Profibrogenic chemokines and viral evolution predict rapid progression of hepatitis C to cirrhosis

Patrizia Farci[a,1], Kurt Wollenberg[b], Giacomo Diaz[c], Ronald E. Engle[d], Maria Eliana Lai[e], Paul Klenerman[f], Robert H. Purcell[d], Oliver G. Pybus[g], and Harvey J. Alter[h,1]

[a]Hepatic Pathogenesis Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; [b]Bioinformatics and Computational Biosciences Branch, Office of Cyber Infrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; [c]Department of Biomedical Sciences, University of Cagliari, 09123 Cagliari, Italy; [d]Hepatitis Viruses Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; [e]Department of Medical Sciences, University of Cagliari, 09042 Monserrato (Cagliari), Italy; [f]Peter Medawar Building for Pathogen Research, University of Oxford, Oxford OX1 3SY, United Kingdom; [g]Department of Zoology, University of Oxford, Oxford OX1 3PS, United Kingdom; and [h]Department of Transfusion Medicine, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD 20892

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Chronic hepatitis C may follow a mild and stable disease course or progress rapidly to cirrhosis and liver-related death. The mechanisms underlying the different rates of disease progression are unknown. Using serial, prospectively collected samples from cases of transfusion-associated hepatitis C, we identified outcome-specific features that predict long-term disease severity. Slowly progressing disease correlated with an early alanine aminotransferase peak and antibody seroconversion, transient control of viremia, and significant induction of IFN-γ and MIP-1β, all indicative of an effective, albeit insufficient, adaptive immune response. By contrast, rapidly progressive disease correlated with persistent and significant elevations of alanine aminotransferase and the profibrogenic chemokine MCP-1 (CCL-2), greater viral diversity and divergence, and a higher rate of synonymous substitution. This study suggests that the long-term course of chronic hepatitis C is determined early in infection and that disease severity is predicted by the evolutionary dynamics of hepatitis C virus and the level of MCP-1, a chemokine that appears critical to the induction of progressive fibrogenesis and, ultimately, the ominous complications of cirrhosis.

hepatitis C virus | slow progressors | rapid progressors | liver fibrosis

Hepatitis C virus (HCV) is an important human pathogen that causes persistent infection in up to 80% of infected individuals (1). It is the most common cause of chronic liver disease in the United States, and the leading indication for liver transplantation (LT) (2). The clinical presentation and outcomes of chronic hepatitis C are highly variable. Although the disease is mild and either stable or slowly progressive in about 70% of chronically infected patients, the remaining 30% develop progressive liver disease culminating in cirrhosis and possibly hepatocellular carcinoma (HCC), leading to death or orthotopic LT (1, 2). Progression to cirrhosis usually takes 20–40 y, but in some patients severe fibrosis can develop rapidly leading to liver-related death within 5–10 y from the onset of infection (3). Presently, it is not possible to predict which individuals will rapidly evolve to severe fibrosis and liver failure.

The mechanisms responsible for the different rates of disease progression in chronic hepatitis C are unknown, but likely reflect differences in the complex interplay between the virus and host immunity. Although HCV infection elicits strong cellular and humoral immune responses (4–7), evidence suggests that the virus has evolved numerous strategies to avoid immune recognition (8). One of the most effective mechanisms relates to the remarkable genetic diversity of HCV, which circulates in vivo as a dynamic distribution of divergent but related genomes termed a "quasispecies" (9). This genomic heterogeneity enables rapid adaptation to selective constraints imposed by the host (10). We previously demonstrated that the evolutionary dynamics of HCV during early infection predicts the outcome of acute hepatitis C, such that a decrease in viral diversity correlates with viral clearance whereas an increase correlates with progression to chronicity (11). These data, subsequently corroborated elsewhere (12–14), are consistent with the concept that aspects of viral evolution reflect the strength of virus-specific adaptive immune responses and therefore the ability of the host to control the virus (8, 15). However, little is known about the correlations among adaptive immunity, HCV evolution, and rates of disease progression once chronic hepatitis C is established.

We have been able to study the complex interplay between virus and host throughout the course of infection in a cohort of patients with acute hepatitis C that progressed to chronicity while under observation during a prospective study of post-transfusion non-A, non-B hepatitis (16); these patients have been followed from the day of transfusion for up to 30 y. Here, we investigate the relationship among biochemical markers of liver damage, viral kinetics, humoral immunity, serum cytokine and chemokine profiles, and HCV evolution in patients with slowly or rapidly progressive disease to obtain insights into the mechanisms underlying variable rates of disease progression.

Results

Acute-Phase Viral Kinetics Differ in Slow and Rapidly Progressive Hepatitis C. Six patients infected with HCV genotype 1 were selected for intensive study because they represented two different clinical outcomes and had serial serum samples available throughout the acute and chronic phases of infection, in addition to clinical and liver pathology data. Disease was mild and stable or slowly progressive for up to 30 y in three patients (slow progressors) and rapidly progressive in the remaining three (rapid progressors), leading to liver-related death within 7 y of the onset of infection (Fig. S1 and Table S1). Strikingly, in two of the three rapid progressors, active cirrhosis was documented within the first 2 y of infection (patients 5 and 6 in Table S1), suggesting that extremely rapid progression to cirrhosis can occur outside the setting of immunosuppression as has been observed following liver transplantation (17) or in HIV-1 coinfected individuals (18). Comparisons were censored at 7 y because all of the rapid progressors had died from end-stage liver disease by that time. In all patients, viral RNA became detectable in serum within 1–2 wk of transfusion, after which viral titers rose very rapidly. The subsequent course of disease could not be predicted by the onset or magnitude of early viremia. However, a difference in viral load between slow and rapid progressors emerged later in acute infection. In slow progressors, HCV viremia became transiently

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undetectable in two of three patients and decreased by 3 logs in the third patient during the first 4 mo of infection; in contrast, none of the rapid progressors lost detectable HCV RNA, although fluctuations in viral level were observed. In slow progressors, serum HCV RNA reappeared at a high level between 6 and 12 mo and subsequently persisted (Fig. 1).

Rapid Disease Progression Correlates with Delayed Alanine Aminotransferase Peak and Antibody Seroconversion. In slow progressors, the decline in viremia coincided with the alanine aminotransferase (ALT) peak (Fig. 1) as previously reported (7, 19, 20). Furthermore, ALT peaked earlier in slow than in rapid progressors, with means of 9 and 15 wk (median 8 and 17) from the time of infection, respectively. ALT levels tended to be higher in rapid than in slow progressors. The temporary loss of detectable viremia seen after the ALT peak in slow progressors was associated with transient ALT normalization, whereas none of the rapid progressors normalized ALT. Because liver damage in acute hepatitis C is believed to be immunologically mediated (7, 8), these findings suggest that slow progressors have an earlier and more effective viral-specific immune response than rapid progressors. Analysis of specific anti-HCV antibodies by enzyme immunoassay (EIA3) documented a delay in anti-HCV antibody seroconversion in rapid progressors (mean, 11.7 wk; median, 12 wk) compared to slow progressors (mean, 8.7 wk; median, 8 wk) from the time of infection (Table S1).

Cytokine and Chemokine Levels in the Acute Phase Predict the Pace of Disease Progression. Because viable cellular samples were not available from this unique historical collection, to evaluate virus-specific cellular immune responses we studied the long-term profile of 39 cytokines and chemokines in serial serum samples during the acute and chronic phase. In both slow and rapid progressors, cytokine and chemokine profiles initially increased, before the rise in ALT levels, and then declined following the ALT peak, with minor fluctuations during chronic infection. The most striking observation of our study was the correlation between cytokine and chemokine expression and the pace of disease progression (Fig. 2 A–E). Overall, slow progressors tended to have higher cytokine and chemokine levels than rapid progressors, but only 5 of the 39 cytokines tested were significantly different between the two groups. We found that during the entire acute phase (both preceding the ALT peak and in the period post-ALT peak until 6 mo), the levels of IFN-γ and MIP-1β (CCL4)—two markers associated with effective, multispecific CD4$^+$ and CD8$^+$ virus-specific T-cell responses (19–22)—were significantly higher in slow than in rapid progressors ($P < 0.05$) (Fig. 2 C and D). By contrast, the levels of MCP-1 (CCL2) (Fig. 2E), IL-8 (CXCL8) (Fig. S2A), and IP10 (CXCL10) (Fig. 2B), three chemokines associated with inflammatory reactions (23–26), became significantly higher ($P < 0.05$) in rapid progressors in the period between the ALT peak and 6 mo post the onset of infection, reflecting a prolonged inflammatory state following the onset of liver injury.

Sustained Elevations of Profibrogenic MCP1 Correlates with Rapid Progression to Cirrhosis. A different pattern was observed during chronic infection. Whereas the levels of IFN-γ and MIP-1β remained significantly higher ($P < 0.05$) in slow progressors (Fig. 2 C and D), MCP-1, a major chemotactic factor for hepatic stellate cells (HSCs) (23), which play a central role in fibrogenesis (26–30), was the only chemokine that remained significantly higher ($P < 0.05$) in rapid progressors until the patient's death from end-stage liver disease. Notably, persistence of MCP-1 elevation was associated with significantly higher ALT levels (Fig. 2B). The relationships among the levels of ALT, HCV RNA, and five cytokines/chemokines (IFN-γ, MIP-1β, IL-8, MCP-1, and IP-10) during the acute and chronic phase of infection in the six patients studied were also investigated by multivariate methods. Q-mode Principal Component Analysis showed a complete separation of slow and rapid progressors and
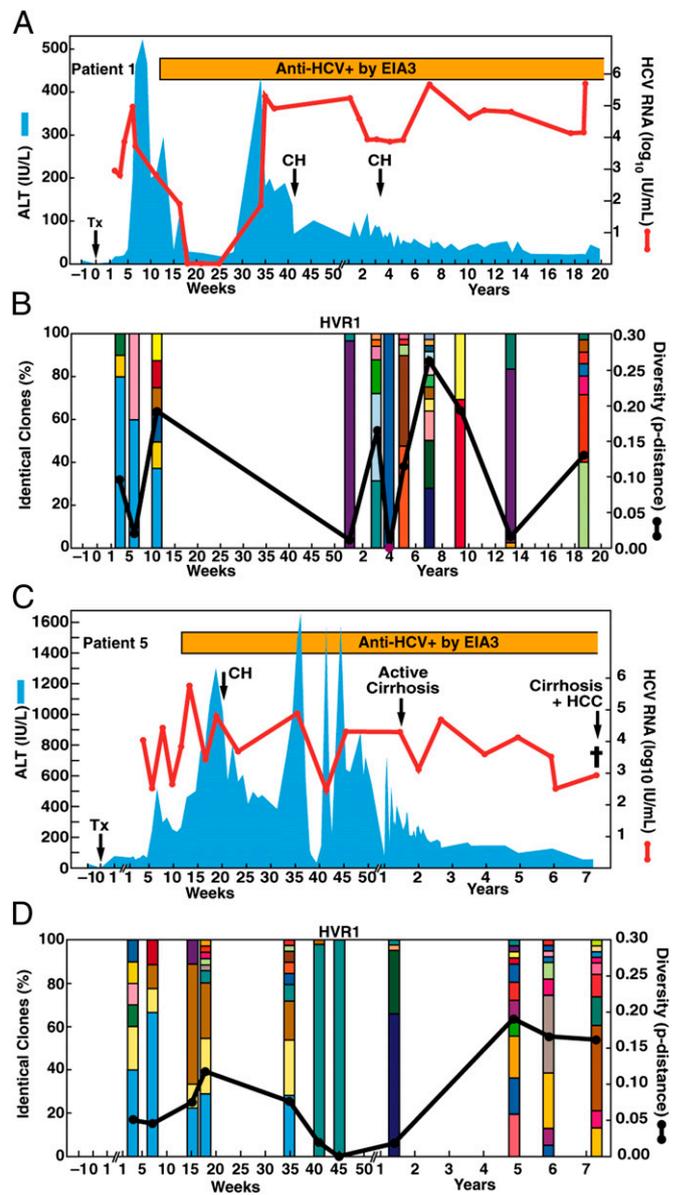

Fig. 1. Long-term clinical course and evolution of HCV in two representative patients followed from the time of infection, one with slowly progressive (patient 1) and one with rapidly progressive hepatitis leading to liver-related death (patient 5). Patient numbers are the same as in Table S1. (A and C) Long-term clinical course of slowly and rapidly progressive chronic hepatitis C. The blue areas indicate ALT levels. The red line indicates the titer of serum HCV RNA, as determined by real-time PCR (TaqMan), on a logarithmic scale. The orange horizontal bars indicate antibodies to HCV detected by third-generation enzyme immunoassay (EIA3). "Tx" denotes the time of blood transfusion. The arrows indicate the time and the results of liver biopsies. "CH" denotes chronic hepatitis and "HCC" indicates hepatocellular carcinoma. In patient 5, the diagnosis of HCC was obtained at autopsy. (B and D) Number of viral strains and diversity (genetic distance among variants) of HCV within the 27 amino acids of the HVR1. The vertical bars indicate the number and the proportion of identical clones. The dominant viral variant found in each patient at the first time point is indicated in blue; other variants are indicated by different colors. The same color denotes identity between viral variants detected at different time points within the same patient and not between different patients. The genetic diversity (black line) was calculated by mean p-distance from the predicted amino acid sequences obtained from each sample (Materials and Methods).

patterns of ALT, HCV RNA, and cytokine/chemokines consistent with the above observations (Fig. 3). A marked separation of slow and rapid progressors was also obtained by hierarchical
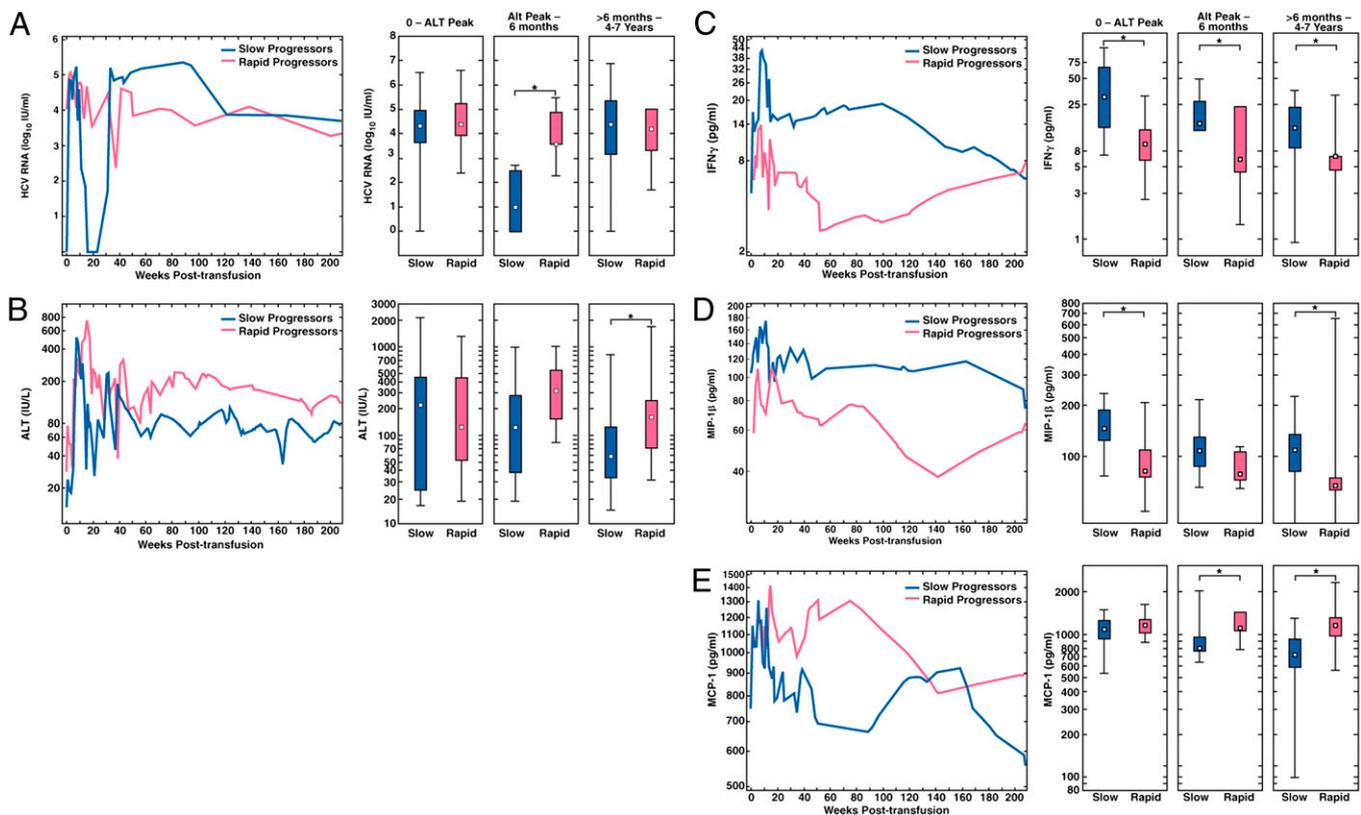
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Fig. 2. Differences in serum HCV-RNA titer and ALT and cytokine/chemokine levels in patients with slowly or rapidly progressive chronic hepatitis C, followed from the time of infection. (*A–E*) Each panel shows the linearly interpolated median curve (*Left*) and boxplots (*Right*) of the median, interquartile range, and minimum and maximum values observed during the early acute phase (from the time of infection to the ALT peak), late acute phase (from the ALT peak to 6 mo from infection), and the chronic phase (from 6 mo to 7 y from infection) of HCV infection. *$P < 0.05$ by Mann–Whitney *U* test between slow and rapid progressors at different time points.

clustering (Fig. 3). Collectively, the results of our study indicate that slow and rapidly progressive liver disease showed distinctive biochemical, virological, and immunological profiles.

Viral Evolution and the Pace of Disease Progression. We subsequently investigated the relationship between HCV molecular evolution and disease progression using powerful analytical methods. Because serum was longitudinally sampled after a known date of infection and none of the patients had received antiviral therapy, this data set allowed for a comprehensive analysis of HCV evolution in relation to disease outcome. Comparisons were censored at 7 y because all of the rapid progressors had died from end-stage liver disease by that time. Viral sequences within the HCV envelope 1 and 2 genes, including hypervariable region 1 (HVR1), were analyzed in all PCR-positive samples during the entire course of infection. DNA amplified by PCR from the E1 and E2 genes was cloned, and a mean of 29 molecular clones for each time point were sequenced, resulting in a total of 1,876 sequences, each 558 nucleotides long. The six individuals analyzed here constituted a subset of those previously selected for a study in which we investigated the evolution of HCV during the first 4 mo of infection and its relationship with the outcome of acute hepatitis C (resolution vs. chronic evolution) (11). In this study, we independently analyzed the genetic diversity and divergence of the HCV quasispecies within E1, HVR1, and E2 outside the HVR1. The greatest diversity and divergence were detected in HVR1 (Fig. 4 *A* and *B*), consistent with the fact that it is a major target for neutralizing antibodies (31), followed by E2 outside the HVR1, and then by E1 (Fig. S3 *A–D*). Both common and outcome-specific changes were observed. During acute infection, HVR1 diversity initially decreased, but then increased dramatically after antibody sero-

conversion, especially in slow progressors, followed again by a sharp decrease between 6 and 12 mo of infection. Then, the levels of HVR1 diversity remained lower in slow progressors until 2–4 y post infection, but not later. From 5 to 7 y post infection, viral diversity dramatically increased in slow progressors, reaching values higher than those seen in rapid progressors before their death (Fig. 4*A* and Fig. S2 *A* and *B*). In both slow and rapid progressors, divergence from the initial viral population increased over time, but a difference in the degree of these changes between the two groups became evident. HVR1 divergence before 1 y was constantly higher in rapid progressors and remained higher throughout follow-up (Fig. 4*B* and Fig. S3 *C* and *D*).

To study the relationship between disease progression and viral evolutionary dynamics, we used the phylogenetic approach implemented in Beast v1.6.2 to reconstruct for each patient the evolution of all E1/E2 sequences, inclusive of the HVR1, which were obtained sequentially over the entire course of the disease. Our data set is unique in that it permitted us to investigate the relationship between long-term disease progression (over several decades) and viral evolutionary dynamics in both acute and chronic hepatitis C. Bayesian molecular clock phylogenies revealed a distinct change in viral evolutionary dynamics during the transition from acute to chronic hepatitis in all patients. During the acute phase, lineages sampled at different times intermingled, with multiple lineages in one sample leading back to multiple lineages in the previous one (Fig. 4 *C* and *D*). However, around 1 y post infection this pattern changed, and a strong genetic bottleneck occurred in all patients with only one, or a few, of the lineages present during acute infection giving rise to the viral population in chronic infection (Fig. 4 *C* and *D*). This dynamic signature indicates that the vast majority
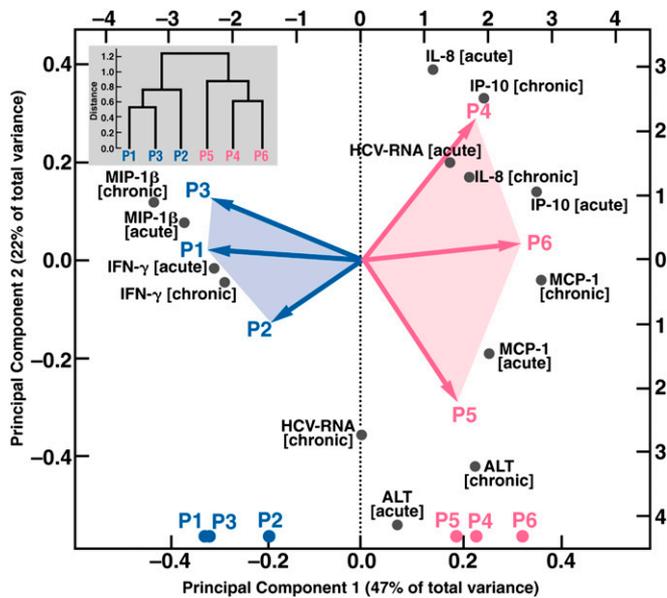
Fig. 3. Q-mode Principal Component Analysis showing the relations among the six patients resulting from the median levels of ALT, HCV RNA, and five cytokines/chemokines (IFN-γ, MIP-1β, IL-8, MCP-1, and IP-10) observed during the acute phase (0–6 mo from infection) and the chronic phase (6 mo–7 y from infection) of the disease. A complete separation of low (blue) and rapid (red) progressors is given by the first principal component, which accounts for nearly 50% of the total variance of the 14 variables included in the analysis. The map of the variables points to an increase of IFN-γ and MIP-1β in slow progressors and of MCP-1, IL-8, and IP-10 in rapid progressors in both the acute and the chronic phase. Higher ALT and HCV-RNA levels are also associated with rapid progressors, although in HCV RNA only during the acute phase. Hierarchical clustering (*Inset*) using the same set of variables confirmed the separation between slow and rapid progressors.

of HCV strains were cleared. The viral nucleotide-substitution rate was estimated independently for the E1, HVR1, and E2 regions of the HCV genome using Beast v1.6.2 (Table S2). Rapid progressors showed higher substitution rates on average compared with slow progressors across all regions. In both rapid and slow progressors, the highest rate was observed within the HVR1. To investigate whether the difference in the evolutionary rates between slow and rapid progressors was due to positive selection, we estimated the mean number of nonsynonymous and synonymous substitutions per nonsynonymous and synonymous site, respectively, across the first 7 y of the Bayesian molecular clock phylogenies. An excess of nonsynonymous substitution with respect to the amount of synonymous substitution was considered to be indicative of positive selection. Overall, the mean number of substitutions per site was greater in rapid progressors, and this effect was especially pronounced for synonymous substitutions (Fig. 4E). Next, we analyzed individual codons for positive selection. The most striking observation from our analysis was the finding of a significantly different distribution of positive selection across the E1/HVR1/E2 region between slow and rapid progressors ($P < 0.01$) (Fig. 4F). The number of codons under positive selection was significantly more concentrated in the HVR1 in slow progressors (9 of 12) than in rapid progressors (3 of 10) ($P < 0.01$), suggesting that host humoral immunity imposes the strongest selection pressure in slow progressors, whereas selection pressures in rapid progressors are less focused (Fig. 4F). Interestingly, none of the slow progressors showed positively selected codons in the E1 region, whereas three positively selected sites in E1 were identified in rapid progressors.

Discussion

Our study provides insights into the clinical, virological, and immunological determinants of slow versus rapidly progressive hepatitis C. Our patients were devoid of confounding factors such as viral coinfections and i.v. drug use or alcohol abuse, and all were Caucasian and infected with HCV genotype 1. Despite the limitations inherent within an historical cohort, particularly the lack of viable cellular samples for analysis of virus-specific T-cell responses and the limited number of patients who could be included in this intensive study, our data indicate that slow and rapid progressors exhibited distinct outcome-specific features that became evident during the acute phase and persisted into chronicity. We found that stable or slowly progressive disease is associated with the induction of earlier and stronger adaptive immune responses against HCV, as documented by an earlier ALT peak and antibody seroconversion, transient clearance or marked reduction of viremia, significant induction of IFN-γ and MIP-1β, along with a significant decrease of proinflammatory chemokines. By contrast, patients with rapidly progressive disease exhibited reduced or delayed adaptive immune responses associated with significantly higher levels of HCV viremia and persistent elevation of proinflammatory and profibrotic chemokines, such as MCP-1, IL-8, and IP-10, beginning in the late acute phase of hepatitis. The higher expression of these chemokines in rapid progressors during the early phase of hepatitis C suggests that these markers might be predictive of rapidly progressive liver disease. Strikingly, MCP-1 persisted at significantly elevated levels throughout the course of the disease. MCP-1 is a critical mediator of liver fibrogenesis via an autocrine loop enacted by HSCs, the primary effector cells in hepatic fibrogenesis (26–30), which both produce and are attracted by this chemokine (26–28). Interestingly, an MCP-1 gene polymorphism was found to be associated with significantly higher hepatic expression of MCP-1 in patients with chronic hepatitis C and more advanced liver fibrosis (32). In addition to its potent profibrogenic action, MCP-1 also recruits macrophages and T cells, thereby further contributing to hepatic inflammation and fibrogenesis (27). In addition, elevated IP-10 and IL-8 levels have been linked to higher necroinflammatory changes and fibrosis in chronic hepatitis C (33–37), as well as in fibrosis progression following liver transplantation (38, 39). Altogether, these results suggest that accelerated fibrogenesis in rapid progressors is secondary to sustained intrahepatic inflammation, leading to iterative hepatocyte injury, consistent with the hypothesis that the liver damage in rapidly progressive hepatitis C is not induced by virus-specific antiviral effector T cells, as occurs during the acute phase, but rather by massive infiltration and activation of nonspecific inflammatory cells and continuous release of proinflammatory and profibrotic soluble mediators.

Although the molecular mechanisms leading to increased liver inflammation in rapid progressors remain to be fully elucidated, the failure of a sufficiently effective virus-specific adaptive immune response during a critical window early in HCV infection appears to play a pivotal role in determining the extent of hepatocellular damage mediated by liver inflammation, necrosis, and early fibrogenesis. In contrast, subjects with stable or slowly progressive disease not only were able to raise a timely virus-specific immune response, but also were able to maintain throughout the course of the infection higher levels of IFN-γ, a cytokine that has been shown to exert antifibrogenic effects (40). Consistent with our findings, patients with intrahepatic CD8^{+} T cells producing IFN-γ were reported to have less fibrosis and lower progression rates than those lacking such cells (41). Although the level of IFN-γ detected in slow progressors was presumably not sufficient to eradicate the virus, it may have effected a degree of viral containment that was clinically relevant and sufficient to alter the course of disease.

This longitudinal study also provided unique insights into the role of viral evolution in disease progression. In all patients, we documented a strong genetic bottleneck that reduced viral

Fig. 4. Viral evolution in slow and rapid progressors during the acute and chronic phase of hepatitis C. (*A*) Genetic variation (diversity) of HCV within the HVR1 in slow and rapid progressors during the entire course of the disease. Median values of amino acid sequence diversity, as measured by the mean P-distance among all sequences within each sample. (*B*) Median values of viral divergence, as measured by the mean P-distance relative to all of the sequences detected in the first PCR-positive sample (founder viral population). Circles denote the mean values for each individual patient. A difference in HVR1 divergence between slow and rapid progressors was seen soon after seroconversion, as well as at 1 y post infection (*P* = 0.028 by Mann–Whitney *U* test when the data from these two times points were combined). (*C* and *D*) Time-structured Bayesian molecular clock phylogenies of all viral nucleotide sequences of the E1/E2 region, including the HVR1, in two representative patients, one with slowly progressive (*C*) and one with rapidly progressive chronic hepatitis C (*D*) leading to end-stage liver disease within 7 y from infection. Patient numbers are the same as in Table S1. In both trees, a red arrow indicates the lineage bottleneck around 1 y post infection. Asterisks along the backbone branches are posterior probabilities >0.95. The shaded areas indicate the acute phase (0–6 mo) of infection. (*E*) Mean number of synonymous and nonsynonymous substitutions per site in slow and rapid progressors during the first 7 y of infection. The bars indicate the means for all patients for each group, and the results are presented separately for the E1, HVR1, and E2 regions. Circles denote the mean values for each individual patient. (*F*) Site-by-site analysis of positive selection, as determined by two-parameter fixed-effects likelihood analysis. The blue box denotes the E1 region, the yellow the HVR1, and the pink the E2. Each bar represents an individual patient who showed positive selection at each codon position. The number of codons under positive selection was significantly more concentrated in the HVR1 in slow progressors (9 of 12) than in rapid progressors (3 of 10) (*P* < 0.01 by Kolmogorov–Smirnov two-sample test). In slow progressors, the analysis at 1 y and during 5–7 y was extended to only two patients because in patient 3 PCR amplification of the E1/E2 region was negative due to undetectable or very low levels of HCV RNA; in rapid progressors, the analysis at 2–4 y and at 5–7 y was also limited to two patients because in patient 6, who died 4 y after transfusion, the amount of serum during the last 2 y was not sufficient for PCR amplification with the set of primers from the E1/E2 region.

diversity around 1 y post infection. Slow progressors also showed a significantly higher positive selection on individual codons within the HVR1, indicative of strong immunologic pressure on this region, which is a major target of neutralizing antibodies (31). It is likely that in slow progressors the recurrence of HCV viremia after a marked, but transient, reduction in viral load during early infection was due to the emergence of immune escape variants (42). In contrast, faster progression correlated with

greater HCV sequence divergence across all regions examined, particularly within the HVR1, and with preponderance of synonymous substitutions as previously reported in HCV and HIV infections (43, 44). This greater accumulation of synonymous substitutions across all of the regions is consistent with a shorter viral generation time and a more rapid turnover of infected hepatocytes, as confirmed by the significantly higher ALT levels detected throughout the disease course. A shorter hepatocyte

life span with continuous replacement of infected cells can explain why the levels of viremia were not significantly higher in rapid progressors (43).

In summary, our data provide a model for the pathogenesis of chronic HCV-induced liver disease whereby rapid disease progression is the consequence of an early ineffective induction of the adaptive immune response followed by the sustained release of chemokine and cytokine mediators of hepatic inflammation and fibrosis. In essence, severe chronic injury may result from an excess of soluble inflammatory mediators rather than from cytotoxic T cells. This model may provide means for the early identification of patients at increased risk of rapid HCV disease progression and for the use of anti-inflammatory and antifibrotic therapy to limit or prevent accelerated liver fibrosis progression.

Materials and Methods

Study Subjects and Design of the Study. Six patients with acute progressing hepatitis C who were enrolled in a prospective study of posttransfusion non-A, non-B hepatitis conducted at the National Institutes of Health (16) were selected for this intensive study because they represented two clearly different outcomes of chronic hepatitis C, had serial serum samples available, and had long-term follow-up. Three patients whom we defined as slow progressors had a mild and stable liver disease for up to 30 y, and three whom we defined as rapid progressors had a rapidly progressive liver disease leading to liver-related death within 7 y of infection (*SI Materials and Methods*).

Methods. Details of serologic and virologic assays including anti-HCV, HCV-RNA extraction, reverse transcriptase reaction, and nested PCR and quantification of serum HCV RNA by TaqMan, HCV genotype, molecular cloning and sequencing, and multiplex cytokine analysis are provided in *SI Materials and Methods*.

Molecular Evolution of HCV. Details on the methods used are provided in *SI Materials and Methods*.

Statistical Analysis. Differences in serum ALT, HCV-RNA, and cytokine/chemokine levels between slow and rapid progressors were assessed by the Mann–Whitney U test and multivariate methods. The distribution of significant positive selection across domains was assessed using the Kolmogorov–Smirnov test. Details are given in *SI Materials and Methods*. Statistical analyses were performed using Statistica (Statsoft) and R software (R Development Core Team, http://www.R-project.org.).

1. Alter HJ, Seeff LB (2000) Recovery, persistence, and sequelae in hepatitis C virus infection: A perspective on long-term outcome. *Semin Liver Dis* 20(1):17–35.
2. Seeff LB, Hoofnagle JH (2002) National Institutes of Health Consensus Development Conference: Management of hepatitis C: 2002. *Hepatology* 36(5, Suppl 1):S1–S2.
3. Di Bisceglie AM, et al. (1991) Long-term clinical and histopathological follow-up of chronic posttransfusion hepatitis. *Hepatology* 14:969–974.
4. Diepolder HM, et al. (1995) Possible mechanism involving T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection. *Lancet* 346:1006–1007.
5. Missale G, et al. (1996) Different clinical behaviors of acute hepatitis C virus infection are associated with different vigor of the anti-viral cell-mediated immune response. *J Clin Invest* 98:706–714.
6. Lechner F, et al. (2000) Analysis of successful immune responses in persons infected with hepatitis C virus. *J Exp Med* 191:1499–1512.
7. Thimme R, et al. (2001) Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J Exp Med* 194:1395–1406.
8. Walker CM (2010) Adaptive immunity to the hepatitis C virus. *Adv Virus Res* 78:43–86.
9. Martell M, et al. (1992) Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: Quasispecies nature of HCV genome distribution. *J Virol* 66:3225–3229.
10. Farci P (2011) New insights into the HCV quasispecies and compartmentalization. *Semin Liver Dis* 31:356–374.
11. Farci P, et al. (2000) The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. *Science* 288:339–344.
12. Laskus T, et al. (2004) Analysis of hepatitis C virus quasispecies transmission and evolution in patients infected through blood transfusion. *Gastroenterology* 127:764–776.
13. Bull RA, et al. (2011) Sequential bottlenecks drive viral evolution in early acute hepatitis C virus infection. *PLoS Pathog* 7:e1002243.
14. Liu L, et al. (2010) Acceleration of hepatitis C virus envelope evolution in humans is consistent with progressive humoral immune selection during the transition from acute to chronic infection. *J Virol* 84:5067–5077.
15. Thomson EC, Smith JA, Klenerman P (2011) The natural history of early hepatitis C virus evolution; lessons from a global outbreak in human immunodeficiency virus-1-infected individuals. *J Gen Virol* 92:2227–2236.
16. Koziol DE, et al. (1986) Antibody to hepatitis B core antigen as a paradoxical marker for non-A, non-B hepatitis agents in donated blood. *Ann Intern Med* 104:488–495.
17. Berenguer M, et al. (2000) HCV-related fibrosis progression following liver transplantation: increase in recent years. *J Hepatol* 32:673–684.
18. Benhamou Y, et al.; The Multivirc Group (1999) Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. *Hepatology* 30:1054–1058.
19. Thimme R, et al. (2002) Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. *Proc Natl Acad Sci USA* 99:15661–15668.
20. Major ME, et al. (2004) Hepatitis C virus kinetics and host responses associated with disease and outcome of infection in chimpanzees. *Hepatology* 39:1709–1720.
21. Betts MR, et al. (2006) HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. *Blood* 107:4781–4789.
22. Deeks SG, Walker BD (2007) Human immunodeficiency virus controllers: Mechanisms of durable virus control in the absence of antiretroviral therapy. *Immunity* 27:406–416.
23. Marra F, et al. (1999) Monocyte chemotactic protein-1 as a chemoattractant for human hepatic stellate cells. *Hepatology* 29(1):140–148.
24. Mahmood S, et al. (2002) Clinical significance of intrahepatic interleukin-8 in chronic hepatitis C patients. *Hepatol Res* 24:413–419.
25. Heydtmann M, Adams DH (2009) Chemokines in the immunopathogenesis of hepatitis C infection. *Hepatology* 49:676–688.
26. Marra F, Aleffi S, Galastri S, Provenzano A (2009) Mononuclear cells in liver fibrosis. *Semin Immunopathol* 31:345–358.
27. Bataller R, Brenner DA (2005) Liver fibrosis. *J Clin Invest* 115:209–218.
28. Friedman SL (2008) Hepatic stellate cells: Protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 88(1):125–172.
29. Seki E, et al. (2009) CCR2 promotes hepatic fibrosis in mice. *Hepatology* 50(1):185–197.
30. Harada K, et al. (2011) Monocyte chemoattractant protein-1 derived from biliary innate immunity contributes to hepatic fibrogenesis. *J Clin Pathol* 64:660–665.
31. Farci P, et al. (1996) Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein. *Proc Natl Acad Sci USA* 93:15394–15399.
32. Mühlbauer M, et al. (2003) A novel MCP-1 gene polymorphism is associated with hepatic MCP-1 expression and severity of HCV-related liver disease. *Gastroenterology* 125:1085–1093.
33. Clément S, et al. (2010) The hepatitis C virus core protein indirectly induces alpha-smooth muscle actin expression in hepatic stellate cells via interleukin-8. *J Hepatol* 52:635–643.
34. Zeremski M, et al. (2008) Intrahepatic levels of CXCR3-associated chemokines correlate with liver inflammation and fibrosis in chronic hepatitis C. *Hepatology* 48:1440–1450.
35. Zeremski M, et al. (2009) Peripheral CXCR3-associated chemokines as biomarkers of fibrosis in chronic hepatitis C virus infection. *J Infect Dis* 200:1774–1780.
36. Mahmood S, et al. (2002) Clinical significance of intrahepatic interleukin-8 in chronic hepatitis C patients. *Hepatol Res* 24:413–419.
37. You CR, et al. (2011) Serum IP-10 levels correlate with the severity of liver histopathology in patients infected with genotype-1 HCV. *Gut Liver* 5:506–512.
38. Berres ML, et al. (2011) Serum chemokine CXC ligand 10 (CXCL10) predicts fibrosis progression after liver transplantation for hepatitis C infection. *Hepatology* 53:596–603.
39. Friedman BH, et al. (2012) Serum cytokine profiles associated with early allograft dysfunction in patients undergoing liver transplantation. *Liver Transpl* 18:166–176.
40. Horras J, Lamb CL, Mitchell KA (2011) Regulation of hepatocyte fate by interferon-γ. *Cytokine Growth Factor Rev* 22(1):35–43.
41. Bonilla N, et al. (2006) Interferon gamma-secreting HCV-specific CD8+ T cells in the liver of patients with chronic C hepatitis: Relation to liver fibrosis—ANRS HC EP07 study. *J Viral Hepat* 13:474–481.
42. von Hahn T, et al. (2007) Hepatitis C virus continuously escapes from neutralizing antibody and T-cell responses during chronic infection in vivo. *Gastroenterology* 132:667–678.
43. Wang XH, et al. (2007) Progression of fibrosis during chronic hepatitis C is associated with rapid virus evolution. *J Virol* 81:6513–6522.
44. Lemey P, et al. (2007) Synonymous substitution rates predict HIV disease progression as a result of underlying replication dynamics. *PLOS Comput Biol* 3:e29.