Antigenic Relatedness of Two Strains of Hepatitis A Virus Determined by Cross-Neutralization

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Cell culture-adapted HM-175 (Australia) and PA-21 (Panama) strains of hepatitis A virus were compared in a cross-neutralization radioimmunofocus inhibition assay. The ratio of antibody titers achieved with two sera against the two viruses differed by less than twofold, indicating a high degree of antigenic relatedness between epidemiologically disparate strains of virus.

Since World War II, when immune serum globulin manufactured within the United States was found to protect American soldiers stationed in the Middle East from developing icteric infectious hepatitis (2), it has been suspected that strains of infectious hepatitis virus occurring in different regions of the world must be closely related antigenically. Additional evidence supporting this hypothesis came from early human transmission studies, which demonstrated cross-immunity between strains of hepatitis virus recovered from individuals infected during a summer camp outbreak in Pennsylvania and from American soldiers serving in the Mediterranean region (6). These early findings were corroborated by subsequent attempts to compare strains of hepatitis A virus (HAV) using primate infectivity to determine HAV neutralization (7), immune adherence hemagglutination (8), or solid-phase radioimmunoassay (5). However, it is only recently that techniques have been developed which permit an accurate determination of the antigenic relatedness of different strains of HAV (4). In this report, we describe the analysis of two epidemiologically disparate, cell culture-adapted strains of HAV by cross-virus neutralization by a radioimmunofocus inhibition assay (S. M. Lemon and L. N. Binn, J. Infect. Dis., in press).

The HM-175 strain of HAV was originally recovered from an infected human in Australia (1) and was isolated in low-passage, African green monkey kidney cells from sixth marmoset passage liver, provided by S. Feinstone, National Institute of Allergy and Infectious Diseases, Bethesda, Md. The PA-21 strain of HAV was originally recovered in Panama (5). Both strains of virus were adapted to growth in continuous green monkey kidney (BS-C-1) cells, and working virus seeds were prepared as previously described (4). HM-175 virus was used at cell culture passage level 11, and PA-21 virus was used at cell culture passage level 6. Although both virus strains were isolated in the same laboratory, all manipulations were carried out in laminar flow hoods, and precautions were taken to ensure that different strains of virus were never handled within the same hood on the same day.

Preinfection and postinfection (day 143) serum specimens were collected from a colony-bred owl monkey experimentally infected with the PA-33 virus (3). (The PA-33 and PA-21 strains of HAV used in these studies were recovered from the feces and liver, respectively, of naturally infected owl monkeys involved in a primate colony-centered epidemic of HAV infection and are considered to represent the same original strain of virus [5]). Preinfection and postinfection (day 127) sera from a chimpanzee experimentally infected with HM-175 virus were the gift of S. Feinstone. All sera were heat inactivated at 56°C for 30 min and diluted in Hanks balanced salt solution containing 5% heat-inactivated fetal bovine serum (HBSS-fbs).

The neutralizing activity of the serum specimens was quantitated for both virus strains by a radioimmunofocus inhibition test. This virus neutralization assay is based on the accurate titration of infectious units of HAV (radioimmunofocus-forming units) which are visualized by the immune autoradiography of cultures of BS-C-1 cells inoculated with virus and overlaid with agarose (4). The method of quantifying infectious HAV is analogous to a virus plaque assay in many ways, and its adaptation to the determination of specific neutralizing antibody is described elsewhere (Lemon and Binn, in press). Briefly, virus was diluted to a concentration of approximately 480 radioimmunofocus-forming units per ml in HBSS-fbs. Fourfold
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dilutions of serum (0.1 ml) were added to 0.9-ml samples of diluted virus, mixed, and incubated at 35°C for 60 min. Duplicate acetone-resistant plastic petri dishes containing confluent BS-C-1 cells were then inoculated with 0.25-ml volumes of the virus-serum mixtures. Control cultures were inoculated with HBSS-fbs alone or with virus mixed with an appropriate volume of HBSS-fbs. After a 120-min adsorption period, cultures were overlaid with medium containing 0.5% agarose and incubated for 14 days at 35°C. The overlay was then removed, and the cells were fixed with acetone and stained with a solution of ¹²⁵I-labeled anti-HAV immunoglobulin G. After several washing steps, the petri dish bottoms were cut out and exposed for autoradiography. This method of detecting infectious HAV is specific, sensitive, and highly reproducible (4). In addition, previous studies have shown that the radioimmunofocus inhibition test is more sensitive than conventional blocking radioimmunoassays in that it is capable of detecting neutralizing antibody at higher dilutions of sera.

The serum-neutralizing antibody titer was defined as the 50% virus survival endpoint, which was estimated by linear regression analysis of the logistic function \[ \log[y/(100 - y)] \] where \( y \) equals the percent reduction in radioimmunofoci relative to virus incubated with HBSS-fbs alone. Statistical analysis was carried out with a Professional Statistical Calculation Package (Ed-Sci Development, San Francisco, Calif.) and an Apple IIe microcomputer.

Results of the cross-neutralization experiment are shown in Fig. 1, which presents the percent neutralization of HM-175 and PA-21 virus strains obtained with fourfold dilutions of sera collected after infection of primates with either HM-175 or PA-33 viruses. No virus-neutralizing activity was detected in preinfection sera. Control cultures, inoculated with virus mixed with HBSS-fbs alone, had a mean of 83 (HM-175) and 82.5 (PA-21) radioimmunofocus-forming units per petri dish. The PA-21 virus seed appeared to be more readily neutralized, both in terms of the degree of neutralization at low serum dilutions and in terms of the titer of neutralizing antibody achieved. However, there was less than a two-fold difference in the ratios of titers achieved with the two sera against the two virus strains (Table 1). Thus, by cross-neutralization, there was no significant antigenic difference detected between these epidemiologically disparate strains of HAV.

The absence of significant differences in the relative ability of HM-175 and PA-33 immune sera to neutralize HM-175 and PA-21 strains of HAV supports epidemiological studies, immune serum globulin trials, human transmission ex-

![Fig. 1](http://iai.asm.org/000000000.png)

**FIG. 1.** Cross-neutralization of HM-175 (A) and PA-21 (B) strains of HAV with fourfold dilutions of postinfection sera collected from primates infected with HM-175 (○) or PA-33 (▲) viruses. Each point represents the mean of neutralization values determined in replicate cultures by the radioimmunofocus inhibition method. Best-fit lines were obtained by linear regression analysis of the logistic function of the percent viral neutralization (see text) obtained with HM-175 (---) and PA-33 (----) immune sera. Regression coefficients varied from \( R = 0.9195 \) to \( R = 0.9809 \), and the slopes of these best-fit lines were not statistically different \( (P > 0.05) \). Control cultures inoculated with virus–HBSS-fbs mixtures contained 83 (HM-175) and 82.5 (PA-21) radioimmunofocus-forming units of HAV, respectively.
TABLE 1. Cross-neutralization of HM-175 and PA-21 strains of HAV by homologous and heterologous primate antisera

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Antisera&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th>Ratio&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HM-175</td>
<td>PA-33</td>
<td></td>
</tr>
<tr>
<td>HM-175</td>
<td>1:1,094</td>
<td>1:2,600</td>
<td>0.42</td>
</tr>
<tr>
<td>PA-21</td>
<td>1:5,176</td>
<td>1:15,523</td>
<td>0.33</td>
</tr>
</tbody>
</table>

<sup>a</sup> Fifty percent radioimmunofocus inhibition titer (see text).

<sup>b</sup> Ratio of reciprocal HM-175 to PA-33 antisera titers against indicated strain of HAV.

experiments, and previous laboratory observations which have suggested that minimal antigenic variation exists between various strains of HAV (2, 5–8). It is noteworthy that the HM-175 and PA-21 viruses were recovered on opposite sides of the world and from different host species (HM-175 from a human and PA-21 from an owl monkey). Although additional comparisons between other cell culture-adapted strains of HAV will be required before any general conclusions can be reached, the lack of detectable differences between the HM-175 and PA-21 strains strongly suggests that a vaccine prepared with any strain of HAV should provide broad protection against other strains from different geographic regions.

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