Hepatitis C virus (HCV) is remarkably efficient at establishing persistent infection, suggesting that it has evolved one or more strategies aimed at evading the host immune response. T cell responses, including interferon-γ production, are severely suppressed in chronic HCV patients. The HCV core protein has been previously shown to circulate in the bloodstream of HCV-infected patients and inhibit host immunity through an interaction with gC1qR. To determine the role of the HCV core-gC1qR interaction in modulation of inflammatory cytokine production, we examined interleukin (IL)-12 production, which is critical for the induction of interferon-γ synthesis, in lipopolysaccharide-stimulated human monocyte/macrophages. We found that core protein binds the gC1qR displayed on the cell surface of monocyte/macrophages and inhibits the production of IL-12p70 upon lipopolysaccharide stimulation. This inhibition was found to be selective in that HCV core failed to affect the production of IL-6, IL-8, IL-1β, and tumor necrosis factor α. In addition, suppression of IL-12 production by core protein occurred at the transcriptional level by inhibition of IL-12p40 mRNA synthesis. Importantly, core-induced inhibition of IL-12p40 mRNA synthesis resulted from impaired activation of AP-1 rather than enhanced IL-10 production. These results suggest that the HCV core-gC1qR interaction may play a pivotal role in establishing persistent infection by dampening T_{H}1 responses.

HCV, a positive-stranded RNA virus of ~9.6 kb, is a serious and growing health threat, having already infected roughly 170 million people worldwide (1, 2). Transmission of HCV has been linked to a blood-borne route (i.e. blood transfusions, organ transplants, and IV drug use) (3). Following a 10–20-year subclinical phase, greater than 80% of patients progress to persistent HCV infection, a leading cause of chronic liver disease associated with the development of cirrhosis, hepatocellular carcinoma, and autoimmune disorders (3–9). The propensity of HCV to persist in the vast majority of infected individuals suggests that the virus has evolved one or more mechanisms of evading host immunity. Unfortunately, the current anti-HCV therapy, IFN-α treatment in conjunction with the nucleoside analog ribavirin, has proven ineffective, and attempts to generate a vaccine against viral proteins have been unsuccessful (10, 11).

The activation of virus-specific cytotoxic T lymphocytes (CTL) is critical for the elimination of virus-infected cells. Virus-specific CTL, both in the liver and periphery, are significantly diminished in chronic HCV patients as compared with those observed for other persistent viruses, such as hepatitis B virus and human immunodeficiency virus (12, 13). This apparent suppression of CTL responses suggests that HCV may interfere with CD4+ helper T cell activation and differentiation, thus allowing for the establishment of chronic infection. IL-12, which is known to regulate proinflammatory T helper (T_{H}1) responses by promoting IFN-γ production, provides an important link between innate and adaptive immunity. Patients chronically infected with HCV demonstrate a diminished capacity to up-regulate IL-12 and generate T_{H}1-type immunity (14–16). In addition to HCV, several other infectious organisms, including measles virus, human immunodeficiency virus, and Legionella pneumophila, prevent the establishment of a T_{H}1 environment by inhibiting IL-12 production (17–19). This suppression of T_{H}1 polarization by infectious organisms may represent a novel mechanism of host immune evasion. Although both lymphocytes and macrophages are targeted for infection by HCV, the rate of infectivity is relatively low (20, 21). Thus, it is unlikely that infection of these cells alone accounts for the immune dysregulation observed in chronic HCV patients, suggesting that the virus may utilize an indirect mechanism, such as the production of an immunomodulatory factor, to persist.

HCV core (21 kDa), which is the first protein to be produced upon viral infection, demonstrates multiple functions, affecting numerous operations within both the host and virus. In addition to forming the viral nucleocapsid, HCV core has been observed to modulate host cellular responses, including apoptosis and immunity (22–25). Studies on mice infected with a panel of recombinant vaccinia viruses expressing the various HCV proteins demonstrated the HCV core protein to be suffi-
fering with AP-1 activation. Importantly, HCV core-mediated inhibition of T cell responsiveness is dependent upon its interaction with gC1qR, suggesting that HCV might usurp gC1qR signaling to down-regulate host immune responses (3). Indeed, gC1qR has been shown to interact with several pathogen-derived proteins in addition to HCV core, including HIV-1 Rev, EBV EBNA-1, and Listeria monocytogenes internalin B, potentially providing these organisms with a novel mechanism of immune subversion (37–39).

Here we sought to determine whether the interaction between HCV core and gC1qR on the surface of monocyte/macrophages (M/Mφ) might suppress IL-12 production, a key cytokine for induction of IFN-γ and Th1 type CD4+ T cell responses. To address this possibility, we examined the effect of HCV core on the production of IL-12 by human M/Mφ stimulated with the toll-like receptor 4 (TLR4) ligand LPS, which is known to induce production of inflammatory cytokines, including IL-12. We demonstrated that HCV core can be bound to the surface of M/Mφ and that this binding involves an interaction with gC1qR. Further, we found that exogenous addition of HCV core to human PBMC stimulated with either LPS or SAC/anti-IL10 antibody results in suppression of both IL-12p70 and IFN-γ production. We subsequently demonstrated that M/Mφ are targeted for IL-12p70 inhibition by HCV core within the bulk PBMC population and that this suppression involves a diminished capacity to up-regulate IL-12p40 mRNA by interfering with AP-1 activation. Importantly, HCV core-mediated inhibition of IL-12p40 mRNA synthesis depends upon its interaction with gC1qR. These results indicate that the HCV core-gC1qR interaction may be significant in the establishment of chronic HCV infection through the suppression of Th1 responses, and, for the first time, report the role of gC1qR in immunomodulation of antigen presenting cell function. Therapies aimed at disrupting this interaction may prove invaluable in the prevention of HCV persistence.

**MATERIALS AND METHODS**

**Cell Cultures—**PBMC were isolated from whole blood or buffy coats of donors (Virginia Blood Services) by Ficoll-Hypaque gradient (Amerham Biosciences). The cells were washed twice and cultured in RPMI 1640 (Invitrogen) containing 10% (v/v) fetal bovine serum (HyClone Laboratories, Logan, UT), penicillin/streptomycin (100 µg/ml; Invitrogen), L-glutamine (2 mM), and 2-mercaptoethanol (55 µM; Invitrogen) at 37 °C with 5% CO2 in a humidified atmosphere. THP-1 cells (American Type Culture Collection, Manassas, VA), a human monocytic leukemia cell line, were cultured in RPMI 1640 containing 2 mM L-glutamine adjusted to contain 0.15% sodium bicarbonate, 0.45% glucose, 10 µM HEPES, 1.0 mM sodium pyruvate, 0.05 mM 2-mercaptoethanol, and 10% fetal bovine serum (Invitrogen) at 37 °C with 5% CO2 in a humidified atmosphere.

**Quantification of Cytokine Production by ELISA—**PBMC (2 × 10⁶ cells/well) were stimulated to produce IL-12p70 and/or IFN-γ by treatment with 100 ng/ml of Staphylococcus aureus Cowen strain (SAC; Sigma) and 10 µg/ml anti-IL-10 antibody (BD Pharmingen, San Diego, CA) in a round-bottomed, 96-well tissue culture plate for 24 h. Primary human M/Mφ (5 × 10⁶ cells/well) were isolated from the bulk PBMC by attachment to a 48-well, flat-bottomed polystyrene tissue culture plate for 20 min at 37 °C. Macrophage maturation of THP-1 cells was driven by incubation of 6 × 10⁶ cells in complete medium containing 1.2% Me2SO for 24 h at 37 °C. These isolated M/Mφ and matured THP-1 cells were presensitized with 100 ng/ml recombinant human (rh)IFN-γ (BD Pharmingen) for 16 h and were stimulated with 5 µg/ml LPS (Sigma) in the presence of varying doses of either β-gal-fused HCV core (Virogen, Watertown, MA) or β-gal control protein for 24 h at 37 °C. The supernatants were obtained to quantify the production of IL-12p70 or IFN-γ by ELISA (BD Pharmingen). For quantification of TNF-α, IL-1β, IL-6, IL-10, and IL-8, primary M/Mφ were activated as above, and cytokine levels were determined using a BD™ cytometric bead array multiplex assay (BD Biosciences), as per the manufacturer’s instructions.

**Flow Cytometric Analysis—**To determine core binding on the cell surface of M/Mφ, 1 × 10⁶ PBMC were incubated for 1 h at 37 °C in the presence or absence of His-tagged HCV core (80 µg/ml). His-purified tagged HCV core was expressed as six histidine residues located at the N terminus of the full-length HCV core protein (amino acids 2–191) and was purified under native conditions as described previously (32). The cells were exposed to fluorescein isothiocyanate labeled anti-Core (Affinity Bioreagents, Inc., Golden, CO) monoclonal antibody or anti-gC1qR polyclonal antibody, prebleed control sera (1:10 dilution; QED Bioscience, San Diego, CA), or soluble His-gC1qR or His-HDFR (prepared in our laboratory) were co-incubated with His-gC1q core and PBMC. Core binding was assessed by flow cytometry as described above.

**Determination of Cytokine mRNA Levels—**RT-PCR was employed to determine the levels of IL-12p40, IL-10, and β-actin mRNA. RNA was isolated using TRIzol (Invitrogen) according to the manufacturer’s instructions. 1 µg of RNA was treated with DNase to digest genomic DNA. 0.27 µg of RNA was then reverse transcribed using murine leukemia virus reverse transcriptase under conditions of 10 min at room temperature, 55 °C for 90 min, 95 °C, and 15–20 µl of cDNA generated in the RT reaction were added to the PCR. PCR was carried out using the following primer pairs: 1) IL-12p40 sense 5′-CAGCAATTTGTTGTCATCTTCTG-3′; antisense 5′-GACGCAGGAAAGCGTACAG-3′; 2) IL-10 sense 5′-ATGCCCAAGTCTGAACCAAC-3′; antisense 5′-GACGTTCAAGGGGCTGCTGTC-3′; 3) β-actin sense 5′-GGCCGAATCTGGCTGATCCACATCT-3′; antisense 5′-GACGCCCGGTTATCAGCATG-3′; 4) GAPDH sense 5′-GGCGGAATATGCTGCTGATCC-3′; antisense 5′-CGGTGTCCTCCGGTACACCT-3′ for 35 cycles of 95 °C for 40 s, 60 °C for 20 s, 72 °C for 40 s, followed by 10 min of incubation at 72 °C.

**Nuclear Extracts and Electrophoretic Mobility Shift Assays—**The nuclear extracts were isolated from suspensions of cells on ice or by lysis in HEPES, pH 7.9, 0.5 M EDTA, 200 µM dithiothreitol, 0.1 M phenylmethylsulfonyl fluoride, complete protease inhibitor mixture (Roche Applied Science), 2.5 µl of Nonidet P-40/ml for 15 min on ice. The cells were centrifuged for 10 min at 800 × g and 4 °C. Supernatant was removed.
and stored as cytoplasmic fraction. The nuclear extracts were prepared by resuspending nuclei in extraction buffer containing 0.1 M NaCl, incubating on ice for 15 min, and centrifuging for 15 min at 16,000 × g and 4 °C. 20% glycerol was added to stabilize extracts, and extracts were mixed with 32P-labeled probes for either NF-κB, 5′-ACTTCTTGGAAATTCCCGAGAGG-3′, AP-1, consensus binding motif 5′-TGACCTCA-3′ (Santa Cruz Biotechnology), or NF-IL-6, 5′-TGACTCA-3′. The mixtures were run on a 4% acrylamide gel, which was prerun for 20 min at 200V. Supernvith was conducted using antibodies specific for the p65 or p50 subunits of NF-κB (Santa Cruz Biologicals). To control for nonspecific binding of the probe, binding was also carried out in the presence of a 10× unlabeled competitor. The gel was dried and exposed to Kodak X-OMAT AR film at −80 °C.

RESULTS

HCV Core Binds the Surface of Human Monocytes/Macrophages in a gC1qR-dependent Manner—We previously demonstrated that HCV core interaction with gC1qR results in suppression of IFN-γ production and T cell proliferation in a mixed lymphocyte reaction (3, 32). To determine whether the HCV core-gC1qR interaction alters the production of inflammatory cytokines (i.e. IL-12) and inhibits IFN-γ production, we first examined the ability of HCV core to bind gC1qR displayed on the cell surface of primary human monocytes/macrophages (M/Mφ). As shown in Fig. 1a, 94.3% of primary M/Mφ were positive for the expression of cell surface gC1qR by flow cytometric analysis. Additionally, when cells were incubated with recombinant His6-gC1qR, 64.0% of primary M/Mφ displayed core protein bound on their surface (Fig. 1b). To assess the role of gC1qR in the association of core with M/Mφ, we determined the ability of α-gC1qR antibody to prevent core binding on the cell surface of M/Mφ. In the presence of specific α-gC1qR antibody, but not prebleed control sera, the percentage of core-bound M/Mφ was reduced by 54%, whereas the mean intensity of staining decreased 68% (Fig. 1c). Likewise, we examined the ability of soluble recombinant gC1qR to antagonize core association with M/Mφ. Excess His6-gC1qR (molar ratio of 6× core) reduced the frequency as well as intensity of core staining in M/Mφ by 42 and 59%, respectively (Fig. 1d). In contrast, an equivalent excess amount of His6-DHFR control protein did not affect core binding to M/Mφ. These results suggest that gC1qR is as a receptor for binding HCV core on the cell surface of M/Mφ.

HCV Core Inhibits IL-12p70 and IFN-γ Production by Activated PBMC—IL-12p70, the biologically functional form of IL-
HCV Core Suppresses IL-12 Synthesis in Human Macrophages

**Fig. 2.** HCV core inhibits IL-12p70 and IFN-γ production by activated PBMC. PBMC (5 × 10^5 cells/well) were plated on a 96-well tissue culture plate and stimulated by 0.006% (v/v) SAC/anti-IL-10 antibody (10 μg/ml) for 24 h at 37 °C in the presence of various dose (25, 50, and 100 nM) of either β-gal core (open circles) or β-gal (closed circles). The supernatants were harvested, and the IL-12p70 (a) and IFN-γ (b) concentrations were determined by ELISA. *, values below the level of detection limit are defined as zero. These results were found to be reproducible in three independent experiments.

**Fig. 3.** HCV core suppresses IL-12p70 production by activated M/Mφ. a, purified human M/Mφ (5 × 10^5 cells/well) were presensitized with 100 ng/ml rh-IFN-γ for 16 h at 37 °C. LPS (5 μg/ml) was added for 24 h at 37 °C in the presence of various doses (12.5, 25, 50, and 100 nM) of β-gal core (open circles) or a β-gal control protein (closed circles). The supernatants were harvested, and IL-12p70 production was determined by ELISA. b, THP-1 (6 × 10^5 cells/well) were plated on a 96-well tissue culture plate for 24 h at 37 °C in medium containing 1.2% MeSO. MeSO-containing medium was aspirated, and medium containing 100 ng/ml rh-IFN-γ was added for 16 h at 37 °C. LPS (5 μg/ml) was added, and IL-12p70 concentration was determined as above. These results were reproducible in three independent experiments.

HCV Core Selectively Inhibits IL-12 Production by Activated Macrophages—Macrophages and dendritic cells are the major sources of IL-12 produced during an inflammatory response (42). Thus, the observed suppression of IL-12p70 by HCV core might be due to a direct interference with macrophage/dendritic cells activation by HCV core. To examine whether HCV core affects macrophage-derived IL-12p70 production, primary M/Mφ were isolated from the bulk PBMC by adherence to polystyrene. Because LPS is a potent inducer of inflammatory cytokine production including IL-12, isolated M/Mφ were activated by LPS in the presence of varying doses of either β-gal core or β-gal. As shown in Fig. 3a, HCV core inhibits IL-12 production by activated M/Mφ in a dose-dependent manner. A 4-fold decrease in IL-12p70 was detected in cells treated with 100 nM core. In contrast, control protein β-gal fails to elicit the inhibitory effect on IL-12p70 production. The HCV core-mediated inhibition of IL-12 production was reproducible in primary M/Mφ derived from 10 different blood donors (data not shown). These results suggest that HCV core interferes with macrophage activation and is able to inhibit IL-12 production. In addition, HCV core also suppressed IL-12p70 production in LPS- or CD40L-stimulated human dendritic cells (data not shown).

To confirm the ability of HCV core to inhibit IL-12 production by primary M/Mφ, we examined the ability of HCV core to
HCV Core Suppresses IL-12 Synthesis in Human Macrophages

**Fig. 4.** HCV core does not affect production of TNF-α, IL-6, IL-1β, and IL-8. Purified human M/Mφ (5 × 10⁶ cells/well) were stimulated with LPS (5 μg/ml) for 24 h at 37°C in the presence or absence of various concentrations of β-gal core after IFN-γ presensitization. The supernatants were obtained, and cytokine production was determined using a cytometric bead array as per the manufacturer’s instructions. These results were reproducible in two independent experiments.

| Concentration (nM) | TNF-α (ng/ml) | IL-6 (ng/ml) | IL-1β (ng/ml) | IL-8 (ng/ml) |
|---------------------|---------------|--------------|---------------|--------------|
| 0                   | 40            | 40           | 25            | 25           |
| 1                   | 35            | 35           | 20            | 20           |
| 10                  | 30            | 30           | 15            | 15           |
| 100                 | 25            | 25           | 10            | 10           |

HCV Core-induced Inhibition of IL-12 Production Is Not Mediated by Overproduction of IL-10—IL-10 is responsible for the negative regulation of IL-12 (40). Thus, IL-10 is a crucial factor for maintenance of a balance between effective resistance against pathogens and detrimental systemic inflammation. To determine whether HCV core-gC1qR engagement affects the production of other cytokines, we examined the effect of HCV core on secretion of TNF-α, IL-6, IL-1β, and IL-8 by LPS-stimulated primary M/Mφ using a cytometric bead array. As shown in Fig. 4, HCV core elicited no significant effect on production of TNF-α, IL-6, IL-1β, or IL-8 in LPS-stimulated primary M/Mφ. This suggests that the ligation of gC1qR by HCV core selectively inhibits IL-12p70 production in macrophages in response to LPS stimulation.

HCV Core-mediated Inhibition of IL-12 Production Occurs at the Level of IL-12p40 Gene Transcription in a gC1qR-dependent Manner—The biologically functional IL-12p70 molecule is a heterodimer comprised of a p40 and p35 subunit, respectively. Purified human M/Mφ heterodimer comprised of a p40 and p35 subunit, respectively.

The biologically functional IL-12p70 molecule is a heterodimer comprised of a p40 and p35 subunit, respectively.

affect IL-12p70 production by the human monocytic leukemia cell line, THP-1. As shown in Fig. 3b, HCV core elicited a similar dose-dependent inhibition of IL-12p70 production by LPS-stimulated THP-1 (10-fold decrease in IL-12p70 at 100 nM core) as observed in primary M/Mφ. It is notable that the amount of IL-12p70 produced by activated THP-1 in this experiment was ~10-fold greater than that observed for primary M/Mφ. In contrast, the β-gal control protein did not affect IL-12p70 production by these cells.

In addition to IL-12, macrophages also produce other inflammatory cytokines including TNF-α, IL-6, IL-1β, and IL-8 followed by the binding of LPS on TLR4 (43). To determine whether HCV core-gC1qR engagement affects the production of other cytokines, we examined the effect of HCV core on secretion of TNF-α, IL-6, IL-1β, and IL-8 by LPS-stimulated primary M/Mφ using a cytometric bead array. As shown in Fig. 4, HCV core elicited no significant effect on production of TNF-α, IL-6, IL-1β, or IL-8 in LPS-stimulated primary M/Mφ. This suggests that the ligation of gC1qR by HCV core selectively inhibits IL-12p70 production in macrophages in response to LPS stimulation.

HCV Core-induced Inhibition of IL-12 Production Is Not Mediated by Overproduction of IL-10—IL-10 is responsible for the negative regulation of IL-12 (40). Thus, IL-10 is a crucial factor for maintenance of a balance between effective resistance against pathogens and detrimental systemic inflammation. To determine whether enhanced IL-10 production is involved in HCV core-mediated inhibition of IL-12, we examined the effect of core on the expression of IL-10 by primary M/Mφ.

As shown in Fig. 5a, secretion of IL-10 from LPS-activated primary M/Mφ in the presence of β-gal core is not enhanced, and in fact, expression levels appear to be slightly decreased by high core concentrations (100 nM). Similarly, treatment of THP-1 cells with β-gal core did not enhance but rather antagonized LPS-activated IL-10 mRNA synthesis (Fig. 5b). These results indicate that suppression of IL-12 by HCV core is not mediated by enhanced expression of IL-10. This is also consistent with results demonstrating that core-mediated suppression of IL-12p70 production occurs in IL-10-depleted culture by treatment of anti-IL-10 antibody as shown in Fig. 2.

HCV Core-mediated Inhibition of IL-12 Production Occurs at the Level of IL-12p40 Gene Transcription in a gC1qR-dependent Manner—The biologically functional IL-12p70 molecule is a heterodimer comprised of a p40 and p35 subunit, respectively. IL-12p40 has been shown to be the regulatory subunit of the p70 heterodimer within macrophage populations (40). Thus, the observed inhibition of IL-12p70 by HCV core might be due to suppression of IL-12p40 mRNA synthesis. To test this possibility, we examined the effect of HCV core on IL-12p40 mRNA synthesis in LPS-stimulated primary M/Mφ by RT-PCR. As shown in Fig. 6a, HCV core significantly inhibits LPS-induced synthesis of IL-12p40 mRNA but not the expression of β-actin. These results suggest that HCV core-induced suppression of
IL-12p70 production occurs at the level of inhibiting IL-12p40 mRNA synthesis. Also it suggests that HCV core-gC1qR engagement may inhibit one or more transcription factors critical for the induction of IL-12p40 mRNA synthesis.

We next sought to determine whether the observed core-induced suppression of IL-12p40 mRNA synthesis in activated M/M6 is gC1qR-dependent. As shown in Fig. 6b, LPS stimulation of THP-1 resulted in strong induction of IL-12p40 mRNA, which was significantly diminished upon the addition of HCV core. Importantly, co-incubation of these core-treated cells with α-gC1qR antibody was able to reverse the core-induced suppression of IL-12p40 mRNA synthesis. In contrast, treatment of prebleed control sera was unable to reverse HCV core-mediated inhibition of IL-12p40 mRNA synthesis. These results suggest that HCV core might usurp gC1qR signaling to influence the LPS-induced inflammatory response. In the absence of LPS, neither prebleed nor α-gC1qR antibody treatment possesses agonistic effect to induce IL-12p40 mRNA synthesis (data not shown).

HCV Core Blocks AP-1 Activation and Delays the Early Activation of NF-κB—The promoter region of IL-12p40 has been well characterized, with several known mechanisms of transcriptional regulation resulting from TLR4 signaling (42, 44–47). AP-1 is known to play a critical role in regulating IL-12p40 gene transcription. In addition to AP-1, NF-κB is involved in transcriptional regulation of IL-12p40, whereas the absolute necessity for NF-κB in IL-12p40 expression remains to be elucidated (44–47). Recent studies indicate two temporally separable NF-κB activation steps in TLR4 signaling: MyD88/TIR domain-containing adaptor protein (TIRAP)-mediated NF-κB activation occurs early after stimulation, whereas TIR domain-containing adaptor inducing IFN-β (TRIF) induces later NF-κB activation (48–50).

To examine whether HCV core may affect the activation of AP-1 and NF-κB and inhibit IL-12p40 mRNA synthesis, we performed electrophoretic mobility shift assay analysis in core-treated THP-1 at various time points (0, 30, 60, 180, and 300 min) after LPS stimulation. As shown in Fig. 7a, HCV core significantly suppresses activation of AP-1 at all time points, when compared with those cells treated with LPS alone. We also examined the effect of HCV core on NF-κB activation using nuclear extracts of LPS-stimulated THP-1 cells by electrophoretic mobility shift assay. As shown in Fig. 7b, NF-κB rapidly accumulated in the nuclei of LPS-stimulated cells by 30 min, dissipating by 180 min. The addition of HCV core to these cells, however, diminished the early activation of NF-κB at 30 min after LPS stimulation, suggesting that core may disrupt the arm of TLR4 signaling responsible for early activation of NF-κB. However, HCV core did not affect the activation of the transcription factor NF-IL-6 (data not shown). Taken together, these results suggest that HCV core disrupts one or more TLR signaling pathways, thus preventing the activation of key transcription factors AP-1 and the delayed activation of NF-κB.

**DISCUSSION**

In this report, we demonstrate that HCV core selectively suppressed IL-12p70 production by activated human PBMC and macrophages following LPS stimulation, whereas levels of TNF-α, IL-1β, IL-6, and IL-8 remained unaffected. Inhibition of IL-12p70 by HCV core led to the suppression of IFN-γ secretion in SAC-stimulated PBMC. In addition, we found this suppression to occur at the transcriptional level through inhibition of AP-1 and the delayed activation of NF-κB.
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IL-12p40 mRNA synthesis. The HCV core-induced inhibition of IL-12p40 mRNA synthesis resulted from the suppression of transcription factor AP-1 rather than the enhanced production of IL-10. Importantly, the HCV core-mediated inhibition of IL-12p40 mRNA synthesis could be reversed by anti-gC1qR antibody treatment, suggesting that an interaction between HCV core and gC1qR is crucial for suppressing IL-12 production. These results suggest that the HCV core-gC1qR-mediated inhibition of IL-12 production may play a pivotal role in establishing and maintaining a Th1 microenvironment in infected individuals.

IL-10 is well established as an important negative regulator of IL-12 production (40). However, we found the core-induced suppression of IL-12 synthesis in LPS-stimulated M/Mo to occur independently of IL-10 induction. Instead, the inhibition of IL-12 by HCV core occurs at the transcriptional level through suppression of IL-12p40 expression. This suggests that the ligation of gC1qR by HCV core might interfere with LPS-induced TLR4 signaling. It is also notable that the suppression of IL-12p40 expression has broader implications for inflammatory cytokine production because IL-12p40 associates with a p19 chain to form a covalently linked heterodimeric cytokine, IL-23 (51). IL-23 is produced by similar cell types as IL-12 and induces IFN-γ production (51). Importantly, IL-23 was shown to induce stronger, more sustained CTL and Th1 immune responses in an HCV vaccination model (52). Given the inhibitory effect of HCV core on IL-12p40 expression, HCV core is also likely to suppress production of IL-23. We are currently investigating the alteration of IL-23 by HCV core.

It is also crucial to point out that core treatment inhibited TLR4-induced activation of the transcription factor AP-1, a key regulator of IL-12p40 mRNA synthesis. However, activation of NF-κB appeared to be delayed rather than abolished. This might explain the selective inhibition of IL-12 production by HCV core while most other cytokines remain unaffected. Recently, two distinct, temporally separable pathways mediate TLR4 signaling: MyD88/TIRAP-dependent and TRIF-dependent pathways. Interestingly, similar to results observed in HCV core-treated macrophages, the early activation of NF-κB was only suppressed, and late activation of NF-κB was sustained in macrophages isolated from TIRAP- and MyD88-deficient mice (49, 50). This suggests that the TIRAP/MyD88-mediated signaling pathway might be responsible for early activation of NF-κB, whereas TRIF-mediated signaling controls later, more sustained NF-κB activity. In addition, TIRAP/MyD88 deficiency completely abolished IL-12p40 production (50, 53, 54). Thus, the binding of HCV core to gC1qR might selectively impair the TIRAP/MyD88-dependent pathway of TLR4 signaling. These results suggest cross-talk between gC1qR ligation and TLR4 signaling.

However, the signaling intermediates of gC1qR have yet to be identified. The ligation of gC1qR by HCV core may lead to induction of negative regulatory molecules, such as the Src homology 2-containing tyrosine phosphatase (SHP-1), which inhibits a distinct TLR4 signaling pathway and thus modulates IL-12 production (55–57). SHP-1 is a protein-tyrosine phosphatase expressed primarily in cells of the immune system. In addition to its functions in hematopoiesis, in vivo studies have revealed that SHP-1 plays an integral role in the negative regulation of immune cell activity (57). SHP-1 was also implicated in the CD46-mediated suppression of IL-12 by measles virus (55). Ongoing research in our lab is focused on determining whether HCV core-gC1qR engagement might induce a negative regulatory molecule, such as SHP-1, to inhibit the proximal events of TLR4 signaling.

IL-12 is known to play a pivotal role in the generation of Th1-type immune responses and provides a critical link between innate and adaptive immunity (42). The establishment of a Th1 environment in the periphery of HCV-infected individuals is thought to be a key determinant of disease progression. Specifically, those who demonstrate Th1 dominance tend toward chronic infection, whereas those with a Th2 phenotype tend to clear the virus (14–16). Despite this apparent immune dysregulation, HCV exhibits a relatively low infectivity rate, suggesting that the virus utilizes an indirect mechanism of immune dysregulation to persist. Because the hepatocyte is the likely primary cell type to support HCV replication and viral persistence, core protein secreted from HCV-infected hepatocyte will bind to gC1qR displayed on macrophages (Kupffer cells) in the local environment (liver) and suppress the inflammatory response mediated by macrophages. These results raise the possibility that HCV core-mediated inhibition of inflammatory response and local innate immune response may play a pivotal role in the establishment of persistent infection. Furthermore, it indicates that the immunosuppressive action of core protein may allow the virus an avenue of escape by down-modulating Th1 responses in favor of switching to Th2 responses.

This immunomodulatory behavior of HCV core may be genotype-specific, because HCV core from certain genotypes have not been found to be immunosuppressive or may represent a generalized mechanism of immune evasion utilized by the virus (58). It is likely that discrepancies in the abilities of these core proteins to affect immune responses might result from a disparity in the magnitude of core secreted during the acute phase of infection, as well as the sequence variability in the gC1qR-binding site within core protein, thus influencing the relative affinity of these core proteins for the gC1qR. In addition, gC1qR polymorphism may play a role in influencing HCV core-gC1qR-mediated immune dysregulation in HCV-infected individuals.

HCV continues to spread and persist among people worldwide, making it a leading indicator for liver transplantation. The ability of HCV core to usurp the complement cascade to afford the virus an indirect mechanism of immune evasion presents a potential therapeutic target. The impaired immune responses by HCV core-gC1qR interaction could occur at two levels. First, the interaction between HCV core and gC1qR on the surface of T cells directly suppresses T cell function (3, 32). Second, the binding of HCV core on gC1qR displayed on the cell surface of antigen presenting cells (i.e., macrophages and dendritic cells) interferes with cell-mediated immune responses by modulating inflammatory cytokine production. The design of molecules aimed at antagonizing the HCV core-gC1qR interaction may provide us with a novel treatment for HCV, allowing the host immune system to effectively clear the virus.

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