MAJOR ARTICLE

Impact of HIV on Host-Virus Interactions during Early Hepatitis C Virus Infection

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Background. Human immunodeficiency virus (HIV) may influence the outcome and natural history of hepatitis C virus (HCV) infection through an impact on acute HCV-specific T cell responses.

Methods. Fifty-five HIV-positive males with acute HCV infection were identified; monoinfected individuals ($n = 8$) were used for peripheral blood mononuclear cell comparison. In 14 coinfected and 8 HCV-monoinfected patients, HCV-specific T cell responses against a range of HCV antigens were assessed using interferon (IFN)–γ enzyme-linked immunospot (ELISpot) and proliferation assays. E1/E2 region genetic diversity and the selection pressure on the virus were measured in 8 coinfected patients by use of cloned sequences over time.

Results. HCV persisted in 52 (95%) coinfected individuals. HCV/HIV coinfection significantly reduced IFN-γ ELISpot responses versus those in HCV-monoinfected individuals, especially against nonstructural proteins (1/10 vs. 5/8; $P = .008$). In coinfected patients, increased HCV genetic diversity was observed between the first and subsequent time points, with no evidence for positive selection in the E1/E2 region sequenced.

Conclusion. HIV coinfection is associated with increased rates of HCV persistence and a lack of critical CD4 T cell responses, with no evidence of immune selection pressure during early HCV infection. Loss of key cellular immune responses against HCV during acute disease may contribute to the failure of early host control of HCV in HCV/HIV-coinfected patients.

The natural history of hepatitis C virus (HCV) in HIV-coinfected individuals is significantly different from HCV monoinfection. Studies among individuals with chronic HCV/HIV-coinfection have revealed important influences of HIV on HCV infection and possibly vice versa [1, 2]. After the introduction of highly active antiretroviral therapy (HAART) for HIV infection and the consequent reduction in HIV-related morbidity and mortality, HCV infection has emerged as an increasingly significant problem in this patient group. HCV/HIV coinfection is associated with accelerated hepatic fibrosis, resulting in increased liver-related morbidity and mortality [3–5]. In the developed world, end-stage liver disease is now a leading cause of death among HIV-infected individuals [6, 7].

Persistence is the most common outcome of acute HCV monoinfection [8]. There is consensus that a successful immune response to HCV requires a strong, broad, and sustained HCV-specific cell-mediated response [9–11]. Significantly, these HCV-specific T cell responses are persistent and have been detected in individuals up to 2 decades after the resolution of HCV infection [12]. It has also been demonstrated that genetic changes in HCV can inhibit CD4 and CD8 T cell recognition as well as promote the evasion of antibody responses [13]. During the acute phase, HCV diversity in hypervariable region (HVR) 1—a major target of the antiviral antibody response—has been linked with clinical outcome [14]. HIV has been associated with higher

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Table 1. Clinical parameters of the coinfected cohort in London and the Italian monoinfected patients analyzed in the natural history and immunological study.

Parameter	RFH cohort, coinfected			Immunological study patients	
	Total ($n = 55$)	Persistent ($n = 52$)	Self-limited ($n = 3$)	Coinfected, RFH ($n = 14$)	Monoinfected, Italian ($n = 8$)
Age, years	35 (24–51)	35 (24–51)	35 (31–38)	35 (24–42)	32 (24–76)
Male sex, no. (%)	55 (100)	52 (100)	3 (100)	14 (100)	5 (63)
Peak ALT level, IU/mL	389 (36–5104)	366 (36–3089)	2258 (1907–5104)	427 (74–5104)	1290 (354–1985)
HCV load, log IU/mL	6.0 (3.0–6.9)	5.9 (3.0–6.9)	6.4 (6.4–6.9)	6.3 (4.6–6.9)	5.3 (4.3–6.7)
Anti-HCV seroconversion, no. (%)	52 (95)	49 (94)	1 (33)	14 (100)	Not available
Non-1 genotype, no. (%)	11 (20)	10 (18)	10 (18)	4 (29)	2 (25)
Transmission factor	Permucosal	Permucosal	Permucosal	Permucosal	Parenteral
Outcome—resolved, no. (%)	3 (5)	0 (0)	3 (100)	2 (14)	3 (38)
Icteric, no. (%)	6 (10)	3 (6)	3 (100)	3 (21)	4 (50)
Length of HIV infection, years	4.6 (0–18.6)	4.5 (0–18.6)	5.8 (0–10.3)	7.1 (0–15.5)	. . .
CD4 cell count, cells/μL	554 (188–1705)	549 (188–1705)	847 (372–1283)	628 (266–1283)	. . .
HIV load, log copies/mL	4.6 (2.6–5.8)	4.6 (2.6–5.8)	5.2	4.4 (2.6–5.7)	. . .
HAART, no. (%)	35 (64)	33 (63)	2 (67)	9 (64)	. . .
Duration of infection, median, weeks	19	. . .	. . .	10	6

NOTE. Data are median (range) values, unless otherwise specified. ALT, alanine aminotransferase; HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; RFH, Royal Free Hospital.

rates of HCV persistence, which, it has been hypothesized, is related to its impact on cellular immune function [15]. Studies of chronic HCV/HIV coinfection have revealed impaired HCV-specific CD4 and CD8 T cell responses [16, 17], but little is known of the early—and critical—events of HCV infection in the presence of HIV.

Recognition of a marked increase in the transmission of HCV among HIV-positive men who have sex with men in London has provided an opportunity to study aspects of the early phase of HCV infection in HIV-positive individuals [18]. The aim of the present study was to describe the natural history of acute HCV in individuals with prior HIV infection, characterizing the early host-viral interactions by analyzing the cell-mediated responses and viral evolution during the acute phase of HCV infection in HIV-coinfected individuals. We addressed whether the poor clinical outcome of HCV/HIV coinfection is related to the early failure of host antiviral immunity.

METHODS

Clinical cohort. The cohort consisted of consecutive HIV-positive individuals diagnosed with acute HCV infection from a London HIV unit between 2000 and 2005. As previously reported, acute HCV infection was defined as documented seroconversion to anti-HCV antibodies or according to clinical and biochemical criteria (acute hepatitis in individuals without preexisting liver disease, excluding other infective, metabolic, toxic, and drug causes, and a serum alanine aminotransferase [ALT] level $\geqslant$10 times the upper limit of normal) along with positive HCV RNA by reverse-transcription polymerase chain reaction (RT-PCR) within 12 months [19]. The HIV unit routinely performed anti-HCV testing at HIV diagnosis and, since 2002, on an annual basis in all patients attending the clinic. Anti-HCV was detected using the Vitros ECI ELISA (Ortho-Clinical Diagnostics), and HCV RNA was quantified using the LCX HCV PCR assay (Abbott), which has a lower limit of detection of 23 IU/mL. HCV genotype was determined using the AutoLIPA assay (Bayer). HIV load was determined using the HIV RealTime assay (Abbott), which has a range of detection of 1.7–7 $\log_{10}$ copies/mL. The date of HCV diagnosis was defined as the first documented positive HCV RNA result, anti-HCV seroconversion, or peak ALT level followed by HCV viremia. Duration of HCV infection was estimated as either the midpoint between the last negative and first positive anti-HCV or HCV RNA result for asymptomatic infection or 6 weeks preceding clinical presentation. Using these estimated dates of infection, comparison of clinical parameters during the pre– and post–HCV-infection periods were made. Self-limited HCV infection was defined as the development of persistently negative PCR results for HCV RNA and normalization of liver function test results without HCV treatment. Persistent HCV infection was defined as HCV viremia after a 12-week observation period from diagnosis. Acute HCV monoinfection is rarely identified; however, peripheral blood mononuclear cells (PBMCs) were available from an Italian cohort of 8 sequentially recruited patients from Vicenza, and these were used as a control group for the immunological experiments.

Cell-mediated responses. PBMCs were collected from whole blood via density gradient centrifugation over Lym-

Table 2. Characteristics of coinfected and monoinfected individuals in immunological and virological studies.

Patient (age in years, sex)	Symptomatic (icteric)	HCV genotype	HCV load, log IU/mL	Peak ALT level, IU/mL	CD4 cell count, cells/μL	Receiving HAART at diagnosis	Outcome (clear)	Estimated duration of infection, weeks
Coinfected								
1 (42, M)	No	1	6.1	235	362	Yes	No	26
2 (34, M)	Yes	1	6.9	1504	821	Yes	No	6
3 (32, M)	No	1	4.6	2428	712	No	No	26
4 (35, M)	Yes	3a	6.4	5104	847	Yes	Yes	6
5 (30, M)	No	1	6.4	74	544	No	No	26
6 (31, M)	Yes	1	6.9	2258	1283	Yes	Yes	6
7 (31, M)	No	3a	6.3	389	437	Yes	No	26
8 (37, M)	No	1	6.0	349	530	Yes	No	16
9 (37, M)	No	1	5.2	498	266	Yes	No	6
10 (29, M)	No	1	6.3	272	844	No	No	6
11 (42, M)	No	3a	6.7	465	862	Yes	No	20
12 (24, M)	No	1	6.3	384	427	No	No	8
13 (39, M)	Yes	1	6.9	1415	337	Yes	No	6
14 (35, M)	No	1	5.8	89	966	Yes	No	12
15 (32, M)	No	1	6.0	412	740	No	No	6
16 (34, M)	No	1	6.9	2282	1008	Yes	No	6
Monoinfected								
1 (42, M)	Yes	1a	5.1	1139	. . .	. . .	Yes	6
2 (24, M)	Yes	1b	5.0	1290	. . .	. . .	Yes	6
3 (32, M)	Yes	3a	6.6	1985	. . .	. . .	No	14
4 (26, F)	No	1a	5.5	354	. . .	. . .	No	9
5 (62, M)	Yes	2	4.3	1725	. . .	. . .	Yes	8
6 (76, F)	No	1b	6.7	695	. . .	. . .	No	6
7 (33, M)	No	1b	Not known	1453	. . .	. . .	No	6
8 (25, F)	No	1a	Not known	1285	. . .	. . .	No	6

NOTE. ALT, alanine aminotransferase; F, female; HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; M, male.

phoprep (Nycomed) and cryopreserved. Peak ALT level was used in an attempt to compare PBMCs from similar time points in different individuals.

Enzyme-linked immunospot (ELISpot) assay. Frozen PBMCs were analyzed for interferon (IFN)–γ–producing cells by ELISpot assay (Mabtech), as described elsewhere [20]. Pooled HCV core–related peptides (20mers overlapping by 10) spanning aa 1–191 (10 μg/mL) and pooled recombinant proteins NS3, NS3/4, NS4, and NS5 (10 μg/mL) (Chiron) were added to separate wells in duplicate. Spots were counted on an ELISpot reader (ELISpot 3.1 SR program; AID Reader System). A test was considered positive if the probability of a spot appearing in the test well was significantly different ($P < .05$) from the probability of a spot appearing in the control well, assuming a Poisson distribution. Typical background was 0–5 spots. Quantification was then done by subtracting the mean background number of spots from the mean number of spots in the test well.

Carboxylfluorescein succinimidyl ester (CFSE) proliferation assay. PBMCs at a concentration of 1×10^7 cells/mL in PBS were incubated at 37°C for 7 min with 0.5 μmol/L CFSE (Molecular Probes). Staining was terminated by adding PBS containing 10% pooled human serum; cells were washed twice and resuspended in culture medium containing 10% human serum at 2×10^6 cells/mL. Stained cells (1×10^6 cells/well; 1 mL) were cultured in 48-well plates with medium alone, phytohemagglutinin as a positive control, core peptide pools 1–4 (10 μg/mL final concentration), and nonstructural proteins (NS3-5; 1 μg/mL final concentration; Chiron). After 6 days of culture, cells for each antigen were transferred into flow cytometry tubes, washed in PBS, and stained at 4°C with the following antibodies: anti–human CD4–allophycocyanin, CD8–phycoerythrin, and, to exclude dead cells, Viaprobe (7-AAD; BD Pharmingen). The number of cells that had proliferated was determined by gating on the lineage-positive $CFSE^{low}$ subset. After normalization for the cell input number, the stimulation index (SI; the ratio of responding cells with vs. without antigen) was calculated, and an $SI > 2$ was considered to represent a positive proliferative response, as defined elsewhere [21]. The CD4 T cell proliferative frequency (percentage of CD4 T cells that are $CFSE^{low}$ after background subtraction) was calculated only for those for which the SI was positive.

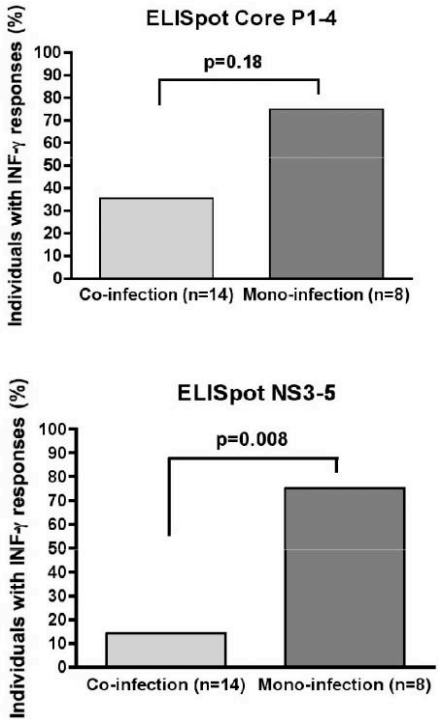

Figure 1. Comparison of interferon (IFN)–γ enzyme-linked immunospot responses in acute hepatitis C virus (HCV)–coinfected and HCV-monoinfected individuals. Shown is a comparison of the percentage of monoinfected and coinfected individuals with detectable IFN-γ responses to pooled core peptides and NS3-5 proteins, by Fisher's exact test.

Cloning and sequencing of the HCV E1/E2 region. Extraction of HCV RNA, RT-PCR, and nested PCR were performed with primers to amplify the E1/E2 section (including HVR1) of the HCV genome, using previously described methods [14, 18].

HCV evolution was explored by cloning the 560-bp E1/E2 (including HVR1) amplicon of the HCV genome (aa 318–503) at longitudinal time points. The E1/E2 amplicon was cloned into a pCR 4.0 TOPO vector (Invitrogen), and individual clones were sequenced. Sequencing was performed by the Sequencing Service (School of Life Sciences, University of Dundee, Scotland; http://www.dnaseq.co.uk), using an Applied Biosystems model 3730 automated capillary DNA sequencer.

E1/E2 sequence diversity and complexity of the within-patient viral population at each time point was determined. For each time point in each patient, the average genetic distance (GD) between each possible pair of sequences was calculated (mean pairwise GD; units are nucleotide changes per site). The model of sequence evolution most appropriate for each patient was selected using MODELTEST [22]. The HKY (Hasegawa, Kishino, and Yano) model was selected for patients 8, 10, 13, 15, and 16; the HKY+γ model was selected for patients 2 and 9; and the F81 (Felenstein 1981) model was selected for patient 12. GDs were calculated using PAUP [23]. The complexity of the HCV quasispecies was defined as the number of different viral sequences observed at each time point for each patient. The mean pairwise GD and complexity values measure the same property and will therefore be highly correlated.

The ratio of the rate of nonsynonymous evolution to the rate of synonymous evolution (d_N/d_S) was calculated for the sequences from each patient. This was done using the program CODEML, which implements the models of codon evolution described by Yang et al. [24]. In each case, a likelihood ratio test was performed to test the hypothesis that $d_N/d_S \leqslant 1$. Rejection of this null hypothesis provides statistically significant evidence for positive selection of the sequences.

Statistics. Clinical parameters within individuals were compared using paired *t* tests. Differences between groups were determined using Student's *t* test, χ^2 test, or nonparametric test (Mann-Whitney *U* test), as appropriate. Statistical analyses were performed using Prism V4 (Graphpad).

Ethical approval. The study was performed under the approval of the local research ethics committee (RFH 6148).

Table 3. Interferon-γ enzyme-linked immunospot responses for individual patients.

Patient	Core 1–4	NS3–5
Coinfected		
1	0	0
2	50	300
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	245	327
12	28	0
13	60	0
14	130	0
Median (range)	0 (0–245)	0 (0–323)
Monoinfected		
1	110	35
2	70	0
3	60	20
4	195	150
5	0	0
6	25	170
7	75	35
8	0	150
Median (range)	65 (0–195)	35 (0–170)

NOTE. Data are spot-forming cells per 1×10^6 peripheral blood mononuclear cells for each monoinfected or coinfected individual for pooled core peptides and NS3-5 proteins.

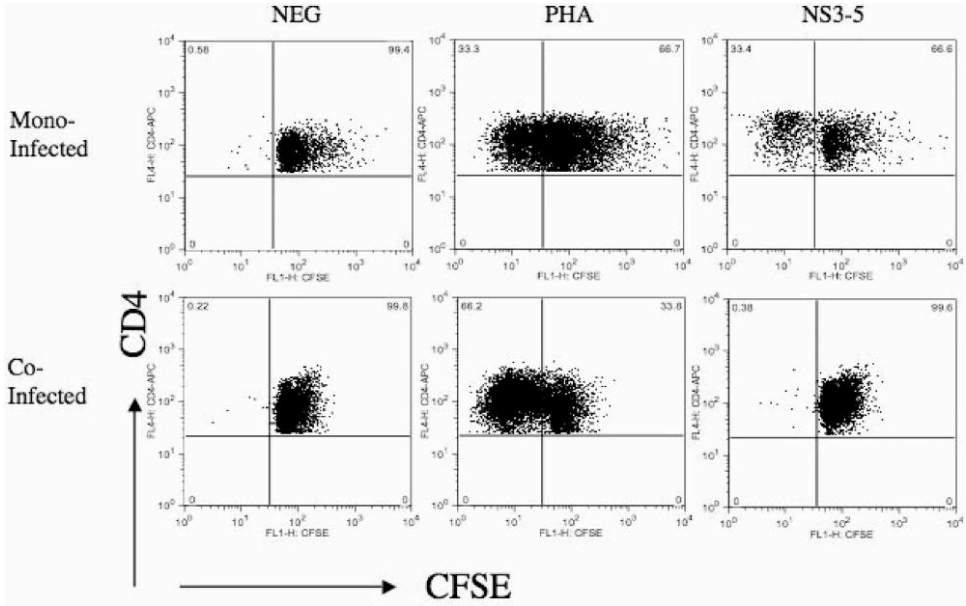

Figure 2. Examples of hepatitis C virus (HCV)–specific CD4 T cell proliferation. Proliferation of HCV-specific CD4 T cells is shown for monoinfected patient 1 *(upper row)* and coinfected patient 5 *(lower row).* Carboxylfluorescein succinimidyl ester (CFSE)–labeled peripheral blood mononuclear cells (PBMCs) on day 6 after stimulation with medium alone (NEG), phytohemagglutinin (PHA), or HCV NS3-5 proteins are shown. Undivided CD4 T cells are detected in the upper right quadrants of each flow cytometry plot, and the CFSE signal is diluted with each cell division as the dye is distributed to the daughter cells. Values in the upper left quadrants of each plot represent the percentage of CD4 T cells that proliferated during the 6-day culture. Cells are gated on CD4-positive and Viaprobe-negative cells.

RESULTS

Clinical natural history. Fifty-five HIV-positive individuals diagnosed with acute HCV infection were enrolled into the study between 2000 and 2005. Serum and/or PBMCs were collected for analysis from 16 coinfected individuals between diagnosis and 6 months after diagnosis. PBMCs were also available for comparison from an Italian cohort ($n = 8$) of acute HCV seroconvertors who were HIV seronegative. The patient parameters are outlined in table 1.

After the diagnosis of HCV infection, coinfected patients were monitored for 12 weeks to allow for spontaneous clearance before initiation of HCV combination pegylated IFN-α2 and ribavirin. The vast majority ($n = 52$; 95%) developed persistent HCV infection. Most individuals ($n = 43$; 78%) were eventually treated, with a median time to treatment of 14 weeks from diagnosis. The clinical parameters of the individuals who spontaneously cleared HCV and those who developed persistent infection are summarized in table 1. The 3 individuals who cleared HCV spontaneously were all jaundiced (3/3 vs. 3/52; $P < .001$) and had a higher median peak ALT level (2258 vs. 366 IU/mL) and CD4 cell count (847 vs. 549 cells/μL) than did those with persistence. The majority (90%) of the cohort were asymptomatic and were diagnosed as a result of the detection of abnormal liver function test results in routine clinical follow-up. Almost all ($n = 52$; 95%) of the coinfected patients seroconverted to anti-HCV during the course of their infection. In the monoinfected control subjects, the spontaneous clearance rate was significantly higher than that in the coinfected patients (37.5% vs. 5%; $P = .004$).

The impact of HCV infection on HIV was explored by comparing CD4 and CD8 T cell counts and HIV loads before and after infection with HCV, in those with available data. There were no significant changes in the median CD4 and CD8 cell counts in patients receiving HAART ($n = 24$) before and after HCV infection (for CD4 cells, 465 vs. 568 cells/μL [$P = .91$]; for CD8 cells, 1108 vs. 1073 cells/μL [$P = .46$]) and in those not receiving HAART ($n = 13$) before and after HCV infection (for CD4 cells, 518 vs. 560 cells/μL [$P = .44$]; for CD8 cells, 1195 vs. 1272 cells/μL [$P = .76$]). The HIV load in patients not receiving HAART ($n = 11$) did not change before and after HCV infection (4.6 vs. 4.9 $\log_{10}$ copies/mL; $P = .82$). There were 8 HIV "blips," defined as a detectable HIV load of between 50 and 400 copies/mL followed by a negative HIV load, in 8 of 18 patients receiving HAART between -103 days and 73 days from the date of HCV diagnosis. There were no reported HIV-related complications during the period after the diagnosis of HCV infection.

Cell-mediated responses. A total of 14 coinfected and 8 monoinfected patients had frozen PBMCs available for immunological analysis. A further 2 coinfected patients (patients 15 and 16) only had serum, which was analyzed for viral evolution. The clinical parameters of each group are outlined in table 1, and individual patient characteristics are shown in table 2. Compar-

Table 4. Longitudinal hepatitis C virus (HCV) evolution in coinfected patients during the early phase of infection.

Patient, time point	Peak ALT level, IU/mL	Days from peak ALT level	Closest HCV load, log IU/mL	Days from HCV load	No. of clones	Overall d_N/d_S	No. of HCV variants	Mean (SD) pairwise GD, substitutions/site	P[a]
2									
1	1504	−1	6.89	−2	10	0.27	5	0.002 (0.002)	
2		33	2.79[b]	−17	8		8	0.012 (0.005)	<.0001
3		61	2.79[b]	11	8		9	0.026 (0.034)	.04
8									
1	249	23	5.95[b]	14	9	3.21	5	0.003 (0.003)	
2		40	5.95[b]	31	10		3	0.002 (0.002)	.01
9									
1	498	−23	2.79	−35	6	0.19	1	0 (0)	
2		30	2.98[b]	−38	4		4	0.014 (0.006)	<.02[c]
3		76	2.98[b]	8	10		10	0.304 (0.272)	.01
10									
1	272	−42	6.34[b]	−105	11	0.32	10	0.02 (0.02)	
2		102	6.34[b]	39	10		9	0.005 (0.003)	<.0001
12									
1	384	−27	6.28	33	11	0.56	4	0.003 (0.003)	
2		103	5.83	−1	12		7	0.003 (0.003)	.29
13									
1	1415	0	6.89	−1	9	0.99	7	0.005 (0.003)	
2		52	5.46	2	7		5	0.009 (0.008)	.003
15									
1	412	48	6.0[b]	0	12	0.081	2	0.001 (0.001)	
2		101	6.0[b]	53	12		10	0.21 (0.19)	<.0001
16									
1	2282	−21	6.89[b]	−1	8	0.26	4	0.004 (0.005)	
2		0	6.89[b]	20	8		5	0.007 (0.007)	.02
3		52	2.56	8	10		9	0.41 (0.067)	.01

NOTE. ALT, alanine aminotransferase; GD, genetic distance; d_N/d_S, ratio of the rate of nonsynonymous evolution to the rate of synonymous evolution.

[a] For mean pairwise GD to previous time point.

[b] Same HCV load measurement for different time points within the same patient.

[c] Wilcoxon signed-rank test.

ison of the IFN-γ ELISpot responses revealed significant differences between the HIV-coinfected and HCV-monoinfected subjects. Fourteen coinfected individuals were compared with 8 monoinfected individuals at the first available time point. The median (range) time of collection of PBMCs was 17 (−5 to 129) days from the peak ALT level. Responses were analyzed against a pool of peptides spanning core and a pool of nonstructural (NS3-5) proteins, which have been demonstrated to elicit CD4 T cell responses in HCV-infected individuals [17, 20, 25]. Strikingly more monoinfected individuals had CD4 T cell IFN-γ responses to the NS3-5 proteins than coinfected individuals (75% vs. 14%; $P = .008$) (figure 1). Given the difference in estimated duration of infection between the cohorts, a subanalysis of HIV-positive individuals (8/14) with a median duration of infection (6 weeks) comparable to that of the HCV-monoinfected individuals was performed. The nonstructural protein responses remained significantly different—responses to core peptides were 50% versus 75% ($P = .61$) and to nonstructural proteins were 12.5% versus 75% ($P = .04$). There was no significant difference in the overall median magnitude of monoinfected individuals' and coinfected individuals' CD4 cell responses to pooled core peptides (65 vs. 0 spot-forming cells/1×10^6 PBMCs; $P = .13$). Combining results from both structural (core) and nonstructural antigens, 7 of 28 responses were detectable in the coinfected group, versus 12 of 16 in the monoinfected group ($P = .002$). Multispecific responses (to both core and nonstructural antigens) were seen in 5 of 8 monoinfected individuals and in 2 of 14 coinfected individuals ($P = .05$) (table 3).

CFSE proliferative assays were used to further analyze these responses in a subgroup of 11 coinfected and 6 monoinfected patients (numbers were limited because of cell availability). Figure 2 gives examples of the CFSE responses for a coinfected and monoinfected individual. These flow cytometry analyses confirmed that HCV-specific responder populations were CD4 T cells in all cases tested (data not shown)—a result validated in a separate study [26]—and confirmed a significant correlation be-

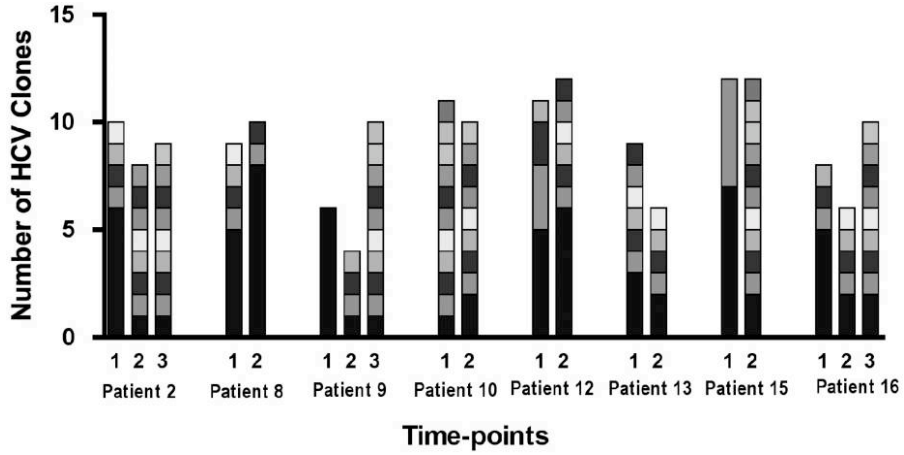

Figure 3. No. of hepatitis C virus (HCV) variants at each time point in each individual ($n = 8$). Each shade represents a different variant within that individual at that time point. The same shade does not represent the same variant between different time points or individuals.

tween the results among those tested by the 2 assays ($r = 0.4$; $P = .026$). A proliferative response to nonstructural proteins was observed in only 1 of 11 coinfected patients (patient 2, who also possessed a strong response to these antigens in the ELISpot assay), compared with 3 of 6 of those tested in the monoinfected group ($P = .1$). Subanalysis comparison between those with shorter-duration HCV infection among the coinfected patients (5/11) versus the 8 monoinfected individuals revealed the following: responses to core, 20% versus 17% ($P = 1$); and nonstructural proteins, 0% versus 50% ($P = .07$).

Combining the results, a response in ELISpot and/or proliferation assays was obtained in 3 of 11 in the coinfected group versus 5 of 6 in the monoinfected group ($P = .05$). Furthermore, looking at response breadth, a response in either assay to both antigens was observed in only 1 of 11 coinfected patients (coinfected patient 2), compared with 5 of 6 monoinfected patients ($P = .005$). These data indicate that CD4 T cell responses to HCV in the coinfected group were less readily detectable than those in the monoinfected group and, where present, were more narrowly directed.

HCV genome evolution and diversity. A total of 175 cloned sequences from 2–3 time points from 8 genotype 1–coinfected individuals were analyzed. The time points studied ranged from −42 to 103 days from the peak ALT level. The median number of clones per time point was 10, comparable to previous studies [14, 27].

The mean pairwise GD (diversity) and number of different viral sequences (complexity) through time in the patients are shown in table 4 and figure 3. The mean pairwise GD increased from 0.005 to 0.069 changes per site ($P = .08$) over the course of subsequent time points. Mean pairwise GD varied greatly, from 0 to 0.304 substitutions per site, and showed a significant increase between the first and subsequent time points in 5 of 8 patients. Two individuals showed a decrease in diversity over time. These changes did not correlate with peak ALT level, CD4 cell count, or HCV load (data not shown). Similarly, the number of different sequences observed at any one time point (complexity) also increased between the first time point and later time points in 5 of the 8 patients studied (figure 3 and table 4).

For each patient, d_N/d_S ratios were calculated. Seven of eight individuals had d_N/d_S ratios <1, implying a lack of positive selection pressure directed against this region in these patients over the time period of study (table 4).

DISCUSSION

HIV infection significantly affects the immune system's ability to control HCV replication. The majority (95%) of coinfected individuals in our study developed persistent HCV infection, which was a significantly higher proportion than that among the Italian monoinfected control subjects (62%) and reported historical control subjects (75%) [8, 19]. This strongly suggests that concurrent HIV infection favors HCV persistence. Furthermore, the median HCV load during the acute phase was higher by ~0.5–1 $\log_{10}$ IU/mL than that among both the Italian and German historical monoinfected control subjects [19], implying poorer virological control of HCV in HIV coinfection during the acute phase of infection. Our data suggest that HIV affects the cell-mediated responses to HCV during the acute phase, contributing to reduced clearance and control of HCV.

In chronic coinfection, HIV significantly impairs the cell-mediated responses to HCV antigens [17]. Although we have demonstrated that CD4 T cell responses to some HCV antigens are detectable during the early phase of infection in coinfected individuals, these were of reduced frequency and lacked the breadth and magnitude that is probably necessary for control of HCV, contributing to the higher rate of viral persistence and higher viral loads in this cohort. The CD4 responses of the coinfected individuals were most obviously defective against NS3-5

proteins. It has been shown that broad CD4 T cell responses, including those directed against the nonstructural proteins, are particularly important for the clearance of HCV [10]. Because of limitations in sampling, we were unable to map the responses down to individual proteins or peptide epitopes, to explore the issue of response breadth (e.g., within the NS3-5 proteins) in further detail.

Importantly—and not previously reported—our analysis reveals that the defect in cell-mediated immunity occurs very early during the course of HCV infection. Comparison of the acute coinfected cohort with a recently analyzed chronically coinfected hemophiliac cohort (analyzed using an identical ELIspot assay) revealed a higher proportion of the acute coinfected cohort had measurable CD4 T cell IFN-γ ELISpot responses, typically observed only against core peptides (35% vs. 7%) [17]. In HCV monoinfection, failure to develop a vigorous, multispecific, and persistent cell-mediated response is postulated to occur as a result of primary T cell failure and/or exhaustion, T cell dysfunction, and viral escape mutation, or a combination of these [28]. Recently, the importance of CD4 T cell responses in acute monoinfection has been shown, without which HCV-specific CD8 T cell and heterologous antibody responses may develop but fail to clear HCV [29]. HIV is likely to affect this at a number of levels, through alterations in CD4 T cell survival, antigen-presenting cell function, and/or disruption of lymphoid architecture.

Replication of HCV is rapid and error prone and has been shown to play an important role in the evasion of host immune responses [30]. However, the complexity of the HCV population (the number of viral variants) and its diversity (mean pairwise GD) during HCV/HIV coinfection has been studied only in the chronic state, and the data conflict, with evidence for both increased and decreased diversity [31–35].

We focused our sequence analysis on the envelope region, because the E1/E2 region (including HVR1) is the most variable region of the HCV genome [36]. Our longitudinal sequence analysis suggested a trend toward increased HCV diversity and complexity during the early postinfection period. However, for the same region, the d_N/d_S ratios remained <1, suggesting that the HCV evolution in this region was not driven by immune selection pressure but more likely was the result of viral mutation and random genetic drift. This is consistent with the lack of measurable cell-mediated immune responses. However, we note that an inability to reject $d_N/d_S \leqslant 1$ in some data sets could reflect low statistical power resulting from low sequence variability. Although we have used a different analysis, our results contrasts strongly with published data on monoinfection, for which there is clear evidence for selection in the same region [14, 37]. Furthermore, although previous data in coinfection have shown a significant humoral response against E1/E2 and an inverse correlation between antibody levels and HCV RNA load [38], our data also argue against a significant selective effect from the humoral immune response, although this was not formally investigated in our study.

A major concern in our study was the comparability between the HIV-infected and monoinfected cohorts. The lack of cell-mediated responses in HIV infection could have been due to the comparison of later-phase HCV infection in this group to earlier infection in the monoinfected group. However, a subanalysis of shorter-duration HCV infection in HIV-positive individuals did not change the significant reduction in responses to nonstructural proteins, strongly supporting the results of the overall comparison. Clinical comparisons need to be cautious as the Italian monoinfected cohort would appear to be more typical of previously described monoinfected groups; the higher clearance rates may represent a selection bias, because symptomatic individuals more likely to clear HCV would also present more frequently to medical care. In acute HCV monoinfection, it has been reported that males and asymptomatic patients (the majority of the HIV-positive cohort) were significantly less likely to spontaneously clear HCV [19].

The data presented here reveal that HIV coinfection has a significant early impact on the clinical, immunological, and virological outcome of acute HCV infection. Persistent HCV infection was the overwhelming outcome in this cohort. Although specific mechanisms were not elucidated, persistence probably relates to a failure of host-adaptive immune responses to HCV rather than viral escape. This defect occurs early during coinfection, emphasizing the importance of CD4 T cells in the control of HCV.

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