Hepatitis C Virus–Specific Immune Responses and Quasi-Species Variability at Baseline Are Associated with Nonresponse to Antiviral Therapy during Advanced Hepatitis C

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Pretreatment hepatitis C virus (HCV)–specific lymphoproliferative (LP) responses, neutralizing antibody (NA) responses, intrahepatic cytotoxic T lymphocyte (CTL) responses, and HCV quasi-species (QS) diversity and complexity were examined in patients with advanced hepatic fibrosis (Ishak fibrosis score of ≥3) and prior nonresponse to interferon (IFN)–α therapy who were enrolled in the initial phase of the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis Trial. Positive baseline HCV E1– and/or E2–specific NA responses (P = .01) and higher baseline HCV QS diversity (P = .01) were more commonly found in patients who did not become sustained virologic responders (SVRs) at week 72 (W72) than they were in those who did. No patients with positive results for both the LP and NA assays achieved a sustained virologic response. Multiple logistic regression analysis revealed that, when the presence of cirrhosis, prior ribavirin therapy, genotype 1 infection, log serum HCV RNA level, and receipt of ≥80% of the prescribed medication were controlled for, a sustained virologic response (W72) was negatively correlated with positive baseline LP assay results (P = .02) and with 1 or more positive assays (LP, NA, or CTL) (P = .02). No differences were noted in baseline intrahepatic CTL activity between SVRs and non-SVRs. Thus, in patients with advanced hepatic fibrosis due to HCV infection, pretreatment HCV-specific immune responses and increased QS variability appear to hinder viral clearance by pegylated IFN-α2a and ribavirin combination therapy.

The role played by hepatitis C virus (HCV)–specific immune responses in the outcome of HCV infection is incompletely understood. Because HCV is believed to be noncytopathic, the immune response has been thought to play a key role in the hepatic damage and ensuing fibrosis that occurs during chronic infection. However, accumulating data from multiple laboratories have indicated that there is an association between enhanced HCV-specific T cell immunity and both recovery from acute infection [1–3] and antiviral therapy–induced viral clearance [4–7]. These data suggest that...
vigorou and broad-based HCV-specific T cell immunity is beneficial to the host and favors viral clearance.

The investigations described here were conducted in the context of the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) Trial, which is a controlled, randomized clinical trial of patients with chronic hepatitis C and advanced hepatic fibrosis (Ishak fibrosis score of 3) for whom interferon (IFN)–α therapy had previously failed [8]. We previously reported that the rate of sustained virologic response to re-treatment with pegylated IFN-α2a and ribavirin in the initial phase of the HALT-C Trial was low (18%) [9] and that immunologic and virologic measurements demonstrated a lack of association with the pretreatment clinical and histologic characteristics of this cohort [10]. In the present study, we simultaneously examined HCV-specific peripheral blood lymphoproliferative (LP) responses, HCV-specific intrahepatic cytotoxic T lymphocyte (CTL) responses, HCV E1 and E2 neutralizing antibody (NA) responses, and HCV E2 hypervariable region (HVR) 1 quasi-species (QS) diversity and complexity before the initiation of antiviral therapy in patients who completed at least 20 weeks of treatment. The goal of the study was to determine how baseline immune responses and viral variability might predict either on-treatment virologic response or subsequent treatment outcome in this group of patients with advanced hepatic fibrosis and prior nonresponse to IFN-α therapy. Finally, we sought to better understand the interactions between different components of the immune response and the virus itself.

**PATIENTS, MATERIALS, AND METHODS**

**Patients.** The design of the HALT-C Trial has been described elsewhere [8]. Briefly, criteria for enrollment were chronic HCV infection with a detectable serum HCV RNA level, prior nonresponsiveness to IFN-α therapy (with or without ribavirin), and the presence of bridging fibrosis or cirrhosis in liver-biopsy samples obtained during the 12 months before enrollment. All liver-biopsy and peripheral blood samples were prospectively collected at 4 of 10 HALT-C clinical sites before the initiation of therapy; the 4 sites that participated did so on the basis of investigator interest. The institutional review boards of all involved institutions approved the study protocols, and written, informed consent was obtained from all study participants. The patients in the present analysis had usable results for any of the 4 substudies (the intrahepatic CTL, peripheral blood LP, NA, or QS substudies). Evaluation of serum HCV RNA levels, HCV genotype, and liver biopsies were performed as described elsewhere [9]. Patients were treated for 20 weeks with pegylated IFN-α2a (180 μg weekly; PEGASYS, Roche Pharmaceuticals) and ribavirin (1000–1200 mg daily, depending on body weight; COPEGUS, Roche Pharmaceuticals) in 2 divided doses. At the week 20 (W20) time point, serum HCV RNA levels were tested in duplicate using the qualitative Roche COBAS Amplicor assay (version 2.0; Roche Molecular Systems). Patients with undetectable serum HCV RNA levels at W20 (virologic responders [VRs]) continued the combination therapy for 28 additional weeks (for a total of 48 weeks) and then were followed for 24 weeks; those with undetectable serum HCV RNA levels at the week 72 (W72) time point were considered to be sustained VRs (SVRs; figure 1). Patients with detectable serum HCV RNA levels at W20 entered the randomized phase of the HALT-C Trial and were considered to be W20 non-VRs as well as W72 non-SVRs. Finally, patients with undetectable serum HCV RNA levels at W20 but in whom serum HCV RNA reappeared at any time point after W20 were considered to be breakthrough/relapse patients and were included in the non-SVR group. Patients who had missing HCV RNA data at W20 or W72 were considered to be non-VRs (intention-to-treat analysis).

**LP, CTL, NA, and QS assays.** Lymphoproliferative responses to superoxide dismutase (SOD)–recombinant HCV genotype 1a protein antigens SOD-c22 (HCV aa 2-120), SOD-c100 (HCV aa 1569-1931), SOD-N55 (HCV aa 2054-2995), Escherichia coli–derived SOD-c33c (HCV aa 1192-1457) and recombinant human SOD (all gifts from M. Houghton, Chiron) were measured in quadruplicate wells using [3H]-thymidine incorporation assays with freshly isolated peripheral blood mononuclear cells (PBMCs), as described elsewhere [10, 11]. T cell lines were derived from fresh liver-biopsy samples (length, ∼0.5 cm) and tested in triplicate wells for cytolyis of autologous 51Cr-labeled B lymphoblastoid cell lines (BLCLs) infected with recombinant vaccinia viruses expressing regions spanning the entire HCV genotype 1a polyprotein—aa 1–339 (C-E1; vv9A), aa 347-906 (E2-NS2; vv1H), and aa 827-3011 (NS2-NS5; vv827), or β-galactosidase as a negative control, as described elsewhere [10, 12, 13]. HCV chimeric envelope glycoprotein (E1-G or E2-G; genotype 1a)/vesicular stomatitis virus (VSVs054; temperature-sensitive mutant of VSV) pseudotype viruses (∼100 pfu) were incubated with serial dilutions of heat-inactivated test sera, and plaques on baby hamster kidney cell monolayers were counted to assess NA activity, as described elsewhere [10, 14, 15].

HCV QS diversity and complexity were evaluated by clonal frequency analysis of the E2 HVR1, as described elsewhere [10, 16]. For each patient, 20 individual QS clones from frozen baseline serum samples were analyzed.

LP assays were scored as positive when the stimulation index (SI) value for any HCV antigen was >4. The SI was defined as the ratio of the mean test antigen cpm/mean control cpm. CTL assays were scored as positive when the percentage of specific lysis of BLCLs infected with HCV-vaccinia recombinant viruses was >10% above the percentage of specific lysis of the control target cells at 1 or more effector-to-target cell ratios. NA assays were scored as positive when ≥50% neutralization against either E1- or E2-pseudotyped viruses was detected. These cutoffs...
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Figure 1. Study design of the initial phase of the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) Trial. All patient samples tested by the lymphoproliferative, cytotoxic T lymphocyte, neutralizing antibody, and quasi-species assays were obtained at baseline, before the initiation of pegylated interferon (IFN-α2a (PEGASYS; Roche Pharmaceuticals) and ribavirin combination therapy. On-treatment virologic response was assessed after 20 weeks of therapy, and patients were categorized as week 20 (W20) virologic responders (VRs) or non-VRs on the basis of the results of serum hepatitis C virus (HCV) RNA testing using the qualitative Roche COBAS Amplicor assay (version 2.0). The W20 VRs continued the combination therapy for 28 additional weeks (for a total of 48 weeks) and then were followed for 24 weeks. Whether serum HCV RNA levels were detectable at week 72 (W72) determined the categorization of the patients as sustained virologic responders (SVRs) or non-SVRs.

Table 1. Characteristics of the study population (n = 367).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, years</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>Male</td>
<td>71</td>
</tr>
<tr>
<td>African American race</td>
<td>12</td>
</tr>
<tr>
<td>Prior ribavirin therapy</td>
<td>74</td>
</tr>
<tr>
<td>Receipt of ≥80% of the prescribed medication</td>
<td>50</td>
</tr>
<tr>
<td>ALT level, mean ± SD, IU/L</td>
<td>104 ± 73</td>
</tr>
<tr>
<td>Serum HCV RNA level, mean ± SD, log IU/mL</td>
<td>6.45 ± 0.56</td>
</tr>
<tr>
<td>Infected genotype</td>
<td></td>
</tr>
<tr>
<td>Genotype 1</td>
<td>87</td>
</tr>
<tr>
<td>Genotype 2</td>
<td>7</td>
</tr>
<tr>
<td>Genotype 3</td>
<td>4</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>31</td>
</tr>
<tr>
<td>Virological Response</td>
<td></td>
</tr>
<tr>
<td>Week 20</td>
<td>35</td>
</tr>
<tr>
<td>Week 72 (sustained)</td>
<td>19</td>
</tr>
</tbody>
</table>

NOTE. Data are percentages of patients, unless otherwise noted. ALT, alanine aminotransferase; HCV, hepatitis C virus.

We also previously reported details on the limited specificity, magnitude, and breadth of immune responses observed in the overall cohort to the genotype 1a antigens used [10]. Positive CTL responses were more frequent in patients with non-genotype 1 infection than they were in patients with genotype 1 infection (30% vs. 20%; P = .31, Fisher’s exact test), but this difference was significant only in the case of CTL responses to...
C-E1 (vv9A) (20% vs. 7%; \( P = .047 \), Fisher’s exact test). Genotype did not appear to influence the frequency of positive LP or NA assay results (data not shown). All analyses were also performed including only those patients with HCV genotype 1 infection; the results obtained were not significantly different from those obtained in the analyses of the entire cohort.

Figure 1 illustrates the study design. In the overall study cohort, 35% (127/367) achieved a virologic response at W20, as defined by an undetectable serum HCV RNA level at that time point (table 1). Nineteen percent (70/367) became SVRs (undetectable serum HCV RNA level at W72). Treatment-response rates were not significantly different among the substudy populations and were similar to those observed in the HALT-C cohort (\( n = 604 \)) [9].

**Similarity of the frequencies of LP, CTL, and NA responses at baseline in W20 VRs and non-VRs.** To investigate the association between baseline immune response and on-treatment virologic response, patients were categorized as either W20 VRs or non-VRs, depending on the absence or presence of detectable serum HCV RNA after 20 weeks of pegylated IFN-\( \alpha \)2a and ribavirin combination therapy. The percentage of patients with positive LP, CTL, or NA assay results at baseline was not different between W20 VRs and non-VRs, as is shown in figure 2A (\( P = .59 \), \( P = 1.00 \), and \( P = .20 \), respectively; Fisher’s exact test). Patients who had missing serum HCV RNA data at W20 (\( n = 25 \)) or W72 (\( n = 10 \)) were classified as non-VRs. Removal of these patients from the analyses did not change any of the results (data not shown).

**More frequent detection of LP and NA responses at baseline in non-SVRs than in SVRs.** To determine whether baseline immune responses to HCV antigens might predict subsequent treatment-induced viral clearance, patients were categorized according to their virologic status at W72, after 48 weeks of pegylated IFN-\( \alpha \)2a and ribavirin combination therapy and 24 weeks of follow-up. For this analysis, all patients with detectable serum HCV RNA levels at W20 as well as W72 were considered to be non-SVRs. The percentage of patients with positive LP, CTL, or NA assay results at baseline among the SVRs and the non-SVRs is shown in figure 2B. Surprisingly, patients with positive NA assay results at baseline were significantly more likely to become non-SVRs (\( P = .01 \), Fisher’s exact test; OR, 0.27 [95% confidence interval [CI], 0.09–0.80]), where an OR of <1.0 indicates a negative correlation between a positive assay and becoming an SVR; negative predictive value [NPV], 93%). Although more non-SVRs had positive LP assay results at baseline than did SVRs, only a trend toward statistical significance was found (\( P = .10 \), Fisher’s exact test; OR, 0.53 [95% CI, 0.25–1.09]; NPV, 87%). No such association was demonstrated for the CTL assay (\( P = .83 \), Fisher’s exact test). It is noteworthy that, when patients had 1 or more positive assay results (LP, NA, or CTL) at baseline, they were also less likely to become SVRs (\( P = .04 \), Fisher’s exact test; OR, 0.38 [95% CI, 0.15–0.96]; NPV, 88%). When patients with positive LP and NA assay results at baseline were compared with those with negative LP and NA assay results at baseline, it was found that no SVRs had positive results for both assays (\( P = .02 \), Fisher’s exact test; NPV, 100%). These findings indicate that the presence of an HCV-specific immune response at baseline—whether determined by the NA assay alone, 1 or more of the assays, or both the NA and LP assays together—was associated with subsequent virologic nonresponse to antiviral therapy.

Because prior ribavirin therapy was a strong predictor of subsequent virologic nonresponse after pegylated IFN-\( \alpha \)2a and ribavirin combination therapy in the overall HALT-C cohort [9], the possibilities that prior ribavirin therapy (1) may have affected the LP and NA assay results at baseline and (2) may have selected for different populations of patients were considered. When the subset of patients without prior ribavirin therapy was examined (\( n = 67 \)), the results were similar to those for the overall cohort. Specifically, non-SVRs were more likely to have positive NA assay results at baseline than were SVRs (\( P = .04 \), Fisher’s exact test; data not shown). Non-SVRs were also more likely to have positive LP assay results at baseline (31%) than were SVRs (17%), although the association was not statistically significant (\( P = .20 \), Fisher’s exact test). Therefore, prior ribavirin therapy did not appear to significantly affect the relationship between baseline LP or NA responses and subsequent virologic response. The median interval from baseline immunologic testing and receipt of the last dose of ribavirin as part of the prior therapy was 25 months (range, 5–62 months).

**Effect of the presence of cirrhosis on the association between baseline LP responses and subsequent virologic response to therapy.** Given our previous finding in this cohort of a possible association between the presence of cirrhosis and a decreased frequency of HCV-specific LP responses [10], we evaluated whether the presence of cirrhosis could affect the association between baseline LP responses and subsequent virologic response to therapy. When the presence of cirrhosis was controlled for, the association between a positive LP assay result at baseline and becoming an SVR was statistically significant (\( P = .04 \), Cochran-Mantel-Haenszel test). Moreover, when patients with cirrhosis (Ishak fibrosis score of 5 or 6) were excluded from the analysis, the association between a positive LP assay result at baseline and becoming a non-SVR was stronger (\( P = .02 \), Fisher’s exact test; OR, 0.38 [95% CI, 0.17–0.86]) (table 2). In the 104 patients with cirrhosis, however, the frequency of positive LP assay results at baseline was low (17/104 [16%]), and a positive result did not predict subsequent virologic nonresponse to therapy (\( P = .67 \), Fisher’s exact test). Similar analyses did not reveal a significant influence of liver histology on the relationship between NA or CTL assay results at baseline with subsequent virologic response to therapy.
Figure 2. Greater frequency of baseline immune responses in patients with virologic nonresponse at week 72 (W72). The percentage of positive lymphoproliferative (LP), cytotoxic T lymphocyte (CTL), and neutralizing antibody (NA) assay results for patients categorized as virologic responders (VRs) or non-VRs at week 20 (W20) are shown in panel A. The percentage of positive LP, CTL, and NA assay results for patients categorized as sustained VRs (SVRs) or non-SVRs at week 72 are shown in panel B. The nos. of patients with positive results by a particular assay differed among categories and are shown in parentheses. The frequencies of positive results for patients who achieved a virologic response and those who did not were compared for each assay using Fisher’s exact test; the P value for each of these comparisons is shown on the corresponding graph. Odds ratios (ORs), 95% confidence intervals (CIs), and negative predictive values (NPVs) for the findings in panel B are as follows: for baseline NA assay results, OR of 0.27 (95% CI, 0.09–0.80) (where an OR of <1.0 indicates a negative correlation between a positive assay and becoming an SVR) and NPV of 93%; for baseline LP assay results, OR of 0.53 (95% CI, 0.25–1.09) and NPV of 87%; for 1 or more positive assay results (LP, NA, or CTL), OR of 0.38 (95% CI, 0.15–0.96) and NPV of 88%; and for positive LP and NA assay results (compared with those with negative LP and NA assay results), NPV of 100% (none of the SVRs had positive responses in both assays, so an OR and 95% CI could not be calculated).

Confirmation, by multivariate logistic regression analysis, of the correlation between baseline immune responses and subsequent virologic response. Apart from the presence of cirrhosis and prior ribavirin therapy, other important factors known to affect the virologic response to antiviral therapy are serum HCV RNA level, HCV genotype, and the amount of
pegylated IFN-α2a and ribavirin received by patients. We therefore performed multivariate logistic regression analysis on data from our cohort, controlling for the presence of cirrhosis, prior ribavirin therapy, genotype 1 infection, log serum HCV RNA level, and receipt of >80% of the prescribed pegylated IFN-α2a and ribavirin during the first 20 weeks of treatment. As is shown in table 3, nonresponse to therapy at W72 was significantly associated with positive LP assay results at baseline (P = .02, χ² test; OR, 0.37 [95% CI, 0.16–0.86]) and with 1 or more positive assays among patients with results for all 3 (LP, CTL, and NA) (P = .02, χ² test; OR, 0.25 [95% CI, 0.08–0.83]). In this model, positive NA assay results at baseline demonstrated only a trend toward an association with virologic response at W72 (P = .07, χ² test; OR, 0.33 [95% CI, 0.10–1.10]).

Increased baseline HCV QS diversity in non-SVRs. Baseline HCV QS diversity (degree of nucleotide heterogeneity) and complexity (number of unique variants) were examined in 83 W20 non-SVRs and 20 W20 SVRs (8 of whom became SVRs) (figure 3A). For consideration of genetic diversity, a value of 1.0 denotes homogeneity; thus, decreasing values correlate with increasing heterogeneity at the nucleotide level [19, 20]. Both QS diversity and complexity were significantly higher at baseline in those who became W20 non-SVRs than they were in those who became W20 SVRs (P = .04 and P = .03, respectively; Wilcoxon rank sum test). Baseline QS diversity was also increased in non-SVRs, compared with that in SVRs (P = .01, Wilcoxon rank sum test) (figure 3B, left panel). Although the median QS complexity was increased in non-SVRs, compared with that in SVRs, only a statistical trend for this association was found (P = .09, Wilcoxon rank sum test) (figure 3B, right panel). When patients were categorized as W20 non-SVRs, breakthrough/relapse patients, and SVRs, a stepwise decrement in QS diversity and complexity was found (for diversity, P = .02; for complexity, P = .02; Spearman’s correlation coefficient) (figure 3C). No association between serum HCV RNA levels and QS diversity or complexity was found (P = .31 and P = .56, respectively; Spearman’s correlation coefficient). Finally, no direct associations could be established between results for the LP, CTL, or NA assays and the HCV QS analysis, even when results for individual antigens were considered.

DISCUSSION

In our cohort of 367 patients tested for HCV-specific immune responses before the initiation of therapy, we found a surprising association between the presence of baseline LP and NA responses and subsequent failure to achieve viral clearance with state-of-the-art antiviral therapy. The unique features of the present study included the large number of samples tested for multiple aspects of the immune response, the specialized cohort of patients with advanced hepatic fibrosis and prior nonresponse to IFN-α therapy, and our focus on the relationship between baseline immune responses and QS variability before the initiation of therapy and subsequent treatment outcome. Multivariate logistic regression analysis that controlled for the presence of cirrhosis, prior ribavirin therapy, genotype 1 infection, log serum HCV RNA level, and receipt of >80% of the prescribed pegylated IFN-α2a and ribavirin revealed that both a positive LP assay result at baseline and 1 or more positive immunologic assay results at baseline predicted subsequent non-SVR status at W72. This is the first report to describe HCV-specific immune responses before the initiation of therapy and relate them to treatment outcome in such a large group of prior non-SVRs with advanced hepatic fibrosis.

Earlier studies have described an association between increased QS variability and nonresponse to therapy, although most [19, 21, 22], but not all [23], studied significantly fewer patients. We similarly demonstrated increased QS variation at baseline in patients who proceeded to virologic nonresponse at both W20 and W72, although ours is the first report of such a correlation in prior non-SVRs with advanced hepatic fibrosis. Moreover, the present study found both increased QS variability and immune responses to be associated with subsequent virologic nonresponse, which is consistent with the notion that immune responses drive QS variation in vivo [24, 25].

However, we were not able to identify direct relationships between LP, CTL, or NA assay results and QS assay results. Our inability to detect these relationships could have resulted from a number of obstacles, such as the relatively small number of overlapping patients analyzed in the QS and immunologic assays, the nature of the cohort studied (all prior non-SVRs with advanced hepatic fibrosis), and the fact that heterologous (rather than patient-specific) antigens were used in the immunologic assays. In addition, the LP assay did not specifically assess responses to the HCV E2 HVR1, which was assessed in the QS assay. Nevertheless, the associations of both increased baseline QS variability and increased baseline LP and NA responses with

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**Table 2. Association between a positive lymphoproliferative (LP) assay result at baseline and the inability to achieve a sustained virological response in patients with hepatic fibrosis but not cirrhosis.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total, no.</th>
<th>LP positive, no. (%)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVRs</td>
<td>56</td>
<td>8 (14)</td>
<td>0.38 (0.17–0.86)</td>
<td>.02</td>
</tr>
<tr>
<td>Non-SVRs</td>
<td>178</td>
<td>54 (30)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVRs</td>
<td>10</td>
<td>2 (20)</td>
<td>1.32 (0.25–6.82)</td>
<td>.67</td>
</tr>
<tr>
<td>Non-SVRs</td>
<td>94</td>
<td>15 (16)</td>
<td>Reference</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval; OR, odds ratio; SVRs, sustained virological responders.
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Table 3. Multivariate logistic regression analysis with the presence of cirrhosis, prior ribavirin therapy, hepatitis C virus (HCV) genotype, log serum HCV RNA level, and receipt of >80% of the prescribed pegylated interferon-α2a and ribavirin as covariates.

<table>
<thead>
<tr>
<th>Assay(s)</th>
<th>Total tested, no.</th>
<th>Responders, no.</th>
<th>Correlation</th>
<th>P</th>
<th>c statistic</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP</td>
<td>338</td>
<td>117</td>
<td>Negative</td>
<td>.16</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>210</td>
<td>68</td>
<td>Negative</td>
<td>.32</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>244</td>
<td>86</td>
<td>Negative</td>
<td>.67</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>One or more b</td>
<td>129 43</td>
<td>Negative .50</td>
<td>0.82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP plus CTL c</td>
<td>120 41</td>
<td>Negative .55</td>
<td>0.82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP plus NA d</td>
<td>145 52</td>
<td>Negative .15</td>
<td>0.79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Boldface indicates a significant association. CI, confidence interval; CTL, cytotoxic T lymphocyte; LP, lymphoproliferative; NA, neutralizing antibody; ND, not done (analysis could not be performed, because no patients with positive LP and NA assay results achieved a sustained virological response); OR, odds ratio.

a No. of responders with positive results.
b One or more positive results among patients with results for the LP, NA, and CTL assays.
c Patients with positive results for both the LP and CTL assays, compared with those with negative results for both assays.
d Patients with positive results for both the LP and NA assays, compared with those with negative results for both assays.

Virological nonresponse lead us to hypothesize that T and B cell responses to HCV may have been sufficient to drive QS diversification but may not have been sufficient to predispose patients to virological clearance. The parameters by which these immune responses are ineffective or counteractive with regard to viral eradication have yet to be defined.

The baseline immune responses present in our eventual non-VR population were clearly inadequate—in terms of magnitude, breadth, and/or specificity (as detailed in Rothman et al. [10])—for the purpose of eradicating HCV. Moreover, the immune responses detected were associated with subsequent nonresponse to antiviral therapy. This finding may appear to conflict with earlier data demonstrating a relationship between enhanced HCV-specific CD4+ T cell proliferative responses and subsequent virologic clearance [4–7]. However, these other studies were performed in treatment-naive cohorts and focused on the development of T cell responses during and after therapy. By contrast, our cohort had longstanding advanced hepatic fibrosis, a history of prior nonresponse to IFN-α therapy, and was studied before the initiation of therapy. It is notable that African Americans, another treatment-resistant population, have recently been shown to more commonly exhibit HCV-specific CD4+ T cell proliferative responses than are Caucasian Americans [26], further supporting the notion that pretreatment immune responses may be predictive of nonresponse to therapy in certain patient cohorts.

The question remains, however, as to why some patients with baseline immune responses were unable to convert them to ones that lead to viral clearance. Several potential hypotheses could explain our results. The persistent activity of immunoregulatory T cells in HCV-infected patients that serves to downregulate the immune response during the chronically infected state [27, 28] could prevent the generation of an appropriate immune response. Other potential mechanisms include host factors (such as cytokine polymorphisms) [29, 30], the absence of an adequate T cell repertoire resulting from immune exhaustion [31], and the phenomenon of “original antigenic sin” [32], wherein the immune responses originally elicited by specific epitopes remain dominant, despite the evolution of variant viral epitopes. Future detailed studies will be required to determine which of these mechanisms is operant. Given the treatment-resistant advanced-disease state of our cohort, the HCV-specific immune responses we detected were, at best, ineffective—and, at worst, they served to impede the development of other, effective immune responses after re-treatment.

In conclusion, we found positive baseline HCV-specific immune responses and increased QS variability to be associated with a subsequent lack of sustained virologic response in a large cohort of patients with advanced hepatic fibrosis and prior nonresponse to IFN-α therapy, indicating an association between HCV-specific immune reactivity and a pretreatment state that is resistant to successful pegylated IFN-α2a and ribavirin combination therapy. Earlier findings from our group [10] and from others [33] have suggested that active intrahepatic HCV-specific CTL responses may be responsible for ongoing liver damage and may play a role in the progression of liver disease. Together, these data suggest that, in certain situations, the presence of an immune response may not necessarily benefit the host. Clearly, the role played by the immune response in the pathogenesis of hepatitis C disease is complex, and the difference between a beneficial and deleterious immune response will need to be carefully dissected as we consider the use of immune-enhancing or -modifying therapies in the future treatment of chronic hepatitis C.
Figure 3. Greater variability of baseline hepatitis C virus (HCV) quasi species (QS) in genotype 1–infected patients with virologic nonresponse. Baseline HCV QS diversity and complexity were analyzed by clonal frequency analysis after genotype 1–infected patients were categorized as virologic responders (VRs) or non-VRs at week 20 (W20; A) and as sustained VRs (SVRs) or non-SVRs at week 72 (W72; B). Panel C shows patients categorized as W20 NRs, breakthrough/relapse patients (BT/Rs), and SVRs. For consideration of genetic diversity, a value of 1.0 denotes homogeneity; thus, decreasing values correlate with increasing heterogeneity at the nucleotide level. Complexity shows the no. of unique variants. The columns indicate the range of values obtained between the 25th and 75th percentiles for diversity and complexity; the error bars indicate the lowest and highest nonoutlier values in each data set; the lines contained within the columns indicate the median value in each data set; and the circles indicate outliers that were >1.5 times the interquartile range from the median. Genetic diversity and complexity were compared between the patients with virologic response and those with nonresponse by use of the Wilcoxon rank sum test, whereas trends were tested by use of Spearman’s correlation coefficient; $P$ values are shown for each comparison. The total no. of patients in each group is shown in parentheses below the corresponding bar graph.
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