Influence of Human Immunodeficiency Virus Type 1 Infection on Acute Hepatitis A Virus Infection

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To assess the possible influence of human immunodeficiency virus type 1 (HIV-1) infection on the clinical course of acute hepatitis A virus (HAV) infection, 15 HIV-1–infected homosexual men and 15 non–HIV-infected age-matched subjects were compared. HAV load was higher in HIV-1–infected than in non–HIV-infected patients ($P < .001$). Duration of viremia in HIV-1–infected patients (median, 53 days) was significantly longer than in non–HIV-infected patients (median, 22 days). HIV–1–infected patients had lower elevations in alanine aminotransferase levels than did non–HIV-infected patients ($P < .05$) but had higher elevations in alkaline phosphatase levels than did non–HIV-infected patients ($P < .01$). Some HIV–1–infected patients still had HAV viremia when clinical symptoms had disappeared and alanine aminotransferase levels had returned to normal (60–90 days after the onset of symptoms). HIV–1 infection was associated with prolongation of HAV viremia, which might cause a long-lasting outbreak of HAV infection in HIV–1–infected homosexual men.

Hepatitis A virus (HAV) infection was prevalent throughout the world before World War II, but its seroprevalence has declined since then in developed countries [1]. The disease itself has been well studied and described in detail in textbooks of infectious diseases [2, 3] and the medical literature [4]. In HAV infection, a large amount of the virus is excreted in the fecal material, mostly during the late incubation period and at the time of onset of symptoms, and excretion continues until peak elevation of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels. The duration of viremia is thought to be quite short, shorter than the period of fecal excretion [5], and limited to the latter half of the incubation period [1], although the use of current molecular techniques has shown that the duration of viremia is in fact longer than previously thought [6–8]. However, information is still very limited; because HAV infection is basically an acute infection, sequential serum samples for long-term follow-up have not been stocked in most clinical settings [9, 10].

The route of HAV infection is almost exclusively fecal–oral transmission [11, 12]. However, HAV is also transmitted from person to person as a sexually transmitted disease among homosexual men [13–15] and as a nosocomial infection in neonatal intensive care units [16]. It can also be transmitted to hemophiliacs through transfusion of blood products [17, 18]. Because there is neither a nonhuman reservoir nor persistent infection in humans, HAV must be transmitted serially from a person with an acute case to susceptible persons. Therefore, epidemics are sporadic in developed countries at
present. However, outbreaks of HAV infection among HIV-1–infected homosexual men can last >1 year [14, 19]. These epidemic data might indicate that fecal excretion is prolonged in the case of concurrent HIV-1 infection. In the present study, we determined the duration of HAV infection on the basis of the level of HAV in serum instead of in fecal material because only sequential serum samples were obtained and stored. We then compared several features of HAV infection in HIV-1–infected and non–HIV-infected patients to determine the influence of HIV-1 infection on the clinical course of acute HAV infection.

**PATIENTS AND METHODS**

**Patients.** From August 1998 through September 1999, 22 homosexual men infected with HIV-1 were diagnosed with acute HAV infection and hospitalized with clinical manifestations and laboratory abnormalities in the AIDS Clinical Center, International Medical Center of Japan [19]. The diagnosis was confirmed by detection of anti-HAV IgM antibodies at the time of documentation of clinical symptoms. To analyze the relationship between HIV-1 and acute HAV infection, patients with hepatitis B and C were excluded. Accordingly, 7 patients were excluded from the study: 5 were coinfected with hepatitis C virus, 1 was coinfected with hepatitis B virus, and 1 did not have sequential stored samples available. For comparison, 15 age-matched HAV-infected non–HIV-infected patients from whom sequential serum samples were obtained and stored from 1990 through 1996 were arbitrarily selected from those at the World Health Organization Collaborating Center on Viral Hepatitis, National Nagasaki Medical Center, Nagasaki, Japan. In the non–HIV-infected group, 8 patients were female (P = .01). Demographic characteristics of the study group are shown in Table 1. All serum samples were stored at −20°C until use. Clinical and laboratory data were reviewed retrospectively after completion of all laboratory tests, including titration and duration of HAV viremia.

Written informed consent was obtained from all HIV-1–infected patients. Verbal consent was obtained from all non–HIV-infected patients at the time that samples were obtained (1990–1996).

**Sero logical analyses and detection and titration of HAV.** Anti-HAV IgG and IgM antibodies in serum samples were determined by use of HAVAB and HAVAB-M kits (Dinabot), respectively. Total RNA was extracted from 80 μL of serum by use of the SUMAI test (Sumitomo), according to the instructions provided by the manufacturer. RNA was resuspended in 12 μL of distilled water, and 10 μL of the suspension was subjected to amplification. The HAV VP1/2A gene was amplified by PCR by use of a One-Step RNA PCR kit (TaKaRa Shuzo) and EX Taq polymerase (TaKaRa Shuzo), according to the manufacturer’s instructions. Sequences of primer sets [16] for reverse transcription (RT) PCR, the first PCR, and the second PCR used in this study with some modifications for Japanese sequences were as follows: for the first PCR, 5′-GGTTTCTATTCAGATTGCAAATTA (positions 2891–2914) and 5′-AGTAAGAAACTCCAGCATCCATCTC (positions 3398–3375); for the second PCR, 5′-TTTAGTTGTTATTTGTCTGTC (positions 2940–2960) and 5′-CATTATTTCATGCTCCTCAG (positions 3284–3265).

Amplification involved 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min. Reamplification of 1-μL aliquots of the first PCR product was then done for 30 cycles under the same reaction conditions. Ten microliters of the resultant PCR product was subjected to agarose gel electrophoresis (2%) in gel that contained ethidium bromide.

### Table 1. Demographic characteristics of HIV-1–infected and non–HIV-infected patients with hepatitis A virus (HAV) infection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV-1–infected (n = 15)</th>
<th>Non–HIV-infected (n = 15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years ± SD</td>
<td>36.7 ± 8.8 (23–54)</td>
<td>34.7 ± 11.7 (18–50)</td>
<td>.58</td>
</tr>
<tr>
<td>Sex, no. of patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>8</td>
<td>.01</td>
</tr>
<tr>
<td>Route of HAV transmission, no. of patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homosexual sexual activity</td>
<td>15</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asymptomatic, % of patients</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CD4 count, mean cells/μL ± SD (range)</td>
<td>448 ± 140 (173–716)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CD8 count, mean cells/μL ± SD (range)</td>
<td>710 ± 218 (461–1142)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Virus load, median cells/μL (range)</td>
<td>3.3 × 10⁶ (&lt;400–1.1 × 10⁸)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Received anti-HIV therapy, % of patients</td>
<td>60</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

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bromide (0.5 g/mL) and the result was visualized with ultraviolet illumination. To avoid contamination during the procedure, RNA extraction and post-PCR analysis were done in separate rooms.

To measure HAV load in serum, the serum sample was sequentially diluted 4-fold, after which nested PCR was performed on each diluted sample. HAV load was semiquantitated and expressed as positive in an aliquot diluted $1 \times 4^n$. For this analysis, we included only those patients for whom samples were stored within 10 days after onset of clinical symptoms. This analysis was done for 8 of the HIV-1–infected and 14 of the non–HIV-infected patients. The mean period [$\pm$ SD] between the onset of symptoms and the analysis of HAV in serum was not significantly different between the 2 groups (HIV-1, 5.4 $\pm$ 2.1 days; non-HIV, 6.4 $\pm$ 1.6 days).

**Definition of duration of HAV viremia.** Duration of HAV viremia was defined as the duration from the onset of clinical symptoms to the time that the last serum sample was obtained that was positive for HAV, provided that after this last positive sample, another serum sample with negative results was obtained in the subsequent 45 days. In some patients, conversion of serum test results to negative was not detected because of the lack of stored samples. These cases were excluded from the analysis of duration of viremia. Therefore, the duration is the shortest estimate. Of the entire group of study patients, 11 HIV-1–infected and 9 non–HIV-infected patients met the criteria for calculation of duration of viremia.

**Sequence analysis of HAV.** To exclude differences in the efficiency of PCR due to mismatch of sequences between primers and clinical isolates for measurement of HAV load, primers of the nested PCR and their flanking regions were sequenced. For the sequence analysis, total RNA (extracted by the same method) was resuspended in 30 $\mu$L of distilled water, and 10 $\mu$L of the suspension was subjected to RT-nested PCR. In both the first and second PCR, the amplification was repeated for 30 cycles of denaturation at 92°C for 1 min, annealing at 53°C for 2 min, and extension at 72°C for 3 min. The primer sets for this analysis were as follows: for the first PCR, 5′-GCAGCAATTCTTCA (positions 3529–3549); for the second PCR, 5′-TCAGATTAGAATCCAGGCT (positions 2800–2819) and 5′-AGATCCACATCCATGGT (positions 3509–3528). After 1% agarose gel electrophoresis, the resultant PCR products were purified with use of SUPREC-01 (Takara Shuzo), according to the manufacturer’s instructions.

Sequencing was done with use of an automatic sequencer (Applied Biosystems) with use of the Big Dye Terminator Cycle Sequence kit (Applied Biosystems). Amino acid sequences were deduced by Genetyx-Win, version 3.1 (Software Development). A phylogenetic tree was constructed from the distance matrix by the neighbor-joining method. These procedures were computed by use of CLUSTAL W, version 1.4. Accession numbers of nucleotide sequences reported in this study were assigned at the DNA Data Bank of Japan under the numbers AB038276–AB038301.

**Statistical analyses.** Laboratory data for HIV-1–infected and non–HIV-infected patients were compared with use of Student’s $t$ test. Nonparametric data, such as titration of HAV viremia and duration of the viremia, were analyzed by use of the Mann-Whitney $U$ test. Categorized variables were compared with use of the $\chi^2$ test with Yates’s correction. $P < .05$ was considered significant.

**RESULTS**

**Comparison of clinical characteristics of HIV-1–infected and non–HIV-infected patients.** In HIV-1–infected patients, there was no correlation among elevation in AST and/or ALT levels, CD4 cell counts, and HIV-1 load. The frequency and severity of clinical symptoms such as fever, nausea, and general malaise in HIV-1–infected patients were similar to those reported for non–HIV-infected patients. As summarized in table 2, serum ALT concentrations were elevated in both groups, but they were significantly lower in HIV-1–infected than in non–HIV-infected patients ($P < .01$). Other measures, such as AST and total bilirubin levels, were not significantly different between the 2 groups, although they tended to be higher for non–HIV-infected patients than for HIV-1–infected patients.
In contrast, serum concentrations of alkaline phosphatase and γ-glutamyltransferase were significantly higher in the HIV-1–infected group than in non–HIV-infected group (P < .001 for alkaline phosphatase and P < .01 for γ-glutamyltransferase).

**Titration of HAV load at onset of acute HAV infection.** HAV load in HIV-1–infected patients was significantly higher (P < .001) than in non–HIV-infected patients (median dilution for a positive result, 1 × 10^−3 vs. 1 × 10^−2; table 3). Sequence analysis of the HAV VP1/2A gene revealed that 13 of 15 strains in HIV-1–infected patients were identical and the remaining 2 were also very closely related (only 1 amino acid substitution), according to the phylogenetic tree analysis (figure 1). The analysis clearly indicated that the outbreak was caused by the same strain, which belonged to subtype 1A [20]. In contrast, HAV obtained from different sites and at different times from non–HIV-infected patients originated from different sources in each case, although all strains also belonged to subtype 1A. We postulated that the outbreak in HIV-1–infected patients was caused by a single strain of HAV, whereas HAV infections in non–HIV-infected patients were caused by various strains, which affected PCR efficiency because of primer-template mismatches and explains the differences in the measured HAV loads between the 2 groups. To exclude this possibility, we analyzed sequences of primer sites and their flanking regions of each virus from non–HIV-infected patients. The analysis revealed some polymorphisms in HAV. However, there were no polymorphisms that affect PCR efficiency, such as primer-template mismatches of >3 bases or some primer-template mismatches at the 3′-terminal base (data not shown) [21].

**Duration of HAV viremia after onset of symptoms.** The duration of HAV viremia after the onset of symptoms was significantly longer in HIV-1–infected patients than in non–HIV-infected patients (median, 53 days vs. 22 days; P < .05; table 3). However, there was no correlation between the duration of viremia and CD4 or CD8 cell counts in HIV-1–infected patients. The duration of HAV viremia also did not correlate with HIV-1 load or the type of anti-HIV treatment received (data not shown).

**Decay of HAV viremia.** The decay pattern for HAV viremia revealed an exponential decrease, like that characteristic of HIV-1 [22]; a straight line for the decay was approximately indicated by at least 3 data points. For such exponential decay, the half-
life of the virus is calculated as \( t = \ln(2)/\text{slope} \) of the decay line. The decay pattern for each patient in the HIV-1–infected group is shown in figure 2A and for the non–HIV-infected group in figure 2B. The median half-life of HAV viremia was 6.4 days for HIV-1–infected patients and 3.7 days for non–HIV-infected patients (table 3). There was no significant difference (\( P = .09 \)) in the decay rate between the 2 groups, although the half-life in HIV-1–infected patients tended to be longer.

**Relationship between ALT values and HAV viremia.** Clinical symptoms disappeared within 30 days for the patients in both groups. The severity of acute HAV infection, as judged by serum ALT concentrations, was less in HIV-1–infected patients than in non–HIV-infected patients (table 2). Figure 3 shows the serial changes in serum ALT concentrations in patients who were negative or positive for HAV after the onset of symptoms. In general, the pattern of the serial changes in ALT was similar for the 2 groups. In the first 30 days after the onset of symptoms, ALT concentrations were elevated in all patients, and all non–HIV-infected patients except 1 were HAV-positive. From day 30 through day 60 after onset, ALT concentrations returned to the normal level in 12 of 25 patients of both groups for whom such data were available. Of these 12 patients, 7 were still positive for HAV. By day 60, serum samples were negative for HAV for all non–HIV-infected patients. In contrast, HAV was still detected in the serum of at least 3 HIV-1–infected patients when ALT returned to normal levels. By day 90, all serum samples were negative for HAV, whereas ALT concentrations were still greater than the normal upper limit for some patients.

**DISCUSSION**

The new findings of this study are that, at the onset of symptoms, HAV load is higher and the duration of HAV viremia is longer in HIV-1–infected patients than in patients without HIV-1, but the elevation of the ALT level is less. It could be postulated that the weak immune system of HIV-1–infected patients may not be able to respond adequately to HAV infection, resulting in a high HAV load and prolonged viremia. There are 2 other possible explanations, on the basis of our finding that the outbreak was caused by a single strain in HIV-1–infected patients but multiple strains in sporadic non–HIV-infected cases. The first explanation is that the difference is artificial, caused by differences in the efficiency of PCR; the second explanation is that infection in the HIV-1–infected patients was caused by a unique strain that was able to escape host immunity. The first explanation was ruled out by the sequence analysis of primer sites of HAV obtained from non–HIV-infected patients. The second explanation is also unlikely because, among our HIV-1–infected patients, the duration of viremia varied from 10 to 89 days. The unique-strain hypothesis could be excluded by analyzing the non–HIV-infected patients infected with this unique HAV strain or by inoculating chimpanzees experimentally with this strain and other strains to compare the duration of viremia and track liver enzyme levels [8].

In this study, more than one-half of the non–HIV-infected

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**Table 3. Titration, duration, and half-life of hepatitis A virus (HAV) viremia in HIV-1–infected and non–HIV-infected patients.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIV-1–infected</th>
<th>Non–HIV-infected</th>
<th>( P^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titration(^b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>( 1 \times 10^{-7} )</td>
<td>( 1 \times 10^{-3} )</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Range</td>
<td>( 1 \times 10^{-3} ) to ( 1 \times 10^{-6} )</td>
<td>( 1 \times 10^{-4} ) to ( 1 \times 10^{-8} )</td>
<td>—</td>
</tr>
<tr>
<td>Duration, days(^c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>53</td>
<td>22</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Range</td>
<td>10–89</td>
<td>12–54</td>
<td>—</td>
</tr>
<tr>
<td>Half-life, days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>6.4</td>
<td>3.7</td>
<td>.09</td>
</tr>
<tr>
<td>Range</td>
<td>1.9–16.4</td>
<td>1.6–9.3</td>
<td>—</td>
</tr>
</tbody>
</table>

* Mann-Whitney \( U \) test.

\(^a\) Dilution at which the test result became positive. Patients with serum samples that were obtained and stored in \( \leq 10 \) days after onset of clinical symptoms were analyzed: 8 HIV-1–infected and 14 non–HIV-infected patients.

\(^b\) Duration from the onset of clinical symptoms to the time that the last serum sample was obtained that was positive for HAV, provided that after this last positive sample, another serum sample with negative results was obtained in the subsequent 45 days; 11 HIV-1–infected and 9 non–HIV-infected patients met these criteria.

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**Figure 2.** Decay pattern of hepatitis A virus (HAV) viremia in patients with (A) and without (B) HIV-1 infection. HAV levels were semiquantitated by use of nested PCR in serum samples diluted \( 1 \times 4^n \). Day 0 was the day when clinical manifestations of acute HAV infection were documented. Linear regression was used to obtain the best-fitting straight line for at least 3 data points. The length of each line was determined by the initial positive point and the last positive point by day 60.
patients were women, whereas all the HIV-1–infected patients were men. However, with respect to the severity and clinical course of acute HAV infection, differences according to sex have not been demonstrated thus far in this well-known disease. Therefore, this demographic difference is unlikely to have affected our results.

Histopathologic studies reveal marked focal activation of cells lining the sinuses and accumulation of lymphocytes and histiocytes in the parenchyma; the latter cells often replace hepatocytes that have been lost by cytolytic necrosis [3]. Cytotoxic T lymphocytes have been reported to be involved in liver injury caused by acute HAV infection [23, 24]. These findings strongly indicate that immune injury is the major pathogenic mechanism of acute HAV infection. Our finding that ALT levels were less elevated in HIV-1–infected patients than in non–HIV-infected patients supports this hypothesis. Therefore, the duration and severity of clinical features and abnormal laboratory test results might also be different for HIV-1–infected patients, depending on their CD4 cell counts. However, we did not find such differences in this study, probably because of the small number of patients studied. In contrast, alkaline phosphatase and γ-glutamyltransferase levels were significantly higher in HIV-1–infected patients than in non–HIV-infected patients. On the other hand, HAV might cause direct injury to the biliary tract, although this hypothesis will have to be tested in future studies.

Only a few studies undertaken since the recent development of PCR techniques have analyzed the levels of HAV viremia [7–10]. Bower et al. [8], who used the RT-nested PCR method, reported that viremia in HIV-1–infected patients lasted 79 days after the peak liver enzyme level was measured, which is the longest duration of viremia previously reported. On the basis of our results, recovery of liver enzyme levels does not always indicate that viremia has resolved. Yotsuyanagi et al. [6] reported that, in some patients, fecal excretion of HAV lasted longer than viremia, and fecal shedding persisted for 3 months, even in the absence of HIV-1 infection. Prolonged viremia may cause prolonged fecal excretion, although there is no evidence whether low-level viremia or fecal excretion can result in transmission of infection.

In regions where HAV prevalence is high, most children are infected early in life, and such infections are generally asymptomatic. However, if infection occurs later in life, it is often associated with severe clinical features. Our data suggest that unexpectedly prolonged HAV viremia, in HIV-1–infected patients, which suggests prolonged fecal excretion of HAV, probably caused the long duration of the outbreak we studied. We have previously reported that high-risk sexual practices among homosexual men are a risk factor for transmission of HAV infection among HIV-1–infected men [19]. Condom use during homosexual intercourse cannot protect against the transmission of HAV. Although the efficacy of the HAV vaccine has been reported to decrease, depending on CD4 T lymphocyte counts, in HIV-1–infected patients [1, 25], vaccination is strongly recommended for homosexual men who test negative for anti-HAV antibody [26], particularly those infected with HIV-1 [27].

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References