Unique Impacts of HBV Co-infection on Clinical and Laboratory Findings in a Recent Dengue Outbreak in China

Yangbo Tang,† Zhihua Kou,† Xiaoping Tang,* Fuchun Zhang, Xian Yao, Shengyong Liu, and Xia Jin*

Department of Laboratory Medicine, Guangzhou 8th People’s Hospital, Guangzhou, China; Department of Medicine, University of Rochester, Rochester, New York

Abstract. High prevalence of hepatitis B virus (HBV) infection in China offers a unique setting to examine HBV’s influence on the presentation of dengue fever. In 398 patients admitted for suspected dengue fever, 89% (353/398) were positive for dengue IgM antibodies. Among dengue-infected patients, 8% (29/353) had chronic HBV co-infection. Only dengue virus serotype 1 was identified by virus isolation and reverse transcriptase-polymerase chain reaction assays. No case of dengue hemorrhagic fever/dengue shock syndrome was diagnosed. In addition to routine clinical tests, interleukin 2 (IL-2), IL-4, IL-6, IL-10, interferon γ (IFNγ), and tumor necrosis factor α (TNFα) levels were measured in the sera of 95% (334/353) of dengue-infected subjects as well as controls. Surprisingly, HBV/dengue co-infected patients made less IL-6 (P < 0.05) and TNFα (P < 0.05) than patients with only dengue infection. Similar levels of IL-4, IL-10, and IFNγ were found in both groups. Thus, HBV co-infection seems to alter the cytokine production pattern when patients contract dengue infection.

INTRODUCTION

Dengue fever is a mosquito-borne disease affecting 50–100 million people annually. It is caused by infection with dengue virus (DENV), a single-stranded RNA virus of the Flaviviridae family that exists as four serotypes: DENV1, 2, 3, and 4.1–6 Primary DENV infection usually presents as a self-limiting febrile illness. Some secondary infections and primary infections in infants can lead to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), possibly caused by a mechanism known as antibody-dependent enhancement (ADE).4,7,8

Since first being reported in the 1770s in Asia, Africa, and North America, dengue fever has spread to more than 100 countries. In China, dengue was first documented in the 1940s in the southern coastal regions of Shanghai city, Fujian, and Guangdong provinces, followed by a major outbreak in 1945 in Hubei province, which was the only in-land epidemic ever reported. No dengue epidemics were documented in the next three decades. Dengue outbreaks have re-emerged since 1978. The most heavily dengue affected area is the Guangdong province. Its warm climate and high humidity are particularly suited for mosquito growth and the spread of dengue virus. All four DENV serotypes have circulated in Guangzhou and surrounding counties. Recent outbreaks since 1995 are all caused by DENV1.5–14 Guangzhou, the capital city of Guangdong, is densely populated by 12 million local residents and migrant workers and is the epicenter of the current dengue epidemic.

Hepatitis B virus (HBV) infection is a leading cause of liver diseases and death in China. Approximately 60% of the 1.3 billion Chinese has a history of HBV infection, and 9.8% of them are chronically infected.15 The manifestation of dengue infection in a population with high HBV prevalence has never been comprehensively studied. In a 2006 dengue outbreak in Guangzhou, we identified 353 patients with dengue fever including 29 with chronic HBV co-infection. We found significant differences in serum cytokine profiles between the HBV-positive and -negative groups. This is the first time that such an observation has been made.

MATERIALS AND METHODS

Study subjects. Three hundred ninety-eight suspected dengue fever patients were admitted to the Guangdong 8th People’s Hospital from August 8 to October 27, 2006. Three hundred fifty-three of them were confirmed as having dengue by either anti-dengue antibody tests or virus isolation in C6/36 mosquito cells. According to World Health Organization (WHO) criteria,16 all patients were diagnosed as having dengue fever and none as having DHF/DSS. Blood, urine, and feces samples were obtained and tested during the hospital stay (ranged from Day 0 to Day 11 after the onset of fever). Sera were collected and stored at −80°C. Sera from 41 healthy donors and 47 chronic HBV-infected subjects were also obtained as study controls. Informed consents were obtained from adult patients or legal guardians of younger patients. Chinese rules and regulations for human subject protection were strictly followed.

Clinical and laboratory tests. Standard tests including complete blood counts, coagulation tests, liver function tests, and occult blood tests were performed in the hospital laboratories. Dengue IgM and IgG capture ELISA kits (Panbio, Brisbane, Queensland, Australia) were used to diagnose dengue infection according to the manufacturer’s protocol.17 For HBV infection, HBs, HBe antigens, IgG antibodies specific for HBs, HBe, and HBc, and IgM antibody for HBc were measured in sera using standard domestic diagnostic ELISA kits (InTec Products, Xiamen, China). A diagnosis of HBV chronic infection was made if a patient was positive for HBsAg and negative for anti-HBc IgM. Serum cytokine levels for interleukin 2 (IL-2), IL-4, IL-6, IL-10, interferon γ (IFNγ), and tumor necrosis factor α (TNFα) were measured using ELISA kits (Diaclone, Besancon Cedex, France) according to the manufacturer’s instructions.

Statistical analysis. Statistical analysis was performed with GraphPad Prism Version 4.0 (GraphPad Software, San Diego, CA). One-way analysis of variance (ANOVA) was per-
formed for group comparisons. Associations between laboratory measurements were analyzed by Spearman rank correlation test. A value of $P < 0.05$ was considered significant.

RESULTS

Epidemiologic features of the 2006 dengue outbreak in Guangzhou. Approximately 90% (353/398) of admitted patients were diagnosed as having dengue, 60% (212/353) of them were positive for anti-dengue IgM, and 40% (141/353) were positive for both anti-dengue IgM and IgG antibodies. In the first 125 admitted patients, only DENV1 serotype was identified by either virus isolation or reverse transcriptase-polymerase chain reaction (RT-PCR) assays. Hence, this was considered a DENV1 epidemic. The dengue cases spread over a wide age range and was equal in both sexes. There were no DHF/DSS cases. Clinical and routine laboratory findings were unremarkable (Table 1) and similar to what was expected for primary DENV1 infection.

Aberrant cytokine secretion in patients with HBV co-infection. Because elevated cytokine level is a regular feature of dengue infection, we decided to compare cytokine levels between 307 subjects with dengue infection only (HBV−DV+), and 27 subjects who have dengue and HBV co-infection (HBV+DV+). These subjects were randomly selected from all subjects in whom we stored sufficient serum. As controls, we also included 41 healthy subjects (HBV−DV−) and 47 patients with only chronic HBV infection (HBV+DV−). The age distributions of these four groups were overlapping, with a median age of ~40 years (Figure 1A). In comparison to healthy controls, alanine aminotransferase (ALT) levels were elevated in patients with either HBV or dengue infection (Figure 1B), but aspartate aminotransferase (AST) levels were only increased in dengue-infected patients, irrespective of HBV status (Figure 1C). Similar abnormal liver function tests were common in patients with dengue.

Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics and laboratory findings of study subjects</th>
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<tbody>
<tr>
<td></td>
<td>Greater than 14 years old</td>
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<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>Cases</td>
<td>180</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.6 ± 14.3</td>
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<tr>
<td>HBV+</td>
<td>19/180 (10.6%)</td>
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<tr>
<td>Hematocrit (%)</td>
<td>40.4 ± 3.5</td>
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<tr>
<td>Petechiae</td>
<td>49/180 (27.2%)</td>
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<tr>
<td>PT (&gt; 14 s)</td>
<td>8/163 (4.9%)</td>
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<tr>
<td>Thrombocytopenia (&lt; 100 x 10^3/mm^3)</td>
<td>145/180 (80.6%)</td>
</tr>
<tr>
<td>Neutropenia (&lt; 2.0 x 10^3/mm^3)</td>
<td>159/180 (88.3%)</td>
</tr>
<tr>
<td>ALT (&gt; 40 U/L)</td>
<td>110/174 (63.2%)</td>
</tr>
<tr>
<td>AST (&gt; 40 U/L)</td>
<td>155/174 (89.1%)</td>
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</tbody>
</table>

FIGURE 1. Age distribution and liver function tests in patient groups. The age distribution (A), and liver functional tests including levels of alanine aminotransferase (ALT, B) and asparate aminotransferase (AST, C) are shown. There were 41 healthy controls (HBV−DV−), 307 patients with only dengue infection (HBV−DV+), 47 patients with only HBV chronic infection (HBV+DV−), and 27 patients with both viral infections (HBV+DV+). The ALT and AST values were compared among groups, and significant differences are marked by their respective $P$ values. The box-and-whiskers show median and 25th and 75th percentiles of data distribution.
FIGURE 2. Comparison of serum cytokine levels in the study groups. Serum cytokines are measured as described in the Materials and Methods in the same subjects shown in Figure 1. Each dot represents an individual patient’s value, which is expressed in picograms per milliliter. The differences in cytokine levels were compared among study groups. Significant differences are marked by their respective P values.

FIGURE 3. Associations between platelet counts and cytokine levels. Linear regression analyses between platelet counts and serum cytokine levels were performed in 307 patients who have only dengue infection (HBV–DV+ group in Figure 1). Platelet counts have significant negative correlations with IL-10 levels (r = -0.1988, P = 0.0005, C), but not with the levels of IL-4, IL-6, IFN-γ, or TNFα (A, B, D, and E).
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Figure 3C) but had no statistically significant correlation with levels of IL-4 ($r = -0.0147$, $P = 0.7968$; Figure 3A), IL-6 ($r = -0.0612$, $P = 0.2852$; Figure 3B), IFNγ ($r = -0.0545$, $P = 0.3410$; Figure 3D), or TNFα ($r = -0.0643$, $P = 0.2615$; Figure 3E). Additionally, AST, but not ALT, levels were positively associated with IL-10 levels ($P = 0.015$, data not shown).

DISCUSSION

By a careful examination of 353 patients with dengue fever, we reconstructed epidemic and immunologic features of a major dengue outbreak in 2006 in the southern China coastal city Guangzhou. For the first time, we discovered that chronic HBV infection led to an aberrant cytokine secretion profile in patients when experiencing dengue infection.

This dengue epidemic was caused by DENV1 infection, which presented clinically as dengue fever only, without a single case of DHF/DSS, although there were thrombocytopenia, neutropenia, and abnormal liver function tests in most patients. These are in agreement with published data that approximately 8% of dengue-infected patients had chronic HBV co-infection, close to the 9.8% national average.

It offers a unique opportunity to examine the effect of HBV on dengue infection. Unexpectedly, those with HBV co-infection made less IL-6 and TNFα, but had similar levels of IL-4, IL-10, and IFNγ in comparison to those with only dengue infection. These intriguing alterations of cytokine levels, as far as we know, have never been previously reported.

In patients with chronic HBV infection, the elevations of these cytokines are usually modest. None of these cytokines are detectable in normal healthy individuals. The magnitude of IL-10, but not IL-4, IL-6, IFNγ, or TNFα, is proportional to the severity of thrombocytopenia ($r = -0.1988$, $P = 0.0005$), similar to a previous report. Moreover, IL-10 levels were positively associated with abnormal AST tests (data not shown), similar to published data.

What causes the aberrant cytokine production in chronic HBV-infected patients when infected by dengue? One obvious reason is that patients with chronic HBV infection tend not to be as well nourished as healthy subjects and hence may be incapable of making some cytokines quickly in response to a viral infection. This is certainly the case for undernourished children in whom DHF/DSS is rarely seen, possibly because of their inability to make the inflammatory cytokines implicated in causing vascular plasma leakage. Further studies may offer mechanistic explanations on why the elevation of some, but not all, cytokines during dengue infection is suppressed by HBV co-infection.

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Authors’ addresses: Yangbo Tang, Guangzhou 8th People’s Hospital, Guangzhou 510060, China, Tel: 0086-20-83710937, Fax: 0086-20-83866127. Zhuhua Kou, Department of Medicine, Infectious Diseases Division University of Rochester, 601 Elmwood Avenue, Box 689, Rochester, NY 14642, Tel: 585-275-3924, Fax: 585-442-9328. Xiaoping Tang, Departments of Laboratory Medicine, Guangzhou 8th People’s Hospital, Guangzhou 510060, China, Tel: 0086-20-83710688, Fax: 0086-20-83828442. Fuchun Zhang, Guangzhou 8th People’s Hospital, Guangzhou 510060, China, Tel: 0086-20-83710912, Fax: 0086-20-83828422. Xian Yao, Guangzhou 8th People’s Hospital, Guangzhou 510060, China, Tel: 0086-20-83710851, Fax: 0086-20-83866127. Shengyong Liu, Department of Medicine, Infectious Diseases Division University of Rochester, 601 Elmwood Avenue, Box 689, Rochester, NY 14642, Tel: 585-275-3924, Fax: 585-442-9328. Xia Jin, Department of Medicine, Infectious Diseases Division, University of Rochester, 601 Elmwood Avenue, Box 689, Rochester, NY 14642, Tel: 585-275-6515, Fax: 585-442-9328.

Reprints requests: Xiaoping Tang, Departments of Laboratory Medicine, Guangzhou 8th People’s Hospital, Guangzhou 510060, China, E-mail: xtping@21cn.com.

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