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Suppression of Host Immune Response by the Core Protein of Hepatitis C Virus: Possible Implications for Hepatitis C Virus Persistence

Mary Kathryn Large,* David J. Kittlesen,* and Young S. Hahn2*†

Hepatitis C virus (HCV) is a major human pathogen causing mild to severe liver disease worldwide. This positive strand RNA virus is remarkably efficient at establishing chronic infections. Although a high rate of genetic variability may facilitate viral escape and persistence in the face of Ag-specific immune responses, HCV may also encode proteins that facilitate evasion of immunological surveillance. To address the latter possibility, we examined the influence of specific HCV gene products on the host immune response to vaccinia virus in a murine model. Various vaccinia/HCV recombinants expressing different regions of the HCV polyprotein were used for i.p. inoculation of BALB/c mice. Surprisingly, a recombinant expressing the N-terminal half of the polyprotein (including the structural proteins, p7, NS2, and a portion of NS3; vHCV-S) led to a dose-dependent increase in mortality. Increased mortality was not observed for a recombinant expressing the majority of the nonstructural region or for a negative control virus expressing the β-galactosidase protein. Examination of T cell responses in these mice revealed a marked suppression of vaccinia-specific CTL responses and a depressed production of IFN-γ and IL-2. By using a series of vaccinia/HCV recombinants, we found that the HCV core protein was sufficient for immunosuppression, prolonged viremia, and increased mortality. These results suggest that the HCV core protein plays an important role in the establishment and maintenance of HCV infection by suppressing host immune responses, in particular the generation of virus-specific CTLs. The Journal of Immunology, 1999, 162: 931–938.
host immune response suppression by hcv core

Effects of specific HCV polypeptides on VV infection. The contributions of specific components of the immune response to VV infection in mice have been well defined, and recovery from VV infection has been demonstrated to be strongly correlated with the generation of a virus-specific CTL response (16, 17).

In the study described here, we examined the virulence of various VV/HCV recombinants in mice and studied the host response induced by recombinant viruses. Surprisingly, we found that a recombinant VV expressing the structural protein of HCV produced a lethal disseminated infection in mice and concomitantly suppressed the VV-specific CTL response and the production of proinflammatory cytokines as well. Using a series of VV recombinants expressing various C-terminally truncated polypeptides, this immunosuppressive effect was mapped to the core protein. These results suggest that expression of HCV core during HCV infection could account for the low frequency of CTLp observed in chronically infected patients and that HCV core may play a critical role in establishing and maintaining persistent HCV infection.

Materials and Methods

Cell lines

The P815 (H-2d) mastocytoma and BSC40 monkey kidney cell lines were maintained in DMEM supplemented with 10% (v/v) FBS and 1% (w/v) glutamine.

Plasmid constructions and generation of VV recombinants

VV/HCV recombinants are designated by the region of the HCV (H strain; genotype 1a) polypeptide sequence expressed. Two recombinants, vHCV-S and vHCV-NS, have been described previously (18, 19). For mapping studies, a nested set of C-terminal deletions was constructed using PCR to introduce a stop codon at or near the end of each individual protein. VV insertion vectors were either pTM3 (20) or pBRTM (19), and the HCV-specific portions amplified by PCR were verified by sequence analysis. The resulting plasmids were then used to rescue the corresponding VV/HCV recombinants by standard methods (21). The salient features of the six VV constructs used in this study are summarized in Fig. 1. The vHCV-S expresses HCV C, E1, E2, p7, NS2, and a portion of NS3. The vHCV-C/p7, vHCV-C/E2, vHCV-C/E1, and vHCV-C express the C to p7, C to E2, C to E1, and C proteins, respectively. The vHCV-NS expresses the majority of NS2 (which begins at residue 809) through the end of polyprotein (NS5B).

VV recombinants were rescued, plaque purified, and expanded in BSC40 cells. Various VV/HCV recombinants were no different in their viral titers when titrated in BSC40 cells. Their titers were determined by standard plaque assay using BSC40 cells. Various VV/HCV recombinants were no different in their viral growth rates (data not shown). The production of the expected HCV-specific proteins was verified by infection of BSC40 cells, radiolabeling, and immunoprecipitation with specific antisera (18, 19) (data not shown). The negative control VV recombinant expressing β-galactosidase, vSC11, has been described (22).

The expression of core protein in mice inoculated with vHCV-C or vHCV-S was confirmed by Western blot analysis in tissues obtained from VV recombinant-infected mice. The core protein was expressed in liver tissue from mice inoculated with vHCV-C and vHCV-S, but was not expressed in liver tissue from mice inoculated with a control VV recombinant (vSC11; data not shown).

Infection of mice and virus titration

Four- to six-week-old BALB/c mice were purchased from Taconic Farms (Germantown, NY). Mice were inoculated i.p. with various doses of the VV recombinants as indicated in the text and figure legends. On days 1–5 after virus inoculation, mice were sacrificed, and liver tissue was harvested to determine recombinant VV titers. Tissue samples were weighed, homogenized in Iscove’s DMEM containing 10% newborn calf serum, and centrifuged at 500 × g for 10 min, and pellets were resuspended in PBS containing 1% FCS and 1 mM MgCl2. Virus was released from the pellets by three freeze-thaw cycles followed by sonication.

To assay for plaque-forming virus, 200 μl of 10−1–10−3 dilutions were incubated with confluent BSC40 monolayers in six-well plates for 1 h at 37°C. Inocula were removed and replaced with DMEM containing 10 μg/ml penicillin/streptomycin, 10% FCS, and 2 mM L-glutamine followed by incubation for 2 days at 37°C. Monolayers were fixed with 7% formaldehyde and stained with crystal violet. Plaques were counted to determine the viral titer of each liver sample, and the viral titer was calculated as log plaque-forming units (pfu) per gram of tissue.

Measurement of primary VV-specific CTL responses

To determine the primary VV-specific CTL response, mice were infected i.p. with 5 × 105 pfu of a vHCV-S recombinant or control recombinant vHCV-NS. On day 5 postinoculation, mice were sacrificed, and spleens were harvested. A single cell suspension of splenocytes was prepared and purified through Isopaque-Ficoll. Cells were harvested from the layer between the Isopaque-Ficoll and medium, and washed twice with medium to remove the residual Ficoll. To measure VV-specific CTL responses, these purified splenocytes were tested using 31Cr-labeled P815 target cells previously infected with wild-type VV. The percentage of specific 31Cr release was determined by standard procedures (23). Values for 31Cr release are the mean of quadruplicate samples; SDs were typically <5%.

Quantitation of VV-specific primary CTL

To determine CTLp frequency by limiting dilution (24), three BALB/c mice per group were infected with 5 × 105 pfu of vHCV-S or vHCV-NS (as a control). At 5 days postinfection, spleen cells from infected mice were pooled and diluted to 5 × 105 to 4 × 106 cells/well. Diluted splenocytes were cultured with VV-infected syngeneic splenocytes as stimulators (103 cells/well). Cells in individual wells were harvested after 5 days, and CTL activity was measured on VV-infected P815 target cells. P815 (H-2d) target cells were infected for 1 h at 37°C with the wild-type VV and were labeled with 150 μCi of 31Cr for 2 h at 37°C. After washing to remove free label, target cells were incubated with VV-specific CTL effectors for 6 h at 37°C, as described previously (23, 25). Calculations using the Taswell method generate a frequency estimate (1/Γ), a corresponding 95% confidence interval, and the χ2 estimate of probability (p) for the frequency estimate. In these studies, p > 0.05 indicates that the frequency estimate is statistically acceptable.

Cytokine analysis of bulk cultures

Primary mixed lymphocyte cultures were prepared using spleens harvested on day 5 from mice infected with vHCV or vSC11 VV recombinants as described above. Splenocytes were restimulated with irradiated VV-infected splenocytes. Responder cells were cultured at 4 × 106 cells/ml with stimulators at a 5:1 responder:stimulator ratio in Iscove’s DMEM containing 10 μg/ml penicillin/streptomycin, 10% heat-inactivated FCS, 2 mM L-glutamine, and 0.05 mM 2-ME. Cultures were incubated at 37°C, and a portion of each culture (culture supernatant and cell pellet) was harvested.
After infection (Fig. 2A), while the vHCV-NS expresses the nonstructural proteins of HCV as the nonstructural protein, NS2, and a portion of the NS3 protein, virus press the HCV structural proteins, i.e., C, E1, E2, and p7, as well as half of the HCV genome (vHCV-NS). The vHCV-S virus expressing the HCV structural genes as a transgene, survived, showed no clinical signs of infection or mortality, their livers, and liver viral titers from these mice were below detectable levels by day 4 after infection. This latter virus is a rVV expressing the pSC11 recombination vector that is used for foreign gene insertion into the VV genome (see Materials and Methods). These findings suggested that expression of one or more HCV structural proteins dramatically increased the virulence of the normally avirulent VV.

To assess the relationship of viral inoculum size to disease severity and mortality, groups of eight mice were infected i.p. with varying doses of the structural protein expressing vHCV-S virus. Lethal infection was demonstrable in a virus dose-dependent fashion. All recipients of 10^8 pfu of vHCV-S virus died by day 5 postinfection, and recipients of 5 × 10^7 pfu of virus also succumbed to lethal infection with 100% mortality evident by day 8 of infection (Fig. 2B). In addition, mice receiving smaller viral inocula, i.e., 10^7 or 10^6 pfu, recovered from infection (Fig. 2B) and showed neither morbidity nor mortality up to 21 days postinfection (data not shown).

To determine the pathological changes associated with lethal infection of vHCV-S-inoculated mice, recipients of 10^8 pfu of VV-specific CTL were generated by a standard method. Two BALB/c mice (6–8 wk old) were infected with 10^7 pfu of recombinant virus (vSC11) encoding a β-galactosidase gene. Spleens were harvested from vSC11-infected mice and were stimulated in vitro with vSC11-infected and irradiated (2000 rad) naive splenocytes. The level of target cell lysis was determined by a standard 51Cr release assay using P815 (H-2d) as target cells. CTL assay was performed as previously described (23). The spontaneous release was <10%. The lysis of 51Cr-labeled VV-infected target cells by VV-specific CTL was inhibited by unlabeled VV-infected target cells (data not shown), suggesting that the VV-specific CTL activity on P815 targets is mediated by class I MHC-restricted CD8+ T cells.

**Results**

**HCV structural gene expression enhances viral virulence**

To evaluate the possible effect of expression of HCV gene products on the virulence of and the host response to VV in the mouse, we infected BALB/c (H-2d) mice i.p. with 10^7 pfu of a recombinant VV (rVV) expressing either the 5' half (vHCV-S) or the 3' half of the HCV genome (vHCV-NS). The vHCV-S virus expresses the HCV structural proteins, i.e., C, E1, E2, and p7, as well as the nonstructural proteins, NS2, and a portion of the NS3 protein, while the vHCV-NS expresses the nonstructural proteins of HCV exclusively (Fig. 1).

Mice infected with vHCV-S became clinically moribund by days 1–2 postinfection, and 90% of these mice died by 5–7 days after infection (Fig. 2A). By contrast, mice infected with 10^8 pfu of the HCV-NS virus, which expresses the HCV nonstructural protein, survived, showed no clinical signs of infection or mortality, and recovered from infection as efficiently as mice infected with the control vSC11 virus (Fig. 2A). This latter virus is a rVV expressing the pSC11 recombination vector that is used for foreign gene insertion into the VV genome (see Materials and Methods).

**HCV structural gene expression is associated with sustained VV replication in vivo**

In view of the necropsy findings described above, we next determined if the HCV structural proteins expressed by the vHCV-S virus were associated with sustained virus replication and elevated virus titers in vivo. Three mice per group were infected with 10^8 pfu of vHCV-S, vHCV-NS, or the control recombinant vSC11 virus. Recipient livers were harvested daily over 5 days, and virus titers in the infected livers were determined. As shown in Table I, after an initial burst of virus replication on day 1 postinfection, recipients of the control vSC11 virus and the vHCV-NS virus expressing the HCV nonstructural proteins rapidly cleared virus from their livers, and liver viral titers from these mice were below detectable levels by day 4 of infection. This finding is consistent with the uniform survival of mice infected with these viruses and suggests a vigorous host response to infection with these rVV.

By contrast, mice infected with the vHCV-S virus expressing the HCV structural proteins failed to clear virus and maintained high titers of infectious virus up to 5 days after inoculation, the time period when we observed the lethality during vHCV-S infection. These results are in agreement with our necropsy findings and...
strongly suggest that these recipients of vHCV-S died from overwhelming infection with this rVV. It is also noteworthy that the effect of HCV structural protein expression on virus replication and presumably the host response to infection was evident early in the infectious process, as virus titers in the livers of vHCV-S recipients were 10-fold higher than titers of vHCV-NS virus or the control VV on day 1 postinfection (Table I).

Suppression of the VV-specific CTL response by HCV protein(s)
The finding that expression of the HCV structural proteins by rVV resulted in the lack of viral clearance suggested that expression of one or more HCV structural proteins drastically inhibited the host response to viral infection. Since virus-specific CTL have been demonstrated to play a critical role in virus clearance and recovery from experimental viral infections, including VV infection (16, 17), it was of interest to determine whether the CTL response to primary VV infection was suppressed in mice infected with vHCV-S virus.

When mice were infected with 5 × 10^7 pfu of the vHCV-NS virus, their immune splenocytes, harvested on day 5 postinfection, exhibited significant cytolytic activity on VV-infected targets in a standard in vitro cytotoxicity assay (Fig. 3A). The magnitude of the in vivo primary VV-specific CTL response in vHCV-NS infected mice on day 5 postinfection was comparable to that in mice infected with the control VSC11 virus (data not shown). As expected, infectious virus was also not detectable in the livers of vHCV-NS-infected mice on day 5 postinfection (Fig. 3B).

By contrast, the in vivo primary CTL response to VV virus was markedly reduced (>5-fold) on day 5 postinfection in the spleens of mice infected with 5 × 10^7 pfu of the vHCV-S virus (Fig. 3A). A comparable inhibition of in vivo primary CTL activity was also observed on day 7 postinfection in vHCV-S-infected recipients that survived to day 7 (data not shown). Commensurate with the low levels of VV-specific CTL activity in the spleens of vHCV-S-infected recipients, the livers of vHCV-S-infected mice had high levels of infectious virus on day 5 postinfection (Fig. 3B). Liver virus titers were at least 1000-fold greater than those of the vHCV-NS-infected recipients on day 5 postinfection. These results suggest that the enhanced virulence of the vHCV-S virus may be due in part to the inhibition of a VV-specific CTL response in infected recipients through the action of one or more HCV structural proteins expressed in vHCV-S-infected cells.

To more precisely determine the degree of suppression of VV-specific CTL activity induced by the vHCV-S virus, we determined the frequency of CTLp in spleens of mice on day 5 postinfection with vHCV-S or vHCV-NS using limiting dilution analysis according to the methods of Taswell (4). We found that mice infected with the rVV expressing the HCV structural proteins had approximately a 10-fold lower frequency of CTLp on day 5 postinfection than recipients infected with virus expressing the nonstructural proteins (Fig. 4). The frequency of CTLp was 1.6 CTLp/10^9 immune splenocytes for the virulent vHCV-S virus and 12.5 CTLp/10^9 immune splenocytes for the avirulent vHCV-NS virus. This result is in keeping with the above findings on the magnitude of the in vivo primary CTL response to these two viruses.

Taken together, these results reinforce the view that the expression of one or more HCV structural proteins in infected cells in vivo leads to inhibition of the host response to viral infection, which is reflected in the suppression of the induction of virus-specific CTL. As discussed below, we also observed a marked inhibition of IFN-γ production by immune splenocytes from vHCV-S-infected mice in response to antigenic stimulation in vitro. Again, this finding suggests that one or more HCV gene products can profoundly alter the host response to VV infection.

Identification of HCV core protein as the gene product involved in immune modulation
Since the HCV-S virus expresses four putative HCV structural proteins, C, E1, E2, and p7, it was important to determine whether the immune suppression induced by infection with the vHCV-S virus was mediated by a specific HCV structural gene product. To approach this question, we constructed a panel of rVV expressing...
progressively larger deletions in the cDNA encoding the HCV structural proteins (see Fig. 1). To evaluate the impact of the incremental loss of HCV structural genes on the host response to infection, we evaluated the in vivo primary VV-specific CTL responses to each of these deletion mutants, to vHCV-S virus, and to the vHCV-NS virus on day 5 after infection. As shown in Fig. 5A, the immunosuppressive activity exhibited by the vHCV-S virus expressing the full complement of HCV structural proteins was also demonstrable in mice infected with a rVV expressing HCV residues 1–192, which encodes the full length of the HCV core protein exclusively. This result strongly suggests that the HCV core protein is the viral gene product that suppresses the host response to infection and enhances the virulence of VV. In agreement with this cytotoxicity data, we also found that mice infected with the rVV expressing only the HCV core protein (vHCV-C) had elevated virus titers in their livers on day 5 of infection (Fig. 5B) and succumbed to lethal infection (data not shown). Virus titers in vHCV-C-infected mice were comparable to those in mice infected with the vHCV-S virus expressing the full complement of HCV structural proteins.

Alteration of cytokine synthesis by HCV gene product(s)

As noted above, along with the assessment of in vivo primary CTL responses to infection, we also compared the in vitro production of IFN-γ and IL-2 in response to wild-type vaccinia-infected APCs of immune splenocytes taken on day 5 after infection with the vHCV-NS virus or the vHCV-S and vHCV-C viruses. IFN-γ production by immune splenocytes from mice infected with the core-expressing vHCV-C virus was profoundly suppressed (Fig. 6). The degree of inhibition of IFN-γ production in vitro was comparable to that observed in splenocytes of mice infected with the vHCV-S virus expressing the full complement of HCV structural proteins. Infection with these core-expressing rVV also resulted in a modest, but significant, inhibition of Ag-stimulated IL-2 production by immune splenocytes from vHCV-C- and vHCV-S-infected mice. Immune cells from mice infected with the vHCV-NS virus produced high levels of both IFN-γ and IL-2 (Fig. 6).

These results suggested that the HCV core protein was responsible for the diminished virus-specific CTL activity and IFN-γ production as well as the enhanced virulence observed in mice infected with the vHCV-S virus. Since activated CD8+ T lymphocytes effectors have been reported to produce high levels of IFN-γ in response to Ag stimulation (28–30), inhibition of the induction of virus-specific CTL by an HCV core-dependent suppressive mechanism would lead to decreased numbers of CTL effectors and diminished in vitro IFN-γ production in response to viral antigenic stimulation.

One potential mechanism by which HCV core could inhibit CTL induction is that HCV core may interfere with viral Ag presentation at a step along the MHC class I processing pathway in cells expressing core protein. To determine the impact of HCV core on viral Ag presentation by MHC class I molecules, we examined the capacity of VV-specific CD8+ CTL generated in response to infection with wild-type VV to lyse target cells displaying VV peptides after infection with the core-expressing vHCV-S, vHCV-C, or with the core-negative vHCV-NS virus. The VV-specific CTL do not lyse uninfected P815 target cells, and the effects of VV-specific CTL are MHC restricted. As shown in Fig. 7, core protein expression had no significant effect on the recognition of VV epitopes by VV-specific CTL. This result suggests that core protein does not disrupt Ag processing and/or presentation of viral Ag to CD8+ T lymphocytes as shown by adenovirus E3/19K protein (8). Therefore, the inhibitory effect of core on the
bulk cultures were assayed for the production of IFN-γ and IL-2 in mice expressing the HCV core protein. Two BALB/c mice per group were inoculated with $5 \times 10^3$ pfu of vHCV-S, vHCV-C, or vHCV-NS as a control. On day 5 after virus inoculation, splenocytes were harvested from two infected mice per group, pooled, and stimulated in vitro with VV-infected splenocytes from syngeneic mice. Supernatants from in vitro splenocyte bulk cultures were assayed for the production of IFN-γ and IL-2 by ELISA. Error bars indicate the SD of quadruplicate wells for cytotoxic assay. Experiments were repeated with twice with similar results. *, and #. Statistically significant difference between the indicated pairs ($p < 0.01$).

host response to viral infection is unlikely to be at the level of CD8$^+$ T lymphocyte recognition of virally infected APCs.

Discussion

In this report we examined the effect of expression of HCV gene products in a rVV on the in vivo virulence of this rVV and on the regulation of the host response to VV in a murine model. We found that the selective expression of the HCV structural proteins in rVV(vHCV-S) dramatically enhanced the virulence of an otherwise avirulent VV strain. This enhanced virulence led to an increase in mortality of mice infected with this VV recombinant and persistently elevated VV titers in primary targets of vaccinia replication in vivo, i.e., liver, spleen, and ovaries. We also observed that this enhanced virulence and the sustained elevation of virus titers in recipients of the vHCV-S were associated with a suppressed in vivo primary cytotoxic T lymphocyte response to VV Ags in these recipients. In addition, immune cells from the spleens of mice undergoing primary infection with this rVV (vHCV-S) produced markedly lower levels of IFN-γ in vitro in response to VV Ags than immunocytes from animals infected with a rVV expressing vHCV-NS. Cells from these animals also exhibited a modest, but significant, decrease in in vitro production of IL-2. Using a series of rVV expressing various deletions in the gene complex encoding the HCV structural proteins, we identified the HCV core protein as the structural gene product that both accounted for the suppression of the host response to infection and enhanced VV virulence.

Viruses have evolved a variety of mechanisms to ensure their replication and survival and to circumvent the host immune response (31). One of the best characterized mechanisms is the inhibition of CD8$^+$ CTL response to the virus by the ability of viral gene products to inhibit the processing of viral proteins and/or the presentation of viral peptide/MHC complexes on the surface of virus-infected target cells (32–34). Indeed, as noted above, expression of the HCV core protein does suppress CTL responses in this model system. However, we could not detect any effect of core protein on the presentation and recognition of processed viral Ag by activated virus-specific CD8$^+$ CTL effectors. Rather, our data from the analysis of the cytolytic activity of primary CD8$^+$ cytolytic effectors taken directly from infected animals as well as from dilution analysis of the frequency of activated CD8$^+$ T lymphocytes giving rise to cytolytic effectors argue for an effect of core protein primarily at the level of CD8$^+$ CTL induction. Therefore, it should be emphasized that one or more HCV gene product(s) may modulate the host response during natural HCV infection for the inhibition of viral Ag processing and presentation by MHC class I molecules. In our murine model, however, we have no evidence that the core protein enhances virulence and suppresses the host response by this mechanism.

Available evidence suggests that core protein has several functions in HCV replication, including viral RNA encapsidation into viral nucleocapsid (35, 36). Because of the localization of the core gene at the 5’ end of the HCV polyprotein transcript, the core protein is likely to be the first viral gene product produced in the virus-infected cells. If, as our data suggest, core protein expression may profoundly suppress the host response to virus infection, the effect of core on the host immune response would be evident in an earlier phase of virus infection before primary virus-specific CD8$^+$ T lymphocyte precursors give rise to CTL effectors. In this connection, it is noteworthy that mice infected with vHCV-C already demonstrate a 10-fold higher titer of virus in the liver as early as day 1 postinfection compared with mice comparably infected with control vaccinia (vSC11) or vHCV-NS (Table I). This finding reinforces the view that HCV core protein acts to suppress the host immune response at an early point in the process of viral infection.

Multiple mechanisms could account for the increase in viral titers observed within 24 h of infection. Both type 1 IFNs, α and β, have been shown to have important and nonredundant roles in the control of VV infection (37). NK cells are also significant to the early control of viral infections, and are greatly stimulated by IL-12 or IFN-α and -β. IL-12 produced by macrophages or NK cells synergize with TNF-α to stimulate the production of IFN-γ by NK cells. IL-12 also appears to be critical for the development of Th1 responses, which can, in turn, augment CTL responses. Therefore, a disruption in the expression or function of any of these cytokines could explain not only the increased early viral titers observed, but also the deficiencies in IL-2, IFN-γ, and CTL as shown here. Precedent for such a mechanism has been demonstrated with the inhibition of IL-12 synthesis by measles virus (38).
The induction of an effective CD8+ CTL response and the production of IFN-γ during infection have been implicated as important factors in resistance to as well as recovery from VV infection in the mouse (16, 17). Available evidence suggests that an effective CD8+ CTL response to virus infection can be mounted in the absence of either IFN-γ production or a functional IFN-γ-γR (27). Therefore, in the murine model of HCV core-mediated immune suppression described here, it is unlikely that core protein acts by inhibiting IFN-γ production early in infection and thereby suppresses an IFN-γ-dependent step in CD8+ T lymphocyte differentiation into activated effectors. Since CD8+ CTL effectors produce high levels of IFN-γ in response to Ag (28–30), it is more likely that the diminished IFN-γ production by immune splenocytes after infection with core expressing rVV reflects a core protein-dependent inhibition of activation of virus-specific CD8+ T cells into IFN-γ-producing CTL effectors. One intriguing mechanism that could account for both the enhanced replication of core expressing VV early in infection and the suppression of the virus-specific CTL response reported here is suggested by the recent report of binding of HCV core protein to the cytoplasmic tail of the human lymphotixin-β receptor (14, 15). If HCV core can directly interact with the intracellular signaling domains of one or more members of the murine TNF receptor family in infected cells and inhibit the function of these receptors, then this inhibitory effect on TNF receptor signaling might account for the suppression of both the early innate response to virus infection and the induction of a specific CTL response observed here.

Although we observed a profound effect of HCV core expression on IFN-γ production by immune splenocytes, the effect of core on IL-2 production was less dramatic. One possible explanation for this result is suggested by the recent finding by Dr. Charles Rice’s laboratory (unpublished observation) that HCV core can up-regulate transcription of the IL-2 promoter. It is not yet clear whether this activity reflects another strategy employed by HCV to dysregulate the host response, but it does point out the potential for this viral gene product to alter multiple steps in the host response to infection with HCV.

In the studies reported here, enhanced virulence of VV was used as a functional readout of HCV core protein-dependent suppression of the host response. Although rVV have been used by other investigators to evaluate the effect of expression of foreign genes on the immune response to VV infection, it was of concern that the immunosuppressive effect of core observed by us in this model system may reflect a suppressive effect dependent upon an interaction between HCV core and one or more VV gene products unique to this class of DNA viruses. Although this possibility cannot as yet be formally excluded, it appears unlikely, since we have been able to demonstrate that HCV core expression enhances the replication and persistence of Sindbis virus (a member of the positive strand RNA alphavirus) in vivo in mice when the core gene is expressed in a recombinant Sindbis virus (Y. S. Hahn, unpublished observation). This result adds support to our view that core protein may likewise have an immunomodulatory effect during HCV infection in humans.

One of the unique features of human HCV infection is the high incidence of development of persistent HCV infection (3). This fact strongly suggests that HCV has evolved one or more strategies to suppress the host response during acute infection and thereby facilitate the development of viral persistence. The results reported here provide compelling evidence that the expression of the HCV core protein may play a critical role in the establishment and possibly the maintenance of persistence during HCV infection in humans.

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References