Proteome-Wide Anti–Hepatitis C Virus (HCV) and Anti-HIV Antibody Profiling for Predicting and Monitoring the Response to HCV Therapy in HIV-Coinfected Patients

Peter D. Burbelo,1 Joseph A. Kovacs,2 Kathryn H. Ching,1 Alexandra T. Issa,1 Michael J. Iadarola,1 Alison A. Murphy,2 Joerg F. Schlaak,4 Henry Masur,2 Michael A. Polis,3 and Shyam Kottilil3

1Neurobiology and Pain Therapeutics Section, Laboratory of Sensory Biology, National Institute of Dental and Craniofacial Research, 2Critical Care Medicine Department, National Institutes of Health Clinical Center, and 3Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; 4University Hospital of Essen, Essen, Germany

We quantified antibody responses to the hepatitis C virus (HCV) proteome that are associated with sustained virologic response (SVR) in human immunodeficiency virus (HIV)/HCV–coinfected patients treated with pegylated interferon and ribavirin. Analysis of pre- and posttreatment samples revealed significant decreases in the combined anti-core, anti-E1, and anti-NS4 HCV antibody titers in those with SVRs but not in those who experienced relapse or who did not respond. Furthermore, anti–HIV p24 antibody titers inversely correlated with treatment response. These results suggest that profiling anti-HCV antibody is useful for monitoring HCV therapy, especially in discriminating between those who experience relapse and those who have SVRs at 48 weeks.

Infection with hepatitis C virus (HCV) is seen in 15%–30% of all human immunodeficiency virus (HIV)–infected individuals in the United States, as a result of the shared routes of viral transmission [1, 2]. The introduction of antiretroviral therapy has improved clinical outcomes in patients infected with HIV. However, liver disease has become a leading cause of morbidity and mortality in this population [3, 4]. HIV/HCV coinfection is also associated with higher HCV levels in serum [5, 6], rapid progression of liver disease [7], and lower efficacy of treatment with pegylated interferon plus ribavirin [5, 8]. Development of biomarkers that can accurately predict therapeutic responses are needed to optimize HCV therapy in this coinfected population. Previously, HIV/HCV-coinfected patients who were not responsive to HCV therapy with pegylated interferon plus ribavirin were found to have a gene-activation signature present before treatment indicative of the activation of many immunorelated molecules, including interferon-stimulated genes [9]. Quantitative and qualitative humoral responses over the course of HCV therapy among HIV/HCV-coinfected subjects have never been studied, to our knowledge. The ability to clearly predict and monitor outcomes of HCV infection in a robust and simple serological test would have obvious clinical utility. Recently, luciferase immunoprecipitation system (LIPS) assays have been used to accurately quantify antibody responses to various viral pathogens [10]. In the present study, we used LIPS profiling of antibodies against the whole proteome of HCV and part of the proteome of HIV to evaluate its utility in predicting and monitoring the response to HCV therapy in HIV/HCV-coinfected individuals.

Methods. This was a prospective, open-label trial performed at the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, Bethesda, Maryland. All 29 patients provided written informed consent approved by the NIAID Institutional Review Board. HIV/HCV-coinfected patients were treated with pegylated interferon alpha-2b at 1.5 μg/kg subcutaneously every week (PegIntron; Schering-Plough) and ribavirin daily (Rebetol; Schering-Plough; 400 mg every morning and 600 mg every evening for those <75 kg or 600 mg twice per day for those ≥75 kg) for 48 weeks and followed up for 24 weeks after the end of treatment. All patients irrespective of virologic response were treated for 48 weeks. One patient discontinued ribavirin at week 24 because of refractory anemia but continued pegylated interferon until week 48.

Patients were eligible for the study if they were >18 years of age and had a CD4 T cell count of >100 cells/μL, an absolute neutrophil count of >1000 cells/μL, an HCV load of >2000 copies/mL, histologic evidence of chronic hepatitis C, and stable HIV disease with or without antiretroviral therapy. Patients with other causes of liver disease, advanced cirrhosis or severe...
liver decompensation, and several other conditions were excluded. These patients included 11 who experienced no response (NR group), 9 end-of-treatment responders who experienced relapse after 48 weeks of therapy (relapse group), and 9 who experienced sustained virologic responses (SVR group). All patients (except for 1 of the patients in the relapse group who was enrolled in the study and 1 of the previous patients in the NR group who was omitted because of lack of a serum sample) have been described elsewhere [9].

*Renilla* luciferase (Ruc) antigen fusions—including HCV core, HCV NS3, HCV NS5A, HIV p24 Gag, and HIV Tat—have been described elsewhere [10]. Four additional HCV proteins were generated as Ruc antigen fusions, including E1, E2, NS3, and NS4. One HCV protein, NS5B, was tested, but it was not found to be useful and was not used further. LIPS assays with these different HCV and other Ruc antigens were performed as described elsewhere [11]. All of the light unit (LU) data represent the average of 2 independent experiments and were corrected for background LU values.

Prism software (version 5; GraphPad) was used for statistical analyses. The Mann-Whitney *U* test was used for comparison of antibody titers between groups, and the Wilcoxon signed rank test was used to evaluate statistical differences between values before and after HCV therapy.

**Figure 1.** Baseline titers of antibodies against hepatitis C virus (HCV) antigens and human immunodeficiency virus (HIV) p24. A, Heat map representation of profiles of antibodies against the 6 HCV antigens in pretreatment samples from HIV/HCV-coinfected patients with differing response to HCV therapy. Antibodies titers were determined for 3 groups of HIV/HCV-coinfected patients, including those who experienced no response (NR) (*n* = 11), relapse (*n* = 9), and a sustained virologic response (SVR) (*n* = 9), as well as for 2 noninfected control subjects. Antibody levels for each serum sample were log10 transformed, and the levels were then color coded as indicated by the scale on the right, in which signal intensities range from high (red) to low (green) antibody titers. The unusual patient in the NR group who did not have anti-core, anti-E1, and anti-E2 antibodies is indicated by the asterisk. B, Anti–HIV p24 antibody titers. Each symbol represents individual samples from patients in the NR, relapse, and SVR groups. Antibody titers are plotted in light units on the *y* axis, and the mean value and 95% confidence interval are shown for each group. The dashed line represents the optimum cutoff determined by receiver operator characteristics for discriminating the SVR from the NR patients. *P* values were calculated using the Mann-Whitney *U* test.

**Results.** Antibody titers in serum samples from all patients and in 2 control samples were evaluated for 6 different recombinant HCV antigens, essentially derived from the whole proteome of HCV. A heat map, constructed with log10-transformed antibody titers, was used to display the differing antibody responses to the 6 antigens in individual samples from these subgroups (Figure 1A). As shown by the heat map, LIPS profiling of responses to these 6 HCV antigens clearly distinguished the 29 HCV-infected serum samples from the 2 uninfected control serum samples. The most useful antibody response was directed against the HCV core, for which all but 1 of the 29 HIV/HCV-coinfected samples was positive. The second most useful antibody response was against NS3 (Figure 1A). The other 4 HCV proteins (E1, E2, NS4, and NS5) showed variable immunoreactivity with HIV/HCV-coinfected serum samples (Figure 1A). Of interest, 1 patient in the NR group was completely negative for anti-core, anti-E1, and anti-E2 antibodies but showed strong immunoreactivity to 3 other nonstructural HCV proteins (Figure 1A). Titers of antibodies against the 6 HCV antigens correlated poorly with each other (*r* > 0.60), suggesting marked heterogeneity in humoral responses (Table 1).

Titers of antibodies against these HCV antigens in pretreatment serum samples showed no significant differences be-
Table 1. Correlation of Titers of Antibodies against 6 Hepatitis C Virus (HCV) Proteins in Pretreatment Samples

<table>
<thead>
<tr>
<th>Protein</th>
<th>NR</th>
<th>Relapse</th>
<th>SVR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>497,200 (95% CI, 279,800–714,700)</td>
<td>483,400 (95% CI, 292,500–674,200)</td>
<td>545,600 (95% CI, 299,400–791,900)</td>
<td>0.65</td>
</tr>
<tr>
<td>E1</td>
<td>2,77 × 10^6, 2.17 × 10^6, 1.71 × 10^6</td>
<td>2.77 × 10^6, 2.17 × 10^6, 1.71 × 10^6</td>
<td>2.77 × 10^6, 2.17 × 10^6, 1.71 × 10^6</td>
<td>0.42</td>
</tr>
<tr>
<td>E2</td>
<td>2.77 × 10^6, 2.17 × 10^6, 1.71 × 10^6</td>
<td>2.77 × 10^6, 2.17 × 10^6, 1.71 × 10^6</td>
<td>2.77 × 10^6, 2.17 × 10^6, 1.71 × 10^6</td>
<td>0.43</td>
</tr>
<tr>
<td>NS3</td>
<td>4,83,400 (95% CI, 292,500–674,200)</td>
<td>2,77 × 10^6, 2.17 × 10^6, 1.71 × 10^6</td>
<td>2.77 × 10^6, 2.17 × 10^6, 1.71 × 10^6</td>
<td>0.70</td>
</tr>
<tr>
<td>NS4</td>
<td>2,77 × 10^6, 2.17 × 10^6, 1.71 × 10^6</td>
<td>2.77 × 10^6, 2.17 × 10^6, 1.71 × 10^6</td>
<td>2.77 × 10^6, 2.17 × 10^6, 1.71 × 10^6</td>
<td>0.43</td>
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This table is available in its entirety in the online version of the Journal of Infectious Diseases.
Figure 3. Informative antibody titers before and after hepatitis C virus (HCV) treatment in human immunodeficiency virus–coinfected patients in the no-response (NR; n = 11), relapse (n = 9), and sustained virologic response (SVR) (n = 9) groups. Shown are anti-core, anti-E1, and anti-NS4 antibodies levels at baseline and after treatment in individual patients. The solid horizontal bars reflect the mean titer in each group for the pre- and posttreatment sample. Statistical differences between pre- and posttreatment values were calculated using the nonparametric Wilcoxon signed rank test. The P values derived from summation of the light unit (LU) antibody titers for the 3 antigens are shown at bottom.

affect the response to HCV therapy. Previously, it has been shown that, among HCV-monoinfected patients, those with SVRs had higher pretreatment anti-NS4A and anti-NS5a antibody titers (without normalization for HCV load) than did those with NRs [12]. It should be noted that our study differs from this published study in that our patient population was coinfection with HIV and HCV, and HCV loads were controlled for. Nevertheless, 1 patient in the NR group completely lacked anti-core, anti-E1, and anti-E2 antibodies but had high levels of other HCV antibodies, possibly explaining the lack of responsiveness to HCV therapy. Given that this patient (infected with HCV genotype 1) had ample antibodies against HIV and nonstructural HCV proteins, it is likely that selective B cell exhaustion or deletion of certain populations of plasma B cells
occurred [13, 14]. Intriguingly, anti–HIV p24 antibodies detected in the pretreatment samples inversely correlated with response to treatment. The highest anti-p24 antibody titers were observed in the NR group, intermediate titers were observed in the relapse group, and the lowest titers were observed in the SVR group. The higher anti-p24 antibody titers in the NR group compared with the SVR group suggests that some of the patients in the NR group who responded poorly to interferon treatment may have had an abnormal immune response to HIV.

Declining anti-HCV core and envelope-specific antibody responses at the end of therapy were observed only in the SVR group, suggesting that these antibody responses could be used to discriminate between those who experience relapse and SVR at the end of therapy. The SVR group showed the largest and most consistent decrease in titers of antibodies against the core, E1, and NS4 proteins after 48 weeks of treatment. In contrast, the NR and relapse groups showed minimal decreases in antibody titers. Despite the less than detectable levels of HCV RNA at the end of HCV treatment in the relapse and SVR groups, significant decreases in anti-HCV antibody titers do frequently occur among patients with SVRs. Given that HCV loads in the relapse and SVR groups were clinically indistinguishable and below the level of detection, it is possible that the decrease in levels of antibodies in the SVR group reflects a marked decline in antigen load in the liver rather than in the plasma. Regardless of the mechanism, the differential response in antibody titers between the SVR and relapse groups at the end of treatment offers a novel tool to predict who will experience relapse after treatment is stopped. This could lead to the development of novel therapeutic strategies, such as extended therapy for those with relapse. Studies addressing whether these antibodies and/or other biomarkers show robust differences at earlier time points may provide practical tools for monitoring therapy.

References