Low Levels of Hepatitis C Virus (HCV) Neutralizing Antibodies in Patients Coinfected with HCV and Human Immunodeficiency Virus

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The hepatitis C virus (HCV) neutralizing antibody (nAb) response in 37 subjects with HIV monoinfection and 37 HCV-infected subjects with well-controlled human immunodeficiency virus (HIV) infection was evaluated using a focus reduction neutralization assay. HCV nAb levels were retrospectively studied in both groups of patients, who were matched on the basis of sex, age, and HCV genotype. The mean HCV nAb level (± standard deviation) among coinfected patients (1.61 ± 0.416) was significantly less than that among monoinfected patients (1.91 ± 0.578) (P = .013). Lower HCV nAb titers in coinfected patients could help worsen the outcome of HCV infection. These results favor starting HCV therapy as soon as possible in coinfected patients.

In today’s era of highly active antiretroviral therapy (HAART), hepatitis C virus (HCV)–related liver disease has emerged as a major cause of morbidity and mortality in patients coinfected with HCV and human immunodeficiency virus [1, 2]. In western Europe, 15%–30% of HIV-infected patients are also infected with HCV [2], and a similar rate has been reported in the United States [3]. In HIV-positive patients, longer survival may permit the progression of HCV-related liver disease and may increase mortality due to its complications, including cirrhosis and hepatocellular carcinoma. Some studies have described the faster progression of HCV-related liver disease and higher HCV loads in HIV/HCV-coinfected individuals [3]. Chronic hepatitis C progressed more rapidly and was more severe in patients with advanced immunosuppression and a greater loss of CD4+ T cells. Control of HCV infection relies principally on a cytotoxic T cell response, as suggested by HCV clearance in patients and chimpanzees lacking a neutralizing antibody (nAb) response [4]. However, the nAb response has been shown to be faster and more intense in patients in whom acute HCV infection resolved [5]. In chronic HCV infection, broadly reactive HCV nAbs and a multispecific T cell response have been reported as only partially controlling the infection, which results in the continuous generation of escape mutants [6]. The progressive loss of viral control could explain the faster progression of hepatitis C in patients coinfected with HIV.

The detection of HCV nAbs was first achieved using an in vitro assay system based on infectious retroviral pseudoparticles that bear HCV envelope glycoproteins. The recent availability of the HCV cell culture model allows use of a standard focus reduction neutralization assay for determining HCV nAbs in patients’ serum specimens [7].

The aim of this study was to determine HCV nAb titers in HIV/HCV-coinfected patients and compare these values with those found in HCV-monoinfected patients. A group of patients with well-controlled HIV infection was selected, and HIV disease in all was at a stage at which HCV therapy could either be started or, if hepatitis C has not progressed, delayed. In this context, the potential role of HCV nAbs was evaluated by measuring HCV viremia in both groups of patients.

Patients and methods. Serum samples from 74 patients with chronic hepatitis C were retrospectively studied. Thirty-seven individuals (24 men and 13 women; mean age, 43.9 ± 7.1 years) were HIV/HCV coinfected, and HIV infection was well controlled (i.e., they had no detectable HIV viremia) by HAART. The HIV/HCV-coinfected and HCV-monoinfected groups were matched according to sex, age, and HCV genotype, as shown in Table 1. Most patients were European and injection drug users. Also, most (i.e. 73.9% and 63.6% of monoinfected and coinfected patients, respectively) presented with moderate fibrosis (defined as stage F2 according to the Metavir score). Of the HIV-infected patients, 1 group of 8 individuals was known to have been infected with HCV before HIV acquisition (the preinfected
group), whereas another group of 8 was known to have been infected with HCV after acquiring HIV (the postinfected group).

The presence of HCV antibodies was determined by a third-generation HCV enzyme immunoassay (AxSYM HCV, version 3.0 [Abbott]) and confirmed by another enzyme immunoassay (Vitros anti-HCV reagent pack [Ortho-Clinical Diagnostics]) or PCR. The level of anti-HCV antibodies was assessed by AXSYM HCV, version 3.0, and expressed in signal/cutoff ratio. HCV RNA was quantified using a commercial assay (Cobas Taqman HCV test, version 2.0 [Roche Diagnostics]), and HCV genotyp-
ing was performed by direct sequencing of the nonstructural protein 5B and 5’ untranslated regions, as previously described. Collection of serum specimens was approved by the local ethics committee, and informed consent had been obtained from all study participants. After collection, all serum samples were stored at -80°C and were not thawed until immediately before the assay was performed.

The serum IgG fraction was purified using protein G-Sepharose (GE Healthcare), as previously described [8]. The IgG concentration was determined using a Bradford assay (Bio-Rad). Purified IgG was stored at -80°C.

The HCV focus reduction neutralization assay was performed in 96-well microtiter plates, as previously described [8]. Serial dilutions (ranging from 1:10 to 1:1280) of purified IgG (10 µg) were established. Each sample was mixed with 100 focus-forming units of HCV JFH-1 strain in 96-well microtiter plates and incubated for 1 h at 37°C in 5% CO2. Huh-7 cell suspension (concentration, 10,000 cells/well) in culture medium was added and incubated for 5 h at 37°C in 5% CO2. After 72 h of incubation, an immunoperoxidase reaction was performed using a primary antibody (obtained from an HCV-positive serum specimen and inactivated at 56°C) and a secondary peroxidase-coupled, Fc-specific anti-human IgG antibody (Sigma). The reaction was developed with DAB peroxidase substrate (Sigma). The number of HCV foci in each dilution was determined. Controls, which consisted of nonneutralized virus and purified IgG from each patient at a 1:10 dilution, were included in each assay. The dilution that neutralized 50% of the virus was calculated by curvilinear regression analysis, using XLSTAT 2006 software (Addinsoft SARL). Each titer was determined as the log value of the reciprocal antibody dilution that reduced the number of viral foci by 50%. The nAb titers were expressed in log units, and mean values (±SDs) were calculated in both groups. The assay cutoff was 1.25 log units.

A paired Student t test was used to perform intergroup comparisons of continuous variables, and a χ² test was performed to compare the distribution of categorical variables. P values of <.05 were considered to be significant.

Results. The HCV focus reduction neutralization assay demonstrated that the mean HCV nAb titer in HIV/HCV-coinfected patients (1.613 ± 0.416 log units) was significantly lower than that in HCV-monoinfected patients (1.912 ± 0.578 log units; P = .013) (table 1). Although it has been previously shown that results of this assay are not dependent on genotype [8], a lower HCV nAb titer in the coinfect ed patients could be confirmed for HCV genotype 1, which was the most prevalent genotype in this study (1.525 ± 0.400 log units, compared with 1.817 ± 0.610 log units in the monoinfected group [n = 23]; P = .05). The coinfect ed population had a greater percentage of patients without a detectable level of HCV nAbs (21.5%) and a narrower nAb titer range (1.299–2.293 log units), compared with HCV-monoinfected patients, among whom only 7.9% had nAbs detected, with a nAb titer ranging from 1.251 to 3.121 (figure 1). HCV genotype 1 was present in 75% and 100% of coinfected and monoinfected patients, respectively, for whom results of tests for detection of HCV nAbs were negative. Titers of global anti-HCV antibodies were also lower for coinfected patients, as determined by the enzyme immunoassay (P = .032).

We also quantified the patients’ serum HCV RNA levels, using a commercial real-time PCR assay. The HCV load in HCV-monoinfected patients (5.851 ± 0.889 log IU/mL) was not statistically different from that in HIV/HCV-coinfected patients (5.989 ± 1.192 log IU/mL). Among HIV/HCV-coinfected patients, the HCV load did not differ significantly between the preinfected group (6.424 ± 0.423 log IU/mL) and the postinfected group (6.466 ± 0.573 log IU/mL). No correlation was found between HCV nAb titer and HCV load in this population with well-controlled HIV infection.

Discussion. The role of HCV nAbs in acute and chronic viral infection is still an important and controversial question. Recently, it was confirmed that the presence of nAbs helps control HCV load and contributes to viral eradication in acutely infected patients capable of clearing the infection [5]. In contrast, chronic HCV infection is characterized by the complete or partial failure to neutralize transmitted virus in the early phase of infection and the delayed induction of nAbs in the late phase of infection [9–11]. Several reports have also shown that cross-reactive nAb responses occurred long after acute infection and then increased in titer and breadth to recognize distant HCV genotypes [6, 11].

Patients with chronic HCV infection and no HIV infection had relatively high HCV nAb titers (range, 1.251–3.121 log
units). Previous studies in which a retroviral pseudoparticle assay was used demonstrated the presence of high HCV nAb titers (>2.50 log) in the serum specimens of HCV-infected patients and broad neutralizing activity against different HCV genotypes [12]. Our present results confirm that most nAbs cross-react with several genotypes, as previously described [8].

The levels of HCV nAbs in patients with HIV/HCV coinfection differed from those determined in monoinfected patients: the coinfected population had lower nAb titers (range, 1.299–2.293 log units) and a greater prevalence of undetectable nAb levels (21.5% of patients). These samples tested positive for specific antibodies by means of an enzyme immunoassay, without detectable neutralizing activity. The high percentage of HCV nAb–negative assays could be explained either by the absence of nAbs in the samples or by the presence of antibodies that did not cross-neutralize with the HCV JFH-1 strain.

Interestingly, our results showed that the HCV nAb titers in the coinfected population were significantly less than those in the monoinfected population (P = .013). Anti-HCV antibodies production seemed already impaired by HIV infection, even in coinfectected patients for whom HIV infection was well controlled by HAART and HIV viremia was not present. Several publications have reported that virus-specific CD4+ and CD8+ T cell responses, combined with HCV nAbs, are important for controlling the acute phase of HCV replication [7, 13]. Chronic infection appears to be associated with the emergence of viral variants that bear escape mutations in CD8+ T cell epitopes [14], independently of a robust, cross-reactive nAb response. However, in the context of HIV coinfection, our results highlight the weakness or even the absence of an HCV nAb response during chronic HCV infection. One possible explanation for this low HCV nAb response is that HIV infection of CD4+ T cells may impair HCV-specific immune functions, including antibody production. Another publication suggested that HCV nAb production was directly linked to the decrease in the CD4+ T cell count [15]. In the present study, HIV infection was well controlled by HAART, with a subnormal CD4+ T cell count and no detectable HIV viremia; there was no correlation between HCV nAb titer and CD4+ cell count.

We refined our HCV nAb analysis with respect to the chronology of viral infection in 2 groups of 8 coinfect ed patients. As expected, the HCV nAb titer was significantly lower in postinfected patients than in postinfected patients. Five patients in each group were injection drug users, but it is possible that additional factors, such as differential exposures to other infectious agents, could have interfered with the immune response. On the whole, these results suggest a significant defect of the CD4+ T cell response in coinfected patients in whom HIV infection was apparently well-controlled. The presence of subnormal levels of CD4+ T cells could be sufficient to impair the immune response and to allow a more rapid progression of the HCV infection. In light of these initial results, further prospective studies are needed to evaluate the contribution of HCV nAbs to HCV disease control. Hence, the results of our present study provide an additional argument that efficient HCV antiviral therapy should be started as soon as possible in HIV-infected patients in order to improve the chance of eradicating HCV.

In conclusion, HCV nAb titers are lower in HIV-coinfected patients. This finding is probably related to the increased immunodeficiency for these patients and may help worsen their prognosis for HCV infection.

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References