NOTES

A Serotyping Assay for Hepatitis C Virus in Southeast Asia

SIRIRURG SONGSIVILAI,* DUANGJIT KANISTANON, AND TARARAJ DHARAKUL
Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

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A serotyping assay for hepatitis C virus (HCV) was evaluated with samples from Thailand, where the distribution of HCV genotypes was different from that in Western countries where the assay was designed and validated. The sensitivity of the assay was low (58%) for HCV RNA-positive samples compared to that of the genotyping assay (95%, P < 0.01). In addition, only 36% of anti-HCV-positive but HCV RNA-negative samples could be serotyped. The serotypes and genotypes were identical in 96% of the samples that could be typed by both methods. Most of the samples with genotype 6, which was common in Southeast Asia, were nontypeable by this serotyping assay.

Genetic heterogeneity of hepatitis C virus (HCV) isolated from different geographical regions was documented, and at least six major genotypes with a series of several subtypes of HCV have been identified to date (2, 11). Infection with different viral genotypes may affect the disease severity and response to treatment (3). In addition, the sensitivity of diagnostic assays for detecting anti-HCV antibodies was influenced by viral genotypes (6, 7). Several HCV genotyping methods have been reported and used to study the epidemiology and clinical significance of the viral genotype (5, 8). However, the genotyping assays were difficult to perform, expensive, and time-consuming and required samples which have to be collected and stored under proper conditions in order to preserve sufficient quality and quantity of viral RNA. Anti-HCV-positive but HCV RNA-negative samples cannot be typed by the genotyping assays. Recently, serotyping methods for differentiating HCV into serotypes which correspond to the viral genotypes were developed. Two HCV serotyping assays have been commercially available: recombinant immunoblot assay HCV serotyping strip immunoblot assay (RIBA HCV serotyping SIA; Chiron, San Diego, Calif.) and enzyme immunoassay (Murex HCV serotyping 1-6; Murex, Dartford, United Kingdom) (1, 4). The former assay was based on selective binding of antibodies to NS4 and core peptides and was able to identify genotypes 1 to 3, while the latter test used competitive enzyme immunoassays with NS4 peptides to differentiate genotypes 1 to 6. Both assays were developed and validated in the United States and Europe. However, little is known about the efficiency of these serotyping assays with samples from other areas of the world where the genotypic distribution of HCV is different from that in the Western countries.

In this study, we evaluated the usefulness of a serotyping assay for HCV by comparing it with a genotyping assay. We used 133 HCV RNA-positive samples from Thailand, where HCV genotypes 3a, 6, and 1b were prevalent (5, 8, 13). In addition, the viral serotypes of 22 anti-HCV-positive but HCV RNA-negative samples whose viral genotype could not be determined were analyzed. All samples were from low-risk blood donors and all tested negative for antibodies to human immunodeficiency virus.

HCV genotypes were identified in samples containing HCV RNA by using the combination of two reverse hybridization assays based on nucleotide sequence heterogeneity in the 5′ noncoding and core regions of the viral genome, as previously described (5). The viral serotypes were determined by using the commercial HCV serotyping 1-6 assay (Murex), and the tests were performed according to the manufacturer’s instruction (1). Briefly, the microtiter plate was coated with a panel of NS4 peptides specific to HCV genotypes 1 to 6. Serotypes were determined by the ability of genotype-specific competing peptides to block the binding of genotype-specific antibodies to the coated peptides. Each sample was incubated in eight wells. The sample was incubated with or without all competing peptides in two wells which served as the blocked and unblocked controls. Each of the remaining six wells was incubated with a panel of competing peptides specific to sets of five HCV genotypes. Based on the manufacturer’s criteria, the serotypes were identified if the optical density (OD) value of a serotyping well which did not contain peptides from a particular genotype divided by the OD of the unblocked control (OD ratio) was greater than 0.4. Samples with an OD ratio less than 0.4 in all serotyping wells were recorded as non-type specific (NTS), whereas samples with a difference in the ODs of unblocked and blocked controls of less than 0.1 were recorded as non-active (NR). Mixed infection was noted if an OD ratio of >0.4 was obtained from more than one serotyping well. This assay could identify HCV serotypes 1 to 6, which correspond to the genotype but not the subtype levels.

The results are summarized in Table 1. Reverse hybridization assays were used to identify viral genotypes in 126 of 133 HCV RNA-positive samples (94.7%). Genotypes 1a, 1b, 3a, 3b, and 6 were found in 12.0, 20.3, 34.6, 3.8, and 24.1% of the samples, respectively. The other seven HCV isolates (5.3%) were unclassified. None of the blood donors were infected with more than one genotype. The genotype distribution pattern was similar to that in our previous report on HCV genotypes in Thailand (5).

In contrast, the serotyping assay did not perform well for...
These HCV serotypes could be identified in only 77 of 133 (57.9%) of HCV RNA-positive samples (P = 0.0014 compared to the genotyping assay, by chi-square test). The sensitivities of the serotyping assay were 68.8% for genotype 1a, 51.9% for genotype 1b, 76.1% for genotype 3a, 60.0% for genotype 3b, and 21.9% for genotype 6. This level of sensitivity was very low compared to those obtained in published studies with samples from the United States and Europe, in which 80 to 90% of the samples could be typed (1, 4, 9, 10, 12, 14). This reflected the heterogeneity between NS4 epitopes of the Southeast Asian HCV isolates and the epitopes of the same genotypes of HCV isolates from the Western countries, isolates which were used for designing the peptides. In particular, the assay performed poorly with the genotype 6 samples, since it was developed based on genotype 6a found in Hong Kong, which is markedly different from the variants of this genotype commonly found in Southeast Asia.

In samples that could be typed by both the serotyping and genotyping assays, almost all samples (96%) showed concordant results. All genotype 1 samples were identified as genotype 1a or 1b. All genotype 3b samples which could be serotyped showed the genotype 3 pattern. However, two genotype 6 samples (6.3%) were identified as genotype 3, and one genotype 3 sample (2.2%) was identified as genotype 1.

In the anti-HCV-positive but HCV RNA-negative samples, the viral genotypes could be identified only by serotyping, the commercial serotyping assay performed poorly and was able to identify the serotype in only 8 (36.4%) of 22 samples. Six of those samples were serotype 1 and two samples were serotype 3.

This is the first report on the applicability of an HCV serotyping assay to samples from a population in which genotypes 3 and 6 are more common than genotype 1. The 70 nonserotypeable samples were identified as mixed infection (15 samples, 21.4%), NR (26 samples, 37.1%) and NTS (29 samples, 41.4%). However, it should be noted that the mixed-infection samples could have been serologically mistyped rather than genuinely infected with more than one HCV genotype because most of these samples had very low levels of antibodies to NS4 peptides or had cross-reactive antibodies to all HCV genotype-specific peptides (data not shown). The nonserotypeable samples could be divided into three groups based on the characteristics of their reactions to the peptides. Twelve samples (17.1%) had anti-NS4 antibodies or non-specific antibodies which could not be blocked by the competing peptides, whereas 20 samples (28.6%) had antibodies that could be blocked by the mixture of peptides from six genotypes but not by any sets of five-genotype-specific competing peptides. These two groups probably represent samples infected with variants of HCV genotypes which were not covered by the competing peptides used in the assays. The remaining 38 samples (54.3%) did not react to NS4 peptides used to coat the plates despite both the presence of antibodies to HCV, as detected by a second-generation assay for anti-HCV antibodies, and the presence of HCV RNA. Collectively, the results demonstrated that the panels of coated and competing peptides used in the serotyping assay were not adequate for the genotypes of HCV found in Thailand.

In conclusion, the commercial HCV serotyping assay had a low sensitivity for all HCV genotypes found in Thailand, where genotypes 3 and 6 were shown to be common. The high concordance of genotyping and serotyping methods for samples that could be serologically typed was observed with genotypes 1 and 3, but not with genotype 6. This study indicated that assays for identifying HCV genotypes should be tailored to viral genotypes common in the geographical area where the assays are used. In its present format, the serotypic genotyping assay has little value for genotype and serotype identification of HCV found in Thailand.

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We thank I. Pike for providing Murex HCV serotyping 1-6 kits and P. Simmonds for helpful discussion.

**TABLE 1. Comparison between HCV genotypes determined by reverse hybridization assays and serotypes determined by enzyme immunoassay**

<table>
<thead>
<tr>
<th>Sample type and genotype</th>
<th>HCV RNA positive</th>
<th>HCV RNA negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) of samples</td>
<td>No. (%) of samples</td>
</tr>
<tr>
<td></td>
<td>Serotype 1</td>
<td>Serotype 3</td>
</tr>
<tr>
<td>HCV RNA positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>16</td>
<td>11 (69)</td>
</tr>
<tr>
<td>1b</td>
<td>27</td>
<td>14 (52)</td>
</tr>
<tr>
<td>3a</td>
<td>46</td>
<td>1 (2)</td>
</tr>
<tr>
<td>3b</td>
<td>5</td>
<td>3 (60)</td>
</tr>
<tr>
<td>6 group variants</td>
<td>32</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Unclassified</td>
<td>7</td>
<td>4 (57)</td>
</tr>
<tr>
<td>Total</td>
<td>133</td>
<td>30 (23)</td>
</tr>
</tbody>
</table>

* Bold indicates samples with the concordant serotyping and genotyping results.

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**REFERENCES**