 1144) of the genomes, including the strains from Martinique and several others from different origins previously characterized (Fig. 2) (7, 10, 23). DENV isolates from Martinique and Sri Lanka were grouped into subtype 3, as defined by Lanciotti (10), which also includes isolates from India, Samoa, Mozambique, Brazil (17), Guatemala (23), and Mexico. The six isolates from Martinique clustered together. An analysis of the deduced amino acid sequences generated a similar phylogram. Altogether, these results could indicate the existence of a genotype specific to Martinique, close to the Brazil isolate. They also indicate a common origin for all of the DENV-3 strains circulating in the American and Caribbean region, their ancestor probably originating from Southeast Asia. New sequences of American DENV-3 strains, encompassing the insertion site, should help to confirm this hypothesis and identify the factors influencing the evolution of the virus in the area, as was recently done for DENV-4 (4).

Two decades ago, DENV-3 strains prevalent in the Caribbean belonged to subtype 4 (20). Viruses belonging to subtype 3 were first reported in Nicaragua and Panama in 1994 (2). They were then identified in Guatemala between 1996 and 1998 and in Puerto Rico, Barbados, and Jamaica in 1998 (15, 23). As was feared, importation of DENV-3 into Martinique occurred rapidly and was first detected in 1999. As the DENV-3 strain isolated in 1999 was the first isolated in Martinique in 20 years, it was likely imported recently. However, the virus seems to have quickly spread and almost completely replaced the previous DENV-1 and -2 serotypes. Alternatively, the persistence of DENV-3 through the 2-year period studied, overlapping epidemic and interepidemic periods, may be due to reimportation of closely related viruses from the neighbor-
ing islands or countries. In any case, the identified virus genotype exhibits great stability and good adaptation to local conditions. In this regard, sequencing of the DENV-3 strains recently detected in Brazil (11), Paraguay (http://www.promedmail.org), or Argentina (http://www.promedmail.org) should lead to specification of the geographic distribution of the Martinique genotype.

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REFERENCES

21. Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W:

