Antibody to the Human T-Lymphotrophic Virus Type 1 (HTLV-1) Envelope Protein Gp46 in Patients Co-infected with HCV and HTLV-1

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Abstract. Human T-lymphotropic virus type 1 (HTLV-1) infection is known to affect hepatitis C virus (HCV) clearance and to accelerate the development of hepatocellular carcinoma in HCV-infected patients. In this study, we found the prevalence and titer of an antibody recognizing the central region of the HTLV-1 Gp46 protein to be associated with the severity of chronic liver disease. The antibody prevalence was significantly correlated with the stage of chronic liver disease (P < 0.0001): 3 (14.3%) of 21 patients with minimal–mild chronic hepatitis, 12 (24%) of 50 with moderate–severe chronic hepatitis, 7 (87.5%) of 8 with liver cirrhosis, and 13 (100%) of 13 with hepatocellular carcinoma. These results indicate that the antibody may be a useful marker of the deterioration of liver disease in patients co-infected with HCV and HTLV-1. This antibody may be useful for the diagnosis of liver diseases and the development of more effective treatments.

INTRODUCTION

Human T-lymphotropic virus type 1 (HTLV-1) is an etiologic agent of malignant CD4 T lymphoproliferation, adult T-cell leukemia/lymphoma (ATLL), and a chronic progressive neurologic disorder termed HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). In addition to playing a pathogenic role in these diseases, HTLV-1 infection has been reported to be associated with a number of other diseases, including uveitis, polymyositis, and chronic inflammatory arthropathy as a result of its immunomodulating effects. HTLV-1 causes impairment of host immunity and induces functional impairment of cellular immune response. The majority of HTLV-1–infected individuals remain asymptomatic during their lifetime, and 5–7% of asymptomatic HTLV-1–infected carriers will develop either ATLL or HAM/TSP, depending on unknown cofactors.

Our previous study showed that the gp46-197 region, corresponding to the Asp197 to Leu216 region of envelope protein gp46 (gp46-197), functions as a binding site for cell-surface receptor molecules in cell-to-cell infection by HTLV-1. The prevalence of the antibody to the gp46-197 region is remarkably increased in the sera of patients with HTLV-1–associated diseases. Also, the antibody titer of ATLL and HAM/TSP patients is significantly higher than that of asymptomatic carriers. The appearance of anti-gp46-197 antibody is independent of the fluctuation of the total anti-HTLV-1 antibody, meaning that this antibody might be a unique predictor for the onset of HTLV-1–associated diseases.

We previously demonstrated that HTLV-1 infection influences hepatitis C virus (HCV) clearance both in the natural course and by IFN-alpha treatment. Notably, the sustained response to IFN-alpha in patients with HCV and HTLV-1 co-infection was significantly lower than in patients with HCV infection alone, and forward logistic multiple-regression analysis indicated that HTLV-1 was a negative predictive marker of IFN-alpha treatment of chronic hepatitis C. It is also well known that patients with HCV infection are at risk of developing chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Boschi-Pinto et al. reported that rates of liver disease and death from HCC were higher in patients with HCV and HTLV-1 co-infection than in those with HCV infection alone.

To search for clues to the mechanisms of the influence of HTLV-1 infection on HCV infection, we tested for anti-gp46-197 antibody among patients with HCV and HTLV-1 co-infection.

MATERIALS AND METHODS

Study procedures. The study area, Iki, is an isolated island in southwestern Japan that has a population of ~32,000. The main sources of income are fishing and farming, and the lifestyle is similar to that of persons residing elsewhere in Japan. This island is endemic for both HCV and HTLV-1. A free public health examination was given that consisted of a general physical examination, a questionnaire, a blood cell count, and a blood chemistry analysis that included antibody to HCV (anti-HCV), HCV RNA, and antibody to HTLV-1 (anti-HTLV-1). Written informed consent was obtained from all residents and patients included in this survey. The study was approved by the Kyushu University Hospital ethics committee and was conducted in accordance with the human experimentation guidelines of the U.S. Department of Health and Human Services. Informed consent was obtained from all participants before the examination.

Subjects. The study included 2,245 residents of Iki Island (773 men and 1,472 women, age range 19–100 years, average age 63.0 years). All were volunteers who underwent a medical examination sponsored by the local public health office in June 2005.

At Mitsutake Hospital, on Iki Island, 243 patients with HCV-related liver disease were also examined (104 men and 139 women, age range 30–92, average age 65.3 years). Of these patients, HCV genotype 1 was found in 195 (80.2%) and genotype 2 was found in 48 (19.8%). Liver biopsy was done for classification of histology using the histologic activity index (HAI). Abdominal ultrasonography and computed tomography revealed that 45 patients had minimal–mild chronic hepatitis (CH) (scores of 1–8), 73 had moderate–severe CH
(scores of 9–18), 14 had liver cirrhosis (LC) (a staging (fibrosis) score of 4 on HAI), and 19 had HCC.

HCV infection was defined as positive for both anti-HCV and HCV RNA for > 6 months, and HTLV-1 infection was defined as anti-HTLV-1 positive. Individuals positive for hepatitis B virus surface antigen were excluded from this study.

Detection of anti-HTLV-1 antibody. Screening for anti-HTLV-1 was done by the passive particle agglutination (PA) method (Fujirebio, Inc, Tokyo, Japan) for all samples. Positive results were confirmed by Western blot analysis (Fujirebio, Inc.), according to the manufacturer’s protocol. Samples for which the results of both methods were positive were classified as positive; therefore, those determined to be positive for anti-HTLV-1 by PA alone were considered to be negative.

Detection and titration of anti-gp46-197 antibody. A modified peptide ELISA without blocking by goat serum and casein was used to detect anti-gp46-197 antibody in all residents and patients with HTLV-1 infection. Briefly, serum samples (1 μL per well) were added to 99 μL of PBS in each well with immobilized synthetic gp46-197 peptide, and the preparation was incubated for 1 h at 37°C. Then, 100 ng of horseradish peroxidase (HRP)-conjugated anti-human IgG (MBL, Nagoya, Japan) in PBS containing 5% BlockAce (Dainippon Seiyaku, Tokyo, Japan) per well was added, followed by incubation for 45 min at 37°C. Color development was done by ortho-phenylenediamine (Sigma-Aldrich, St. Louis, MO), and absorbance at 492 nm after addition of 50 μL of 2.5 M H₂SO₄ per well was measured in all anti-gp46-197 antibody-positive serum samples. The titer was calculated by multiplying the dilution value by the corresponding absorbance value.¹³

Testing for HCV markers. Anti-HCV was examined by ELISA (HCV EIA 2; Abbott Laboratories, North Chicago, IL) in all serum samples.¹⁴ All anti-HCV-positive samples were tested for serum HCV RNA by two-stage polymerase chain reaction (PCR).

HCV RNA was extracted from 50 μL of serum by Sep Gene RV (Sanko Junyaku, Tokyo, Japan). Complementary DNA was synthesized by use of random primers and reverse transcriptase (Super Script; Life Technologies, Gaithersburg, MD). HCV RNA was detected by two-stage PCR with primers from the 5’ NC of the HCV genome, as described elsewhere.¹⁵,¹⁶

The serum HCV RNA level was determined by the second-generation Cobas Amplicor HCV Monitor assay (COBAS v2.0, Roche Diagnostics System, Meylen, France (Amplicor monitor). The range of the linear relationship provided was 5–5,000 kIU/mL for the Amplicor monitor.

The HCV RNA genotype was determined by two-stage PCR, using universal and type-specific primers from the putative C gene of the HCV genome, with modifications of the methods of Okamoto et al.¹⁷ and Hayashi et al.¹⁶

Blood cell counts and blood chemistry analysis. Platelet count and alanine aminotransferase (ALT) were determined by a commercial blood chemistry analysis machine in a professional laboratory.

Statistical analysis. The Mann–Whitney U-test and χ² test were used to analyze the characteristics of patients co-infected with HCV and HTLV-1. The Cochran–Armitage test was used to compare differences in the prevalence and titer of anti-gp46-197 antibody between patients with liver diseases and residents who had undergone a medical examination. For all tests, P < 0.05 was considered to have statistical significance.

Correlations between the anti-gp46-197 antibody titer and the ALT level and platelet count were analyzed by BMDP statistical software for the IBM 3090 system computer (BMDP Statistical Software, Inc., Los Angeles, CA).

RESULTS

We examined the age- and sex-specific prevalences of HCV and HTLV-1 of the 2,245 residents of this HTLV-1–endemic area. HCV RNA was detected in 2.7% (N = 61) and anti-HTLV-1 in 20.3% (N = 456) of the residents. The prevalence of both HCV and HTLV-1 increased significantly with age. The prevalence of HTLV-1 infection was significantly higher among women (22.1%) than among men (16.2%; P = 0.0009), but the difference in HCV infection among men (4.2%) and women (1.9%) was not (P = 0.577).

Of the 2,245 residents, the prevalence of HCV infection alone was 2.2% (N = 49), HTLV-1 infection alone 19.8% (N = 444), and HCV and HTLV-1 co-infection 0.5% (N = 12). Moreover, of the 243 patients with HCV-related liver disease, the prevalence of HCV infection alone was 62.1% (N = 151), and that of HCV and HTLV-1 co-infection was 37.9% (N = 92). We tested for anti-gp46-197 antibody in residents and HCV-related liver disease patients with HTLV-1 infection. Of 444 residents with HTLV-1 infection alone (121 men, 323 women), only one resident was positive for anti-gp46-197 antibody. Of 12 residents with HCV and HTLV-1 co-infection (7 men, 5 women), this antibody was detected only in a 70-year-old female resident (8.3%) who had mild liver abnormality but no hematological abnormalities. Of 92 HCV-related liver disease patients with HTLV-1 co-infection (37 men, 55 women), this antibody was detected in 35 patients (16 men, 19 women, 38.0%). The anti-gp46-197 antibody was not found in any of the patients without HTLV-1. None of the 456 residents or 92 HCV-related liver disease patients with HTLV-1 infection had HTLV-1–associated diseases. Anti-gp46-197 antibody-positive patients showed no deviation in age, sex, or HCV RNA level.

Of 195 genotype 1 patients and 48 genotype 2 patients with HCV-related liver disease, HTLV-1 was detected in 39.5% (N = 77) and 31.3% (N = 15), respectively. Of 77 genotype 1 patients and 15 genotype 2 patients with HCV and HTLV-1 co-infection, anti-gp46-197 antibody was detected in 36.3% (N = 28) and 46.7% (N = 7), respectively. Among 92 chronic liver disease patients with HCV and HTLV-1 co-infection, the HCV RNA level was 1,010.2 ± 192.5 kIU/mL in 35 anti-gp46-197 antibody-positive patients and 1,336.1 ± 184.5 kIU/mL in 57 negative patients (P = 0.164). The status of HTLV-1 and anti-gp46-197 antibody were not related to the status of HCV infection.

The relationships of anti-gp46-197 antibody with the ALT level and platelet count were analyzed (Figure 1). In 92 HCV-related liver disease patients with HTLV-1 co-infection, the ALT level was not different between the 35 anti-gp46-197 antibody-positive patients (60.1 ± 8.7 IU/L) and the 57 negative patients (51.0 ± 5.6 IU/L) (P = 0.12). On the other hand, a positive correlation between the titer of anti-gp46-197 an-
The prevalence of HCV RNA in the present study was followed at the single hospital. Therefore, most residents with HCV infection reported to hospitals for testing. Most of the HCV-infected residents with liver abnormalities in the present study were followed at the single hospital. Therefore, the prevalence of HCV RNA was only 2.7% in the present study, despite the area being endemic for HCV.

We also reported that the higher rate of HCV RNA positivity found among Iki Island residents who tested positive for anti-HTLV-1 and anti-HCV may be due to an immunosuppressive effect of HTLV-1. Moreover, the significantly lower rate of sustained response to interferon treatment in patients with HCV and HTLV-1 co-infection, compared with that in those with HCV infection alone, and the results of forward logistic multiple regression analysis showed that HTLV-1 negativity was predictive of the success of interferon treatment of chronic hepatitis C, indicating that HTLV-1 infection might affect the elimination of HCV.

Chronic hepatitis, cirrhosis, and HCC are known sequelae of chronic HCV infection. We also reported that persistent liver damage plays an important role in the development of HCC in patients with chronic HCV viremia, as shown by results indicating that HCC developed more often in patients with consistently abnormal ALT levels than in those with consistently normal ALT levels. Moreover, HTLV-1 infection contributes to the development of HCC in patients with HCV infection. It seems possible that HTLV-1 affects the deterioration of liver disease in chronic hepatitis C patients. However, we have no evidence concerning the precise mechanisms by which HTLV-1 influences HCV infection.

The gp46-197 region on the gp46 envelope protein of HCV and HTLV-1 co-infection: (A) Our earlier study indicated that blood samples obtained from patients with minimal-mild chronic hepatitis; □, patients with moderate-severe chronic hepatitis; ■, patients with liver cirrhosis; ○, patients with hepatocellular carcinoma.

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** Relationship between anti-gp46-197 antibody titer and alanine aminotransferase level (ALT) (A) and platelet count (B) in anti-gp46-197-positive patients with hepatitis C virus and human T-lymphotropic virus type 1 co-infection: (A) $P = 0.008$, $r = 0.552$; (B) $P = 0.644$, $r = -0.086$; □, patients with minimal-mild chronic hepatitis; ○, patients with moderate-severe chronic hepatitis; ■, patients with liver cirrhosis; ○, patients with hepatocellular carcinoma.

### DISCUSSION

We previously demonstrated a high prevalence of both HCV and HTLV-1 infection among 2,280 residents of Iki Island in a study done between 1996 and 1999. The prevalence of anti-HCV was 13.9% and the prevalence of anti-HTLV-1 was 23.2%. Our earlier study indicated that blood transfusions could be a common route for the spread of both HCV and HTLV-1. Anti-HCV is the most sensitive marker for HCV infection, including past infection, and the percentage HCV RNA-positive to anti-HCV-positive is generally 80–85%. The prevalence of HCV RNA in the present study seems to be lower than the usually estimated rate. Because we have informed the residents of the risk of HCV infection and the importance of testing and treatment, most residents with HCV infection reported to hospitals for testing. Most of the HCV-infected residents with liver abnormalities in the present study were followed at the single hospital. Therefore, the prevalence of HCV RNA was only 2.7% in the present study, despite the area being endemic for HCV.

The relationship between the titer of anti-gp46-197 antibody and the histologic status of 92 HCV-related liver disease patients with HTLV-1 co-infection is shown in Table 1. The average titers of anti-gp46-197 antibody were 4,715 ± 529 in minimal-mild CH, 6,343 ± 830 in moderate-severe CH, 7,835 ± 2,045 in LC, and 8,132 ± 1,127 in HCC. The anti-gp46-197 antibody titer increased with the severity of liver disease.

### TABLE 1

<table>
<thead>
<tr>
<th>HAI category</th>
<th>Total</th>
<th>Anti-gp46-197 antibody positivity (%)</th>
<th>Anti-gp46-197 antibody titer (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM–CH</td>
<td>21</td>
<td>3 (14.3)</td>
<td>4715 ± 529</td>
</tr>
<tr>
<td>MS–CH</td>
<td>50</td>
<td>12 (24.0)</td>
<td>6343 ± 830</td>
</tr>
<tr>
<td>LC</td>
<td>8</td>
<td>7 (87.5)</td>
<td>7835 ± 2045</td>
</tr>
<tr>
<td>HCC</td>
<td>13</td>
<td>13 (100)</td>
<td>8132 ± 1127</td>
</tr>
</tbody>
</table>

* MM–CH, minimal-mild chronic hepatitis; MS–CH, moderate-severe chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; data ± SE are shown.
HTLV-1 is the essential domain for cell-to-cell infection. The anti-gp46-197 antibody is a biomarker for the development of adult T-cell leukemia and HTLV-1-associated myelopathy from a carrier state. The biologic correlates and pathologic significance of this antibody production are not clear at the present time. In the present study, we assessed the relationship between the prevalence of anti-gp46-197 antibody and the deterioration of liver disease in patients with HTLV-1 and HCV co-infection. The anti-gp46-197 antibody was found significantly more often in patients with HCV-associated liver diseases (35 of 92, 38.0%) than in residents (1 of 12, 8.3%) co-infected with HCV infection and HTLV-1. On the other hand, 1 of 444 (0.2%) residents with HTLV-1 infection alone showed positive for gp46-197 antibody. This prevalence was significantly lower than that of patients with HTLV-1 infection alone (7 of 23, 30.4%; data not shown). Notably, in this study, individuals in whom the antibody was detected did not have any HTLV-1-related diseases. The antibody seems to appear with the onset of liver disease in patients co-infected with HCV and HTLV-1.

The anti-gp46-197 antibody titer was closely associated with the severity of the liver disease. Patients co-infected with HCV and HTLV-1 had liver deterioration with lower HCV RNA levels than did patients with HCV alone. This suggests that HTLV-1 co-infection might influence the onset of liver disease and deterioration, meaning that the expression of HTLV-1 structural protein gp46 accelerates the activity of the host immune system in viral carriers.

Many investigators have reported that antibody production does not directly damage the liver and that immune response of cytotoxic CD8+ T lymphocytes (CTL) is the main reason for the development of hepatitis. Although the exact mechanism of HCV-associated liver damage has not been established, it is widely accepted that immune-mediated mechanisms, particularly HCV-specific CTL and helper CD4+ T lymphocytes, are associated with the pathogenesis of HCV-induced liver damage. We previously reported that the frequency of IFN-γ producing CD4+ (Th1) and CD8+ (Tc1) T cells is increased in the peripheral blood of chronic hepatitis C patients, suggesting that these Th1 and Tc1 cells are involved with liver damage by HCV infection. As for HTLV-1, cell-mediated immune response, especially CTL response to HTLV-1, is involved in the severity of HTLV-1–associated diseases. Interaction between the responses to HCV and HTLV-1 is involved in the severity of HTLV-1–associated diseases.

These results indicate that the appearance and titer of the anti-gp46-197 antibody may be practical markers of deterioration of the liver disease of patients co-infected with HCV and HTLV-1. This antibody may be useful for diagnosis and for increasing the effectiveness of treatment of liver diseases. In the future, it will be important to examine direct and/or indirect influences of the production of this antibody on the deterioration of liver disease associated with HCV/HTLV-1 co-infection. To further clarify the role of the anti-gp46-197 antibody, we intend to do an extensive cohort study to examine the relationship between the time of appearance of the antibody and the timing of seroconversion in both prospective and retrospective studies.

Received October 24, 2006. Accepted for publication January 15, 2007.

Acknowledgments: In addition to the authors, the following investigators were involved in the present study: Yoko Tomonari and Hiroshi Manabe, Fukuoka Red Cross Blood Center; Chikushino, Fukuoka, Dr. Arahito Mitsutake, and Dr. Takeshi Kuga, Mitsutake Hospital, Iki, Nagasaki.

Financial support: The research was supported by a grant from the 21st Century COE Program of the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan.

Disclaimer: None of the authors have a commercial or other association that might pose a conflict of interest.

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