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

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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 alfa-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
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 (LU)</th>
<th>Relapse (LU)</th>
<th>SVR (LU)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>297,900</td>
<td>292,500</td>
<td>299,400</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>E1</td>
<td>483,400</td>
<td>674,200</td>
<td>545,600</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>E2</td>
<td>483,400</td>
<td>674,200</td>
<td>545,600</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NS3</td>
<td>279,800</td>
<td>292,500</td>
<td>299,400</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NS4</td>
<td>279,800</td>
<td>292,500</td>
<td>299,400</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NS5A</td>
<td>279,800</td>
<td>292,500</td>
<td>299,400</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

This table is available in its entirety in the online version of the Journal of Infectious Diseases.

monitoring HCV therapy. The Wilcoxon sign rank test revealed that 4 of the 6 HCV proteins (core, E1, E2, and NS4) showed statistically significant (P < .05) decreases in antibody titer between the pre- and posttreatment samples (Figure 3). In contrast, antibody responses to the NS3 and NS5A antigens did not significantly change between before and after treatment (P > .44). Substratification by treatment outcome revealed that the SVR group showed the most consistent and largest decrease (P = .02) in titers of antibodies against the 3 most informative antigens (core, E1, and NS4) after treatment (Figure 3). In contrast, the NR and relapse groups had relatively stable titers of antibodies against these 3 antigens between before and after treatment (P = .70 and P = .43, respectively) (Figure 3). Anti-HIV p24 and anti-BRLF2 Epstein-Barr virus antibody titers also did not change with HCV therapy (data not shown). There was heterogeneity in response to the different HCV antigens in the SVR group, in which some patients showed the largest decrease in anti–HCV core antibody titers, whereas other patients showed more pronounced decreases in anti-ENV1 and anti-NS4 antibody titers. Because decreasing levels of antibodies against these 3 HCV antigens was a common feature of the SVR group versus the NR and relapse groups, the relative decrease in antibody titer between the pre- and posttreatment samples was the most useful approach for distinguishing SVR from NR and relapse. With an antibody titer decrease of >1.5-fold between the pre- and posttreatment samples used as a marker of HCV therapy success, 6 of the 9 patients in the SVR group were positive, compared with only 1 of the 9 patients in the relapse group and none of the patients in the NR group. Overall, this LIPS assay measuring differences in titers of antibodies against these 3 HCV antigens between the pre and posttreatment samples showed an 86% positive predictive value in identifying a response to therapy.

**Discussion.** Our study suggests that highly quantitative HCV proteome–wide antibody responses can be a valuable tool for monitoring and predicting HCV therapeutic responses among HIV-coinfected patients. Few studies have examined the utility of anti-pathogen antibodies for predicting and monitoring drug therapy. Our LIPS assay provided a clearer summary of the marked patient variability in humoral responses to the whole HCV proteome than has been previously reported. None of the baseline antibody responses to the 6 different HCV proteins predicted response to HCV therapy. This suggests that preexisting host humoral responses to HCV generally do not

Titters of antibodies against the panel of HCV proteins were also evaluated before and after treatment for their value in between the NR, relapse, and SVR groups (figure 2). For example, the mean anti-core antibody titers in the NR, relapse, and SVR groups were 497,200 LUs (95% confidence interval [CI], 279,800–714,700 LUs), 483,400 LUs (95% CI, 292,500–674,200 LUs), and 545,600 LUs (95% CI, 299,400–791,900 LUs), respectively, and the Mann-Whitney U test showed no statistically significant differences (P > .65). These results suggest that anti-HCV antibody titers in the pretreatment HIV/HCV-coinfected serum samples have no obvious predictive value for response to treatment.

Because of the known effect that HIV/HCV coinfection has on HCV therapy, antibody responses to several HIV proteins were also evaluated in the 3 groups. As shown in Figure 1B, all 29 pretreatment serum samples were robustly seropositive for anti–HIV p24 Gag antibodies by previously determined cutoffs [10], whereas the 2 uninfected control samples were negative. The mean anti-p24 antibody titers in the NR, relapse, and SVR groups were 2.77 × 10^9, 2.17 × 10^9, and 1.71 × 10^9 LUs, respectively. The anti-p24 antibody titer in the relapse group did not significantly differ from that in either the NR or SVR group (P > .47, Mann-Whitney U test); the NR and SVR groups showed a statistically significant difference in anti-p24 antibody titer (P = .023). Anti-p24 antibody titers did not correlate (P > .05) with HIV or HCV load, genotype, or CD4 T cell count (data not shown). However, anti-p24 antibody titers paralleled the cumulative group scores for the interferon-associated gene-expression signature previously reported by Lempicki et al [9] for the same patients. Titers of antibodies against the Tat protein of HIV did not significantly differ between the NR, relapse, and SVR groups (P > .26) (data not shown). Because statistically higher anti-p24 antibody titers were detected in the NR group versus the SVR group and correlated with failure of HCV therapy, a cutoff based on 2.2 million LUs was determined to optimally separate these 2 groups. By this approach, 9 of the 11 patients in the NR group were above the cutoff, compared with only 2 of the 9 patients in the SVR group (Figure 1B). On the basis of this analysis, anti-p24 antibody titers provide 82% positive predictive value in identifying patients who will experience therapy failure. Although anti-p24 antibody titers had value only in distinguishing NR from SVR (and none for relapse), there is little practical predictive value for this test in HIV/HCV-coinfected individuals.

Titters of antibodies against the panel of HCV proteins were also evaluated before and after treatment for their value in...
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 coinfect ed 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 SRVs. 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