Full-Breadth Analysis of CD8⁺ T-Cell Responses in Acute Hepatitis C Virus Infection and Early Therapy

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Multispecific CD8⁺ T-cell responses are thought to be important for the control of acute hepatitis C virus (HCV) infection, but to date little information is actually available on the breadth of responses at early time points. Additionally, the influence of early therapy on these responses and their relationships to outcome are controversial. To investigate this issue, we performed comprehensive analysis of the breadth and frequencies of virus-specific CD8⁺ T-cell responses on the single epitope level in eight acutely infected individuals who were all started on early therapy. During the acute phase, responses against up to five peptides were identified. During therapy, CD8⁺ T-cell responses decreased rather than increased as virus was controlled, and no new specificities emerged. A sustained virological response following completion of treatment was independent of CD8⁺ T-cell responses, as well as CD4⁺ T-cell responses. Rapid recrudescence also occurred despite broad CD8⁺ T-cell responses. Importantly, in vivo suppression of CD3⁺ T cells using OKT3 in one subject did not result in recurrence of viremia. These data suggest that broad CD8⁺ T-cell responses alone may be insufficient to contain HCV replication, and also that early therapy is effective independent of such responses.

Hepatitis C virus (HCV) infection affects 170 million people worldwide and represents a major public health problem in many countries (24). After acute infection the majority of individuals develop chronic disease, which may result in hepatic failure and liver cancer (34). Persons who clear HCV infection spontaneously usually display vigorous and multispecific cellular immune responses (8, 14, 26, 37), but the mechanisms by which the virus evades immune responses in humans developing chronic infection remain unclear. CD8⁺ T cells are critical in antiviral defense (35), playing a crucial role in a number of acute and persistent virus infections, such as influenza virus (12), human immunodeficiency virus (HIV) (16, 30, 33), Epstein-Barr virus (EBV) (5, 6, 36), and cytomegalovirus (15). In the chimpanzee model strong and multispecific CD8 T-cell responses have been associated with spontaneous control of HCV (8), and the emergence of escape mutations has been associated with the development of viral persistence (11, 38). Studies addressing this important question in humans are limited, often to responses restricted by a single allele, such as HLA A2 (18, 25, 37, 39), and the critical relationship between the breadth and magnitude of CD8⁺ T-cell responses in the acute phase of infection and disease outcome remains to be defined.

Once chronic HCV infection is established, therapy with alpha interferon (IFN-α) and ribavirin leads to successful virological outcomes in about 50 to 75% of cases (13, 27), depending on viral genotype and pretreatment viral loads. Recent studies have suggested that therapy during the acute phase of infection is much more effective, with up to 95% of patients becoming virus-free after a relatively short course of alpha interferon monotherapy (19, 29). It has been suggested that cellular immune responses, which are strong in acute disease but much weaker in chronic infection, might be boosted through and/or synergize with antiviral therapy (19, 21, 40) and therefore mediate viral clearance and prevent relapse at the end of treatment. However, recent studies have been controversial in their findings (28) for both CD4⁺ (21, 32) and CD8⁺ (32, 41) T cells and their relationship to treatment outcome of acute HCV infection.

We present here a comprehensive analysis of T-cell responses in acute HCV infection in a group of patients who subsequently received early therapy. In contrast to the earlier studies noted above, we examined immune responses by multiple parameters and at an individual epitope level, using assays based on function as well as direct visualization of T cells.

MATERIALS AND METHODS

Study subjects. Eight subjects with acute HCV infection were recruited in Boston (A1 to A5, A7, and A8) and in Bochum, Germany (A6). Informed consent in writing was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval from the local institutional review boards. HCV

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seropositivity was defined as the confirmed presence of anti-HCV antibody (third-generation enzyme immunoassay). PCR positivity was defined as the detection of HCV RNA by PCR (detection limit, 600 HCV RNA IU/ml of plasma; version 2.0 Amplicor assay; Roche Diagnostics, Somerville, NJ). Diagnosis of acute HCV infection was based on seroconversion for HCV antibodies and the presence of HCV RNA by PCR. All subjects were negative for HIV antibodies and HBV surface antigen. Additional clinical information about the individuals and the treatments they received is given in Table 1.

**PBMC preparation.** Fresh peripheral blood mononuclear cells (PBMC) from all study subjects were obtained from centrifugation of blood (drawn into heparin or acid citrate dextrose tubes) over Ficoll solution (Sigma-Aldrich) and washed three times (5 min; 1,000 rpm; 25°C) in RPMI 1640 (Sigma-Aldrich) supplemented with 50 μl/ml penicillin, 2 mmol/literL-glutamine, and 50 μg/ml streptomycin. Cells were processed fresh or frozen and stored in liquid nitrogen.

**HCV-derived peptides and recombinant proteins.** Peptides corresponding to the amino acid sequence of the HCV-1a strain, spanning the entire HCV polyprotein, were synthesized as free acids using the 9-fluorenemethoxy carboxyl method. The 301 peptides used in the initial screening assay were 20 amino acids (aa) in length, overlapping adjacent peptides by 10 aa. The 83 optimal epitope peptides were 8 to 10 amino acids in length. Additional truncated peptides were synthesized to determine the optimal epitope sequence.

The recombinant HCV proteins used in this study were expressed as carboxy-terminal fusion proteins with human superoxide dismutase in *Saccharomyces cerevisiae* or *Escherichia coli* and were kindly provided by Michael Houghton (Chiron Corporation, Emeryville, Calif.). These proteins were derived from the HCV-1 sequence and encoded core (C22-3 aa 2 to 120), NS3 (C33C aa 1192 to 1395), NS5 (aa 2054 to 2995).

**HLA typing.** HLA typing was performed by the Tissue Typing Laboratory at the Churchill Hospital, Oxford, and the Massachusetts General Hospital Tissue Typing Laboratory using standard molecular techniques (4).

**ELISpot assay.** Polyvinylidine 96-well plates (Millipore, Billerica, MA) were coated with 2.5 μg/ml recombinant human anti-IFN-γ antibody (Endogen; Pierce Biotechnology, Rockford, IL) in phosphate-buffered saline (PBS) at 4°C overnight. Fresh or previously frozen PBMC were added at 200,000 cells/well in 140 μl R10 medium (RPMI 1640 [Sigma-Aldrich Corp., St. Louis, MO], 10% fetal calf serum [FCS; Sigma-Aldrich], and 10 mM HEPES buffer [Sigma-Aldrich] with 2 mM glutamine and antibiotics [50 U/ml penicillin-streptomycin]. Peptides were added directly to the wells at a final concentration of 10 μg/ml. The plates were incubated for 18 h at 37°C, 5% CO2. Plates were then washed, labeled with 0.25 μg/ml biotin-labeled anti-IFN-γ (Endogen), and developed by incubation with streptavidin-alkaline phosphatase (Bio-Rad Laboratories, Hercules, CA) followed by incubation in 5-bromo-4-chloro-3-indolylphosphate-nitroblue tetrazolium (Bio-Rad) in Tris buffer (pH 9.5). The reaction was stopped by washing with tap water, and the plates were dried prior to counting on an ELISpot reader (AID, Strassberg, Germany). For quantification of ex vivo responses, the assay was performed in duplicate, and background was not more than 15 spot-forming cells (SFC)/105 PBMC. Responses were considered significant if the number of spots per well minus the background was at least 25 SFC/105 PBMC (23). Phthyoethylglutamin served as a positive control for T-cell stimulation.

**HLA class I-peptide tetramer staining.** HLA class I-peptide tetramers were prepared as previously described (2) and included tetramers specific for three HCV epitopes restricted by HLA A2 and one HCV epitope restricted by HLA A1, HLA B8, and HLA B35, respectively (for HLA A2, NS3 peptide 1073-1081 [CINGWVCVT]; NS4 peptide 1406-1415 [KLYILGINAV]; and NS5B peptide 2594-2650 [ALYDVVTKL]; for HLA A1, NS3 peptide 1435-1443 [ATDLMTGY]; for HLA B8, NS3 1395-1367 [HSKKKCDDEL]; and for HLA B35, NS3 1359-1367 [PHNIEVEVL]). A total of 0.5 to 1 million PBMC were stained as described (26). Briefer, tetramer staining was performed for 20 min at 37°C. After washing for 5 min with PBS containing 1% FCS at room temperature, cells were pelleted and directly stained with CDs-peridinin chlorophyll protein fluorochrome (Becton Dickinson, Mountain View, CA) for 20 min at 4°C. Cells were then washed as described above and fixed using PBS–1% formaldehyde. Flow cytometric analysis was performed with a BD FACS Calibur, and data analysis was performed using the CellQuest (BD) software. Staining was considered positive if tetramer-positive cells formed a cluster distinct from the tetramer-negative CD8+ T-cell population and the frequency of tetramer-positive cells was greater than 0.02% of the total CD8+ population.

**Bulk stimulation of peripheral blood mononuclear cells.** In order to establish CD8+ T-cell lines, cryopreserved or fresh PBMC (4 × 10^6) to 10^7 cells in 1 ml R10 (RPMI 1640–10% FCS; Sigma-Aldrich) in 6-well U-bottom plates (Costar) in 200 μl of R10-HAB medium (RPMI 1640–10% human AB serum) and 10 mM HEPES buffer (Sigma-Aldrich) with 2 mM glutamine and antibiotics (penicillin-streptomycin; 50 U/ml Sigma-Aldrich) and the designated proteins in quadruplicate wells. After a 6-day incubation at 37°C and 5% CO2, wells were pulsed for 6 h with 1 μCi of [3H]thymidine (NEN, Perkin-Elmer, Boston, MA). Cells were then collected on filters, and the amount of incorporated radiolabel was measured with a beta counter. For the purposes of data interpretation, a stimulation index of 5 or more was considered significant.

**Statistical analysis.** Statistical analysis was performed using GraphPad Prism (GraphPad, San Diego, CA). All data were compared using the nonparametric Mann-Whitney test with a *P* value of <0.05 considered significant.

For patients with pretreatment HCV-specific T-cell responses, ELISpot and tetramer analysis data during antiviral therapy were fitted with a one-phase exponential decay curve to examine the decay dynamics of CD8 T-cell responses during therapy (*y = A·e^{-(kX) + B}; half-life = 0.693/k*).

**RESULTS**

Eight individuals (A1 to A8) with acute HCV infection followed by early treatment were studied. The clinical details of the cohort are presented in Table 1. Early therapy was initiated in all cases within 6 months (range, 5 to 18 weeks) of acute HCV infection and led to a sustained virological response in
four of eight (Table 1). One patient (A1) with a relapse after therapy achieved a sustained virological response after retreatment with pegylated interferon. In another subject (A7) a brief period in which the patient interrupted therapy occurred, but otherwise all completed a full initial course of therapy ranging from 16 to 44 weeks.

**CD8⁺ T-cell responses in acute infection.** In these persons, we performed a detailed assessment of the breadth of the pretreatment CD8⁺ T-cell responses using a comprehensive screening gamma interferon ELIspot (23), allowing us to define the epitopes targeted by each individual (Fig. 1a). In addition, for specific HLA alleles we retested samples with HLA class I-peptide tetramers once epitope-specific responses had been defined (Fig. 1b). The first available sample from each subject was evaluated using the full-genome screening ELIspot, as well as PBMC from additional later time points (Fig. 2). Five of eight individuals studied showed responses prior to or at the end of treatment was not related to the virological outcome (P = 0.29 and P = 0.41, respectively; Mann-Whitney test). Importantly, in individuals with multiple responses, all followed similar kinetics. In addition, no significant broadening of the T-cell response was observed when comprehensive screening was applied at time points during or after therapy. Gamma interferon ELIspot assay and tetramer stains showed very similar profiles over time (Fig. 2), indicating that the decline under therapy is not a result of decreased functionality but rather a true decline in effector frequencies. No additional HCV-specific T-cell responses were detected using tetramer analysis, arguing against...
FIG. 2. Clinical course and immune responses in subjects A1 to A8. (a and b) ALT values and HCV viral load were followed in all subjects (upper panels). Areas shaded in gray denote administration of antiviral treatment. The IFN-γ ELISpot assay employing the full set of overlapping peptides covering the whole HCV polyprotein was used to screen for CD8⁺ T-cell responses at multiple time points (arrows in middle graphs of panel a and in the lower graph in panel b). All peptides eliciting positive responses in the screening ELISpots were then tested for all available time points (middle graphs [a] or lower graphs [b]). For four subjects, we were able to test selected CD8⁺ T-cell responses also by using class I tetramers (A1 to A4) (lower graphs in panel a). Further details of the immune responses and clinical course in A1 are shown in Fig. 3, but data are also included here for comparison with the rest of the cohort. For subjects with HCV-specific pretreatment responses, we also determined the decay of the ELISpot and tetramer response by fitting with a one-phase exponential decay curve (c). Half-lives of responses are given in weeks.

The two episodes of therapy that A1 received were analyzed separately [A1(1) and A1(2)]. Since therapy was interrupted for A7, we only examined the responses in the second longer phase after interruption of treatment. Results were similar for ELISpot and tetramer results, with half-lives between ~3 and 34 weeks.
the presence of completely anergic T-cell populations that might be missed in the screening ELISPOT assay.

**CD8⁺ T-cell responses during viral rebound after treatment of acute HCV infection.** In four of the eight subjects (A1 to A3 and A8), virus rebounded after the end of the treatment course, and in subject A7 viremia also reemerged during a brief treatment interruption prompted by increasing side effects. Overall, in those subjects where virological recurrence occurred, a significant increase in T-cell responses compared to the end-of-treatment values was seen ($P = 0.025$; mean rise, 300 spots per million PBMC). In contrast, in the four persons with sustained virologic responses to treatment, no similar rise was seen after stopping therapy (Fig. 2).

A similar response was also seen using tetramers. For example, during viral relapse in subject A1, there was a dramatic flare of the HCV-specific T-cell response, with two responses expanding 20-fold compared to the end of treatment and cells targeting these two epitopes together totaling more than 5% of CD8⁺ T cells, shortly after the time of the rise in alanine aminotransferase (ALT) (Fig. 2). Thus, at this stage of infection virus-specific CD8⁺ T cells can maintain the capacity to reexpand in vivo in response to antigen exposure.

**Persistent treatment-induced clearance of HCV viremia despite immunosuppressive therapy.** The above-described kinetics of the T-cell responses suggest that treatment-induced clearance of acute HCV infection is not dependent upon augmentation of cellular immune responses and does not require persistent immune surveillance. We were given a unique opportunity to assess this further in subject A1 (Fig. 3). Shortly after the end of the initial treatment course, classified as an end-of-treatment virological response, viremia recurred, and this was accompanied by a strong flare in ALT. Therefore, treatment was reinitiated in week 40, using pegylated alpha interferon, and continued for a further 16 weeks, with successful control of virus. One week after cessation of interferon treatment, the patient received a living donor renal transplant.
As part of the immunosuppressive protocol, the patient was given prednisone and azathioprine, as well as a course of the anti-CD3 monoclonal antibody OKT3 (7), during the first post-operative week for early rejection. The patient has remained HCV PCR negative in blood since this time for a follow-up period of more than 20 months.

During the second course of treatment in this person, we observed the same phenomenon of declining HCV-specific CD8$^+$ T-cell responses after IFN-mediated viral control was achieved (Fig. 2c and 3). After treatment with the anti-CD3 antibody OKT3, which results in complete downregulation of the T-cell receptor of CD4$^+$ and CD8$^+$ T cells, rendering them immuno-incompetent (7), these HCV-specific T-cell responses completely disappeared for several weeks (and were also not expandable in vitro) and then reemerged, but only to levels just around the detection limit of the tetramer assay. However, after the OKT3 treatment had been terminated, they were nevertheless present and functional, as they were readily detectable after a single round of peptide stimulation in vitro (data not shown), indicating the recurrence of an expandable memory cell population. As a control, an HLA A2-restricted EBV-specific response was maintained during both treatment episodes and reemerged to previous levels following OKT3 therapy (data not shown). The lack of recrudescence of HCV viremia with OKT3 treatment provides additional evidence that HCV is not under immunological control by T cells when virus remains undetectable following treatment. It suggests that HCV can either be fully eradicated under therapy or remains under control through mechanisms other than the T-cell response.

**CD4$^+$ T-cell responses.** CD4 proliferative responses were also analyzed in all eight subjects (Fig. 4), with no significant responses detected prior to therapy. Even after initiation of antiviral therapy, vigorous and broadly directed proliferative responses were observed in only two of eight persons, in contrast to the uniform detection of these responses in persons who spontaneously control viremia long term (10, 14). Both subjects A1 and A7 developed responses against nonstructural HCV proteins; however, of the two, only subject A7 achieved a sustained virological response. In the other subjects only a modest or no significant CD4 proliferative response was detected.

In subject A1 we were able to closely follow the proliferative response during viral relapse, retreatment, and immunosuppressive therapy (Fig. 3). After induction of strong responses during the first course of therapy, proliferative responses were again undetectable during the early phase of relapse but then reemerged and stabilized during retreatment. Finally, the proliferative responses were completely lost during OKT3 therapy and the entire subsequent follow-up period.

**DISCUSSION**

The majority of persons with acute HCV infection develop chronic infection characterized by persistent viremia, while a minority of patients are able to spontaneously control viremia after acute infection, which is associated with strong virus-specific CD4$^+$ and CD8$^+$ T-cell responses (26, 37). Recently it has been shown that early treatment of acute HCV infection leads to resolution of viremia in the majority of persons studied thus far, but the potential role of virus-specific adaptive immune responses to this outcome is not clear. Here we performed a detailed analysis of T-cell responses to all expressed HCV proteins in persons with acute infection and on early therapy. Our results indicate that virus-specific CD8$^+$ T-cell responses uniformly decrease with successful treatment and increase transiently with recrudescence of viremia in those who fail to achieve a sustained virologic response. Moreover, the data indicate that when treatment is successful, it is not associated with persistent augmentation of immune responses but rather with sustained decline in CD8$^+$ T-cell responses. Since virus did not recrudesce even when T-cell responses were rendered immuno-incompetent by treatment with OKT3, the data also indicate that sustained immune control is not necessary for persistent clearance of plasma viremia.

This study was designed to examine cellular immune responses in much greater detail than previous studies, which have led to disparate conclusions. This included the use of HCV-peptide tetramers as well as simultaneous measurement of multiple epitope-specific CD8$^+$ T-cell responses and concurrent measurement of virus-specific CD4 T-cell responses. The study involved persons with acute infection who did not rapidly control viremia—it remains possible that some of the individuals may have controlled infection at a later time point without therapy, although in the absence of proliferative responses this...
would be unusual, as CD4 help has been shown to be critical for successful control of HCV (14, 17). In two subjects, CD8 T-cell responses were not detected at any time point, which opens the possibility that primary failure to generate HCV-specific T cells is one mechanism for HCV to persist. As a caveat, it is not feasible to test each subject with peptides matching the autologous HCV sequence, and so the possibility remains that responses were present but not detected in our assays. The possibility of missing responses by using genotype 1 reagents was greatest in subjects A5 and A6 with genotype 2 infections; however, in subject A5 we nevertheless detected responses against four class I epitopes. Sequencing of virus as it evolved in two patients (A2 and A3) has revealed evidence of immune escape during this acute period, as recently reported (38). Escape does not, however, appear to be uniform, as sequencing of the dominant epitopes in subject A1 did not reveal evolution of escape mutants over the study period (data not shown).

The cohort was heterogeneous in three important parameters: the time of treatment initiation, the treatment protocol, and the treatment outcome. However, despite this, strong similarities were seen in the impact of therapy—a decline in all cases where responses were previously present and no boosting or broadening of the response throughout the cohort. In addition, these findings were true in cases where therapy was successful and also unsuccessful. Indeed, in patient A1 the impact of therapy upon the immune responses was similar during the first (unsuccessful) and second (successful) treatment period. In this context, the unexpectedly high relapse rate in this study provided an important control group. The reasons for this low response rate in our cohort are not easily defined. However, two of the four subjects who relapsed (A1 and A8) were infected through blood transfusions with large inocula, which might establish a more robust infection early on and, hence, one more difficult to eradicate. In addition, two subjects with treatment failure were infected by the same virus (A3 and A8), raising the possibility that this infecting strain is especially resistant to therapy.

The decline of HCV-specific CD8 T-cell responses during therapy is likely to be due to the withdrawal of antigen and argues against treatment-induced immunological containment of ongoing viral replication. An alternative explanation would be that T cells redistribute to the liver during therapy and therefore decline in frequency in PBMC. However, the fact that strong T-cell responses are typically detected in PBMC of subjects after spontaneous resolution of HCV infection suggests that a successful immune response is typically not limited to the liver. A decline in responses during therapy is also observed during HIV infection, with a similar exponential decay, although in that situation an initial transient boost after the introduction of antiretroviral treatment may be seen (31). Treatment withdrawal through structured treatment interruption in HIV infection also leads to a rise in viral load followed by a boost in T-cell responses (1). In both cases, therefore, the CD8 T-cell responses left at the end of successful therapy have shrunk to a core of cells, which is believed to be less dependent on antigen for its maintenance. That these cells readily proliferate is demonstrated not only by the expansion of these cells during viral relapse, but also by the fact that we were able to expand HCV-specific T cells in vitro in all subjects.
with detectable responses during early infection, sometimes several years after the end of therapy (data not shown). The relationship between antigen load and CD8+ T-cell responses is not simple, however, in either infection. In HCV, even by utilizing comprehensive screening approaches, we have been unable to find a correlation (inverse or positive) between viral load and magnitude of the response in chronically infected patients (22, 42). Nevertheless, an important distinction between those who control virus spontaneously and those who do not is that responses tend to be stronger and broader in the...
long term in the absence of viremia (22). Thus, although in acute disease and in relapse from early therapy virus may drive the cytotoxic T-lymphocyte responses, these responses are clearly not maintained in the periphery during chronic disease. The case of subject A1 is especially informative as to the role of CD8+ and CD4+ T cells in vivo for maintaining long-term control. Treatment with the anti-CD3 antibody OKT3 was started within the first month after the cessation of the second interferon treatment. As a result, functional T cells were completely absent for at least 2 weeks and, as a consequence, HCV-specific CD4+ and CD8+ T-cell responses were completely abolished during that time. However, this was not associated with viral relapse. Together with the results analyzing the T-cell response under therapy, this demonstrates that therapy in acute disease does not boost antiviral CD8+ T-cell responses and, furthermore, that HCV-specific CD4+ and CD8+ T-cell responses are not required for long-term maintenance of an end-of-treatment response in this setting.

The effects of treatment on CD4+ T-cell responses have been studied mainly in those with chronic infection (3, 9, 20, 21), often detecting proliferative responses once virus was suppressed under therapy. Similarly here, CD4+ proliferative responses were not detected prior to therapy but emerged in three cases once virus was controlled. This may in part be due to direct effects of therapy (alpha interferon and ribavirin) and in part to reduction in viral load. We also detected a small number of HCV-specific CD4+ T-cell responses using the ex vivo ELIspot (data not shown). These showed the same kinetics as ex vivo CD8+ T-cell responses with declining frequencies during therapy, suggesting that neither CD4+ T-cell numbers nor the proliferative capacities of these cells were significantly boosted. As a previous study of chronic infection suggested (3), we did not find that emerging T helper cell proliferative responses were directly linked to a sustained virological response. Early therapy in subject A1 did indeed boost CD4+ proliferative responses, but it did so during both the first (unsuccessful) and second (successful) treatment. And while one subject mounting vigorous CD4+ proliferative responses (subject A7) indeed cleared HCV, other individuals achieved a sustained virological response despite weak proliferative responses. Furthermore, similar to the argument for CD8+ T cells, the subsequent OKT3 treatment also demonstrates that the CD4+ T-cell response is not absolutely required for long-term treatment success.

Recently, three studies have addressed the impact of early HCV therapy on antiviral immune responses, although with conflicting results ranging from treatment-induced augmentation of T-cell responses (21, 41) to blunting of responses (28). The present study demonstrates the decline in T-cell responses in treated acute HCV infection not only using functional assays but also using HCV-peptide tetramers to directly visualize effector cells, in order to rule out the possible presence of functionally altered cells. Overall, our more detailed study is most consistent with that of Rahman et al. (32) in that no clear linkage between immune responses and outcome was seen. Our data of the dynamics in subjects with a viral relapse add importantly to the results of a single subject with therapy failure reported in the study by Rahman and colleagues.

In summary, our results define firstly the breadth of the CD8+ T-cell response at early time points in human HCV infection. Whether breadth alone will distinguish between successful and unsuccessful immune responses was not addressed in this study and is currently under investigation in a larger group of individuals without early treatment. However, it does appear that viremia may be sustained, and virus may even recrudesce in the face of multispecific CD8+ T-cell responses—suggesting that broad responses alone may be insufficient to control HCV replication. For example, in the case of subject A1, the rapid reemergence of virus from undetectable levels in blood occurs despite the presence of a strong, multispecific, and apparently functional CD8+ T-cell response. Possibly, as shown in the lymphocytic choriomeningitis virus model of viral persistence in the mouse, failure of other arms of the immune response has knock-on effects leading to an overall failure of control. Nevertheless, it does appear that a successful treatment effect is achieved without broadening the cellular immune response, and without a long-term requirement for cellular immune control. Treatment of virus at a stage when its specific adaptation to the environment of an individual host is less complete (including possibly evasion of innate immunity and establishment of infection in niche cell populations) may potentially tip the balance in favor of more effective containment.

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