Dengue hemorrhagic fever in a Japanese traveler who had preexisting Japanese encephalitis virus antibody

Rumi Sato,* Nobuyuki Hamada,** Takahito Kashiwagi,** Yoshihiro Imamura,** Koyu Hara,** Yoshiko Naito,* Natsuko Koga,* Munetsugu Nishimura,* Tomoko Kamimura,* Tomohiko Takasaki,*** Hiroshi Watanabe** and Takeharu Koga*

*Department of Internal and Respirology Medicine, Asakura Medical Association Hospital, Japan; **Department of Infection Control and Prevention, Kurume University School of Medicine, Japan; ***Laboratory of Neuroviruses, Department of Virology 1, National Institute of Infectious Diseases, Japan

Corresponding author: Rumi Sato, M. D., Ph. D, Department of Internal and Respirology Medicine, Asakura Medical Association Hospital, Fukuoka 838-0069, Japan

E-mail: sato.rm@asakura-med.or.jp

Tel: +81-946-23-0077 Fax: +81-946-23-0076

Running title: dengue hemorrhagic fever as a primary infection
Abstract: A patient, an adult Japanese traveler who had just returned from Thailand, had developed dengue hemorrhagic fever (DHF). A primary infection of dengue virus (DENV) was confirmed, in particular, DENV serotype 2 (DENV-2) via the detection of the virus genome, a significant increase in its specific neutralizing antibody and the isolation of DENV-2. DHF is often observed following a secondary infection from another serotype of dengue virus, particularly in children, but this case was a primary infection of DENV. Japan is a non-endemic country of dengue disease. Instead, only Japanese encephalitis (JE) is known to be an endemic flavivirus family. In this study, IgG antibody against Japanese encephalitis virus (JEV) was detected. JEV belongs to the family of dengue virus and prevails in Japan, particularly in Kyushu. Among many risk factors for the occurrence of DHF, a plausible candidate could be a cross-reactive antibody-dependent enhancement (ADE) mechanism by JEV antibody. This indicates that most Japanese travelers, who live in non-endemic areas of dengue, particularly in Kyushu, should pay attention to the occurrence of DHF.

Key words: dengue hemorrhagic fever, Japanese encephalitis virus antibody,
cross-reactive antibody, imported infection, petechiae, thrombocytopenia,

antibody-dependent enhancement (ADE)
Dengue virus (DENV) is a mosquito-borne virus that is common in tropical and subtropical areas. The prevalence of dengue disease has widely expanded geographically in recent decades [1]. Imported DENV infection is increasing in Japan [2]. DENV infection results in a sub-clinical infection, dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). Although most cases are self-limiting febrile illnesses, DHF is fatal unless its plasma leakage is treated early. There are four different antigenetic serotypes of DENV (DENV-1, DENV-2, DENV-3, and DENV-4). A primary infection with a single serotype leads to an antibody production that cross-reacts with all serotypes. Despite the cross-reactivity, produced antibody do not protect against infection from different serotypes [3]. Epidemiological studies suggest that pre-existing cross-reactive antibodies may enhance the severity of the disease following secondary infection with a different DENV serotype. Antibody-dependent enhancement (ADE) has been as an explanation for the mechanism underlying DHF/DSS. DENV also serologically cross-reacts with Japanese encephalitis virus (JEV) and both belong to the virus family *Flaviviridae*. Although the clinical implications of
JEV/DEV cross-reactivity remain undefined, some evidence suggests that infection with pre-existing JEV antibody may be linked to the severity of a subsequent DENV infection [3, 4]. Herein we report the case of a primary infection with DENV-2 presenting with DHF with a pre-existing JEV antibody.

CASE REPORT

A 64-year-old male Japanese patient complained of diarrhea, fever (38.2°C) and rash. He was admitted to the Asakura Medical Association Hospital six days after the onset of disease. The patient remembered that he had received many mosquito bites nine days before the onset of symptoms during his visit to Bangkok, Thailand. The patient presented with a minimal degree of thrombocytopenia (Fig. 1) and a petechial rash on the body, with the exception of his face. A tourniquet test was positive. The patient also showed liver dysfunction (AST: 72 IU/l, ALT 44 IU/l) and an increase in atypical lymphocyte count (Fig. 1). Hematocrit values were 48% on admission and 40.6% on the third day of admission. Rapid tests for antibodies (SD BIOLINE Dengue IgG/IgM kit, Standard Diagnostics Ltd.,
Yongin, Korea) and antigens (NS1 Ag Strip, Bio-Rad, Hercules, CA) to DENV were both positive. The patient’s physiological parameters on admission were as follows: blood pressure 105/61 mmHg, respiration rate 15/min, and PaO₂ 40.4 mmHg. Neither leukocytosis (white blood cells, 4,800/ml) nor inflammation (C-reactive protein, 0.4 mg/dl) was observed. A treatment of intravenous rehydration and rest was prescribed. A significant increase in the white blood cell count was observed on days eight to nine after the onset of disease (Fig. 1). Four days after admission, the leukocytosis had disappeared. The patient’s platelet count gradually recovered to a normal level. The patient was discharged on day ten after the initial hospitalization.

We diagnosed the patient with DHF, grade 1.

Since the DENV genome was undetectable using a conventional single RT-PCR [5], we designed new specific primer sets and developed a nested RT-PCR [6]. Total RNA was extracted using whole blood samples from day seven after the onset of disease, which was subjected to RT reaction using a mixture of three primers: (DNGRT_3048: CTYTCTATCCARTAVCCCAT, DNGRT_2943: ADCCATATRTTGGTHGTGAA, and DNGRT_2657: TCDKWKGHTATTGTYTTCACA). The RT products were applied to the
first PCR using DNGRT_2657 and a FW primer mixture (see below). The second PCR was performed using each type of specific primer pairs; (DNGFW_1: CATCCTGGGAGACACTGCATGGGA and DNGRV_1: TGGGAATTTATATGACTCTGTCCA (for DENV-1), DNGFW_2: CATTTTGGGCAGACACGCGCTGGGA and DNGRV_2: TGGGAATTTATATGACTCTGTCCA (for DENV-2), DNGFW_3: CATCTTGGGAGACACGCGCTGGGA and DNGRV_3: TGGGAATTTATATGACTCTGTCCA (for DENV-3), DNGFW_4: CATCTTAGGTGAAACAGCTTGGGA and DNGRV_4: TGGGAATTTATATGACTCTGTCCA (for DENV-4)). The cycle conditions were as follows: 45°C for 1 hr (RT step); 40 cycles of 92°C for 1 min, 53°C for 1 min, and 72°C for 1 min (first and second PCR steps). The DENV-2 was identified by amplicon (351 bp) sequencing.

Although the ELISA test to detect IgG for DENV (Dengue IgG indirect ELISA, Panbio Ltd, Sinnamon Park, Queensland, Australia) was negative on day four after the onset of disease, the test was positive on day seven after the onset of disease (Table 1). We isolated dengue virus type 2 from a whole blood sample, which was collected on the fourth day after the onset of disease.
Using a plaque reduction assay in C6/36 mosquito cells, we found the neutralization antibody titer to be significantly increased (four-fold, Table 2) only against DENV-2 antigen, but not against DENV-1, 3 and 4 antigens. Thus, we confirmed a primary infection with DENV, particularly DENV-2 for this patient. It was also noted that the patient had pre-existing IgG antibody to JEV (Table 1).

**DISCUSSION**

Between 1 and 3% of all cases of dengue disease exhibit DHF/DSS, and 95% of DHF/DSS occurs in children [7]. The risk of developing DHF is higher with a secondary DENV infection compared with only a primary DENV infection. The patient in the present study developed DHF, although he was confirmed only with a primary infection from DENV. Epidemiological data reveals several risk factors for severe dengue, which are age, sex, high body-mass index, MHC-I related sequence B, and phospholipase C epsilon 1 [1, 8]. Also secondary infection by a different serotype has also been listed as a risk factor. A highly pathogenic dengue virus strain was suggested [9]. Among those factors the induction of a previous heterotypic dengue antibody
may cause severe dengue through an antibody-dependent enhancement (ADE) mechanism. This mechanism could work even when the previous antibody is derived from a similar virus, for example JEV. We similarly detected a pre-existing JEV antibody (Table 1). There is at least one report of an unusual case where antibodies of DENV and JEV coexisted and presented several suggestive interpretations [10]. The author suggested that co-invasion could occur in an area that is endemic for both viruses. On the contrary, only JE is endemic in Japan. Therefore, the cross-reactivity between DENV and JEV suggested that the pre-existing JEV antibody might have been associated with DHF upon primary DENV infection, for example via an ADE mechanism.

The DENV genome can be detected only during the early days of infection before 0-fever day of DF. The detection rate via RT-PCR is reported to be 33% [11]. The virus titer of DHF patient is supposed to be lower than that of DF patient [12]. We tested a whole blood sample on the seventh day of disease, and by using a nested RT-PCR with a new design of primers we were able to detect the DENV genome whereas a conventional single RT-PCR could not. This nested RT-PCR can be applicable to the detection of DENV
genome in DHF patient samples.

Recently, dengue infections of people who travel to endemic areas have increased in Japan [13]. Japanese encephalitis (JE) is almost the only endemic flavivirus disease in Japan, particularly in southern regions [14, 15]. An estimated 2% of population are believed to have been naturally infected with JEV. Vaccination programs have increased the number of people receiving neutralization antibody against JEV, and people who are less than 50 years of age have been targeted. Therefore, some Japanese travelers may have an increased risk of developing DHF via the ADE mechanism. In the United States, it is West Nile fever that is endemic compared with JE in Japan [16]. West Nile virus (WNV) is a member of the JE serocomplex of the family Flaviviridae, genus Flavivirus. WNV and DENV have common epitopes [17]. In the United States, imported dengue fever is also increasing [18]. American travelers may have a latent risk of manifestation of DHF through a mechanism that is similar to the case described in this paper. In fact, an unusual manifestation of WNV infection (hemorrhagic fever) was reported in the United States [19]. In that case, a high titer of neutralization antibody against DENV-2 was retrospectively found. Although this case was
a reverse example, which shows the relationship between WNV infection and
the DENV antibody, the severe symptoms caused by WNV could have been
modified by the DENV antibody through, for example, an ADE mechanism.

In conclusion, with increases in the global spread of the dengue virus,
the risk of DHF or of modified symptoms of indigenous flavivirus infection
caused by dengue infection might increase in countries that are non-endemic
to dengue. Therefore, people in all countries should take precautions against
such a risk.

Conflicts of interest: None
Figure legend

Figure 1: Clinical course of dengue hemorrhagic fever.

Left axis: platelet counts x 10^4 / μl (□-); Right axis: white blood cells / μl (●-). % value in the parenthesis shows atypical lymphocytes. Level changes of fever, rash/petechiae, and diarrhea are shown by illustration using distorted chevron.
References


14. Konishi E, Kitai Y, Tabei Y, et al. Natural Japanese encephalitis virus infection among humans in west and east Japan shows the need to continue a vaccination program. Vaccine


Fig. 1

![Graph showing changes in PLT and WBC over time with markers for Onset, Admission, and Discharge. The graph highlights days 1-18 with specific counts and percentages of PLT and WBC, indicating fever, rash, and diarrhea.](image-url)
Table 1: Virus detection and IgG titers.

<table>
<thead>
<tr>
<th>Disease days</th>
<th>Virus isolation (DENV)</th>
<th>Real-time PCR*</th>
<th>RT-PCR using newly designed primers</th>
<th>ELISA IgG (DENV) Index**</th>
<th>ELISA IgG (JE) P/N ratio***</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>+(type 2)</td>
<td>+</td>
<td>nt</td>
<td>-(0.63)</td>
<td>+(7.16)</td>
</tr>
<tr>
<td>7</td>
<td>nt</td>
<td>nt</td>
<td>+(type 2)</td>
<td>+(2.11)</td>
<td>nt</td>
</tr>
<tr>
<td>8</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>+(2.35)</td>
<td>nt</td>
</tr>
<tr>
<td>10</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>+(2.45)</td>
<td>nt</td>
</tr>
<tr>
<td>49</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>+(2.93)</td>
<td>+(13.7)</td>
</tr>
</tbody>
</table>

* RNA was extracted from isolated dengue virus type 2 [11]. **Index value more than 1.1 means existence of IgG antibody against dengue virus. ***P/N ratio not less than 2.0 means existence of IgG antibody against Japanese encephalitis virus. nt: not tested.
Table 2: Titer of neutralization antibodies against four dengue virus serotypes.

<table>
<thead>
<tr>
<th>serotype</th>
<th>disease day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>&lt;10 x</td>
</tr>
<tr>
<td>2</td>
<td>&lt;10 x</td>
</tr>
<tr>
<td>3</td>
<td>&lt;10 x</td>
</tr>
<tr>
<td>4</td>
<td>&lt;10 x</td>
</tr>
</tbody>
</table>