Dengue hemorrhagic fever (DHF) is the main cause of mortality in dengue virus infections. Its pathogenesis is not very well defined; however, available data strongly suggest that most cases are caused by immunopathologic mechanisms. Antibodies to dengue virus have been reported to mediate three biologic functions in vitro which may contribute to prevention of or recovery from dengue virus infection (neutralization, complement-mediated cytolysis, and antibody-dependent cell-mediated cytotoxicity) (5, 6). However, antibodies can also augment dengue virus infection through the phenomenon called antibody-dependent enhancement. Despite the antigenic relatedness of viruses in the dengue virus complex, two or more virus types may sequentially infect one host. When this occurs, the antibody response to the sequential infection is markedly different from that elicited by the primary infection (3, 4).

Dengue virus proteins can stimulate antibody production; however, few studies have been conducted to characterize this response and to define how these antibodies are related to the recovery from or severity of dengue infections.

The objective of this study was the definition of the immune response to dengue virus structural and nonstructural proteins. Serum samples taken within 5 to 7 days of disease onset from 20 serologically confirmed dengue cases from Colombia were studied. Dengue immunoglobulin M (IgM) antibodies were detected in all samples. In three DHF cases dengue virus type 2 (Den-2) was detected by PCR. Sera from 5 dengue fever patients (all with a primary dengue infection) and 15 DHF patients (5 primary and 10 secondary infections) were examined by means of Western blotting (8). Den-2 and Den-4 antigens were prepared as described by Falconar (2). Briefly, viruses were inoculated in Vero cells and incubated at 37°C until 70% of the cytopathic effect was evident (4 to 5 days). Cultures were centrifuged at 3,000 rpm for 30 min. Proteins in the supernatant were precipitated over 18 h at 4°C using 7% ammonium sulfate. Following 40 min of centrifugation at 15,000 rpm, the pellet was resuspended in sample buffer (25 mM Tris-HCl [pH 6.8], 3% sodium dodecyl sulfate, 10% glycerol, 0.05% bromophenol blue-xylene cyanol). Ten percent polyacrylamide gel electrophoresis was performed according to the classical method of Laemmli with two modifications: 2-mercaptoethanol was omitted from the sample buffer, and the antigen was heated for 2 min at 80°C (1, 7). After electrophoresis, proteins were transferred to nitrocellulose membrane (Schleicher & Schuell), and Western blotting was performed using an anti-human Ig conjugated with peroxidase (Amersham). Human sera were diluted 1/40 and 1/80. Dengue polyclonal and monoclonal antibodies and serum from a nonimmune dengue virus-infected individual were used as controls. Noninfected cells were included as a negative control. No visible bands appeared in lanes containing noninfected cells.

Total Igs to at least one or two proteins of Den-4 antigen were demonstrated in 9 of 10 (90%) of primary cases and in all of the secondary cases. No antibodies to NS1 could be detected in any sera of the primary cases; however, 4 of 10 secondary cases (40%) had anti-NS1 antibodies. Responses to envelope (E) and NS5 proteins were consistent in both primary and secondary cases, being more intensive in the latter. A greater reactivity to the different proteins was observed in secondary cases (Table 1). No anti-NS3 antibody could be detected in primary or secondary cases.

A more limited response was observed with Den-2 antigen. Again, a wide anti-E antibody response was observed in both primary and secondary cases, and anti-NS3 antibody could be detected in all secondary cases. The response to NS1 was again observed only in secondary cases but was higher (80%) and more intensive than that observed with Den-4 antigen.

Humans infected naturally with a dengue virus have a rapid, potent antibody response that is easily measured by many serological tests. However, the qualitative antibody response to dengue viruses has not been widely studied. Our results are in general agreement with previous reports (1, 5, 9). In either primary or secondary cases, anti-E antibody is the most frequently detected. Probably this fact is related to the role of E protein, which is the major virion surface protein and the most important viral antigen in terms of virus biology, humoral immunity, and protection. It is also very interesting to note the wide antibody response to NS5 protein (similar to E), as this is a nonstructural protein with a polymerase activity.

Some authors have reported a significant antibody response to NS3 protein in both primary and secondary cases (1); how-
ever, our results show a significant and specific antibody re-
sponse in secondary cases, probably due to specificity of these
antibodies according to the infecting serotype (all secondary
cases presented anti-NS3 antibody to Den-2 antigen). NS3
(which is considered the viral protease) is an important target
for human T cells. These results confirm, once again, that NS3
and NS5 proteins (produced during the first steps of viral
replication) are able to elicit specific antibodies, whose role is
unknown at present.

Anti-NS1 antibodies were detected only in patients with a
secondary type of infection. It has been suggested that anti-
NS1 antibodies may be associated with DHF, as they have been
frequently detected in sera from DHF patients with a second-
ary infection (5). In our study, we also found the anti-NS1
antibody in sera of eight DHF patients with a secondary infec-
tion; however, no anti-NS1 antibody was detected in five DHF
patients with primary infections. The role of NS1 protein in
replication and morphogenesis is not clear; however, it is found
at high titers in animals infected or immunized with virus. The
anti-NS1 antibody detection in DHF secondary cases suggests
a role in the etiopathogenesis of DHF.

Our results demonstrate that the antibody response in acute-
phase samples from primary and secondary cases suggests the
possibility of implementing new diagnostic assays with higher
sensitivity for E, NS3, and NS5 antigen detection that allow the
eye early diagnosis of dengue fever and DHF cases.

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REFERENCES

1. Churdboochart, V., N. Bhamarapravati, S. Peampramprecha, and S. Siri-
navin. 1991. Antibodies against viral proteins in primary and secondary
ical Medicine, London, United Kingdom.
In D. Gubler and G. Kuno (ed.), Dengue and dengue hemorrhagic fever—
fever in the Americas: guidelines for prevention and control. Scientific pub-
lication 548. Pan American Health Organization, Washington, D.C.
9. Se-Thoe, S., M. Ng, and A. Ling. 1999. Retrospective study of Western blot

TABLE 1. Antibodies to Den-2 and Den-4 proteins in primary and secondary cases

<table>
<thead>
<tr>
<th>Infection and antigen</th>
<th>C</th>
<th>preM</th>
<th>E</th>
<th>NS1</th>
<th>NS3</th>
<th>NS5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Den-2</td>
<td>ND</td>
<td>1 (w)</td>
<td>6 (w)</td>
<td>1 (w)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Den-4</td>
<td>2 (w)</td>
<td>3 (w)</td>
<td>9 (7w, 2s)</td>
<td>7 (w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary Den-2</td>
<td>ND</td>
<td>6 (w)</td>
<td>10 (2w, 8s)</td>
<td>10 (w)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Den-4</td>
<td>7 (4w, 3s)</td>
<td>9 (2w, 7s)</td>
<td>10 (s)</td>
<td>4 (3w, 1s)</td>
<td>10 (1w, 9s)</td>
<td></td>
</tr>
</tbody>
</table>

a C, capsid; preM, M precursor; E, envelope; NS1, NS3, and NS5, nonstructural proteins. The intensity of reaction is shown in parentheses (w, weak; s, strong).
b ND, not done.