Hepatitis C Virus Core and Nonstructural Protein 3 Proteins Induce Pro- and Anti-inflammatory Cytokines and Inhibit Dendritic Cell Differentiation

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*J Immunol* 2003; 170:5615-5624; doi: 10.4049/jimmunol.170.11.5615
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Hepatitis C Virus Core and Nonstructural Protein 3 Proteins Induce Pro- and Anti-inflammatory Cytokines and Inhibit Dendritic Cell Differentiation

Angela Dolganiuc,* Karen Kodys,* Andrea Kopasz,* Christopher Marshall,* Twan Do,* Laszlo Romics, Jr.,* Pranoti Mandrekar,* Maria Zapp,† and Gyongyi Szabo2*

Antiviral immunity requires recognition of viral pathogens and activation of cytotoxic and Th cells by innate immune cells. In this study, we demonstrate that hepatitis C virus (HCV) core and nonstructural protein 3 (NS3), but not envelope 2 proteins (E2), activate monocytes and myeloid dendritic cells (DCs) and partially reproduce abnormalities found in chronic HCV infection. HCV core or NS3 (not E2) triggered inflammatory cytokine mRNA and TNF-α production in monocytes. Degradation of IκBα suggested involvement of NF-κB activation. HCV core and NS3 induced production of the anti-inflammatory cytokine, IL-10. Both monocyte TNF-α and IL-10 levels were higher upon HCV core and NS3 protein stimulation in HCV-infected patients than in normals. HCV core and NS3 (not E2) inhibited differentiation and allostimulatory capacity of immature DCs similar to defects in HCV infection. This was associated with elevated IL-10 and decreased IL-2 levels during T cell proliferation. Increased IL-10 was produced by HCV patients’ DCs and by core- or NS3-treated normal DCs, while IL-12 was decreased only in HCV DCs. Addition of anti-IL-10 Ab, not IL-12, ameliorated T cell proliferation with HCV core- or NS3-treated DCs. Reduced allostimulatory capacity in HCV core- and NS3-treated immature DCs, but not in DCs of HCV patients, was reversed by LPS maturation, suggesting more complex DC defects in vivo than those mediated by core or NS3 proteins. Our results reveal that HCV core and NS3 proteins activate monocytes and inhibit DC differentiation in the absence of the intact virus and mediate some of the immunoinhibitory effects of HCV via IL-10 induction.

production during the alloantigen-induced T cell proliferation. Our data also demonstrated that HCV core and NS3 proteins inhibit DC differentiation rather than maturation. These results suggest that HCV viral proteins regulate activation of the innate immune system in the absence of direct viral infection of monocytes or DCs.

**Materials and Methods**

**Patients and controls**

A total of 16 healthy controls (serologically negative for HCV, HBV, and HIV, ages 35 ± 9; 10 females and 6 males) and 15 chronic HCV-infected (anti-HCV Ab and PCR positive), untreated patients (ages 38 ± 7; 7 females and 8 males) was included. The study was approved by the Committee for the Protections of Human Subjects in Research at the University of Massachusetts Medical School.

**Monocyte isolation and myeloid DC generation**

Monocytes were separated from peripheral blood by centrifugation on Ficoll-Hypaque (Amersham Pharmacia, Uppsala, Sweden) density gradient, as previously described (22). Mononuclear cells (10 × 10^6 cells/well) were incubated in six-well plates in RPMI 1640 supplemented with 15% FBS (HyClone, Logan, UT) for 3 h, and nonadherent cells were removed by gentle wash. Adherent monocytes were cultured in RPMI 1640 with 10% FBS and rested overnight before stimulation.

DCs were generated, as described (23). Briefly, adherent blood monocytes were treated in DC medium (RPMI 1640 with 10% FBS, 2 mM l-glutamine, 50 μM 2-ME) with IL-4 (0.1 μg/ml) and GM-CSF (0.01 μg/ml; PeproTech, Rocky Hill, NJ) in the presence or absence of HCV proteins (core, aa 2–192, and NS3, aa 1450–1643; from Mikrogen GmbH, Martinsried, Germany). HCV E2 protein (aa 374–715) was tested in a one-way MLR with normal, allogeneic T lymphocytes (23). According to the manufacturer recommendations. CellQuest (BD Immunocytometry Systems) programs. Apoptosis was determined using the FlowJo (Tree Star, San Carlos, CA) and statistical analysis showed 98% CD3 staining by FACS analysis.

**DC surface marker analysis and apoptosis**

DCs were labeled with FITC- or PE-labeled mAbs (anti-human CD1a, CD3, CD11c, CD14, CD19, CD56, CD80, CD81, CD83, HLA-ABC, HLA-DR, or relevant isotype controls; BD PharMingen, San Diego, CA), according to the manufacturer’s directions. Briefly, cells (1 × 10^7) were incubated with 20 μl of Ab in a total volume of 100 μl (2% FBS-PBS) for 60 min at 4°C in the dark, extensively washed, and resuspended in 500 μl of 1% paraformaldehyde in PBS. Using a FACSCalibur (BD Immunocytometry Systems, San Diego, CA), flow cytometry, cells were gated according to their size (forward light scatter) and granularity (side light scatter), and surface marker expression of gated large and granular cells was analyzed using the FlowJo (Tree Star, San Carlos, CA) and CellQuest (BD Immunocytometry Systems) programs. Apoptosis was determined using the annexin V FITC kit (BioSource International, Camarillo, CA), according to the manufacturer recommendations.

**Quantitation of allostimulatory activity**

Allostimulatory capacity of day 7 irradiated DCs (3000 rad; stimulator cells) was tested in a one-way MLR with normal, allogeneic T lymphocytes (2 × 10^5 cells/well; responder cells) at various stimulator/responder cell ratios (1:20–1:80) in triplicates in 96-well plates (Corning Glassware, Corning, NY) (23). [3H]-Thymidine (DuPont-NEN, Boston, MA) incorporation was evaluated during the final 16 h of the 5-day assay. Results were expressed as mean cpm ± SE. Rat anti-human IL-10 mAb (clone JES3-9D7; BioSource International) was used at 15 μg/ml, or rIL-12 (PeproTech) at 15 ng/ml concentrations, where indicated, was added for the entire duration of the MLR, as described (7).

**Immunoblotting**

To isolate the cytoplasmic cell fraction for immunoblotting, the cell pellets (1–10 × 10^6 cells) were suspended in 250 μl of ice-cold lysis buffer (10 mM HEPES; 10 mM KCl; 0.1 mM EDTA; 0.1 mM EGTA; 1 mM PMSF; and aprotinin, leupeptin, and antipain, all 10 μg/ml) and kept on ice for 20 min. After this incubation, 25 μl of 10% Nonidet P-40 (1% final concentration) was added, the lysate was centrifuged at 14,000 × g for 30 min, and cytoplasmatic fraction was separated from pelletted nuclei and used for subsequent protein determination (Bio-Rad, Hercules, CA). A total of 30 μg of protein from cytoplasmatic fraction was separated in a 7.5% polyacrylamide gel, transferred to a nitrocellulose membrane (0.45 μm pore size; Schleicher & Schuell, Keene, NH), and blocked with 10% nonfat dry milk. Immunostaining was performed with anti-iκBα rabbit polyclonal primary Ab, followed by incubation with secondary Ab conjugated to HRP (Santa Cruz, Santa Cruz, CA). Immunoreactive bands were visualized using ECL reagents (NEN Life Science Products, Boston, MA). To confirm the equal protein loading in all samples, the blot was stripped for 30 min at 50°C in a stripping buffer (100 mM 2-ME, 1 M Tris-HCl, and 2% SDS), washed extensively, and relabeled with anti-β-actin Ab and secondary HRP Ab.

**RNAse protection assay**

A total of 2 × 10^6 DCs was stimulated, as indicated in figure legend, for 12 h. Total cytoplasmic RNA was isolated using Tri-reagent (Molecular Research Center, Cincinnati, OH), according to manufacturer’s instructions. Antisense probes were labeled with [32P]UTP (NEN Life Science Products) using the Riboquant in vitro transcription labeling kit (BD PharMingen), according to manufacturer’s instructions. A mixture of probes, Riboquant hCK-2b (BD PharMingen), was used to facilitate the simultaneous quantification of several RNA species. The antisense probes generated using this probe set included the controls, GAPDH and L32, and the human cytokines IL-10, IL-12, IL-1α, and IL-1Ra. The RNAse protection assays were performed using the Riboquant RNAse protection assay kit (BD PharMingen), according to manufacturer’s instructions. In brief, molar excesses of labeled probes were incubated with RNA derived from cells in hybridization buffer supplied by the manufacturer for 12–16 h at 56°C. Hybridized samples were then digested with RNase A + T1 mixture for 45 min at 30°C. Subsequent to digestion, the protected fragments were separated from digested probe by electrophoresis on an 8 molar urea, 5% polyacrylamide Tris borate EDTA gel. The gels were then vacuum dried, exposed directly to film, and developed. The band intensities were quantitated using the Science Lab Image Gauge V3.41 software. For each cytokine, the mRNA levels were adjusted according to L32.

**Cytokine analysis**

Supernatants were collected on day 4 of the MLR and stored at −80°C until analyzed for IL-10, IL-2, TNF-α, or IL-12 (p40 + p70) using specific cytokine ELISA kits (BD Immunocytometry Systems).

**Statistical analysis**

The Wilcoxon nonparametric analysis was used in the Statview (SAS Institute, Cary, NC) program on a Mac G4 computer (Apple, Cupertino, CA).

**Results**

**HCV core and NS3 proteins trigger cytokine production in monocytes**

Innate immune cells, particularly monocytes and DCs, play a pivotal role in recognition of invading viral pathogens (25, 26). The activation of innate immune cells occurs in a virus-specific manner and leads to induction of inflammatory and immunomodulatory cytokines. In this study, we investigated the hypothesis that HCV proteins activate monocytes in the absence of the intact virus or viral replication. Normal human blood monocytes were exposed in vitro to HCV core, NS3, and E2 proteins, and the production of TNF-α, IL-12 (p70), and IL-10 was assessed. Data in Fig. 1 demonstrate that HCV core and NS3, but not E2 proteins triggered TNF-α and IL-10 production in monocytes, while IL-12 (p70) was not induced by any of these HCV proteins. LPS, a potent monocyte activator, resulted in induction of all three cytokines. The HCV proteins were free of endotoxin, as determined in a Limulus amebocyte assay (data not shown), and suggested by the lack of their IL-12 induction in monocytes. During HCV infection, HCV proteins, particularly core, enter the circulation at detectable levels that are lower than the HCV protein concentration used in our in vitro experiments (15). Thus, we evaluated the effect of decreasing doses of core and NS3 proteins on monocyte TNF-α and IL-10 production.
induction and found a dose-response effect on induction of these cytokines (Fig. 1). These results suggested that HCV core and NS3 proteins trigger naive monocytes to produce both proinflammatory (TNF-α) and immunoinhibitory (IL-10) cytokines.

Next, we sought to determine whether monocytes of chronically HCV-infected patients that are constantly exposed in vivo to HCV core and NS3 proteins would exert abnormal cytokine production. In monocytes of patients with chronic HCV infection, high levels of TNF-α and IL-10 were induced upon stimulation with HCV core and NS3 proteins or with the positive control, LPS (Fig. 2). The TNF-α and IL-10 levels were significantly higher in the HCV patients’ monocytes compared with control monocytes, suggesting their in vivo preactivation. Although NS3 induced high levels of TNF-α (p = 0.03) and IL-10 (p < 0.001), HCV core induced only IL-10 (p < 0.001) to greater levels in patients’ monocytes than in normals.

Monocyte activation by HCV core and NS3 proteins was further evaluated at the mRNA levels. HCV core and NS3 proteins increased mRNA levels for IL-10 and IL-12 p40 in normal monocytes (Fig. 3). We found induction of IL-12 p40, but not p35 mRNA, which observation was consistent with the lack of biologically active IL-12 (p70) induction at the protein level in core and NS3-stimulated monocytes (Fig. 3a). Activation of proinflammatory pathways in monocytes is mediated via activation of the nuclear regulatory factor NF-κB that involves rapid phosphorylation and degradation of the cytoplasmic IκBα (27). In this study, we found that similar to LPS stimulation, both HCV core and NS3 proteins resulted in decreased cytoplasmic levels of IκBα, suggesting involvement of NF-κB activation (Fig. 3b). Furthermore, activation of monocyte-derived immature DCs with HCV core and NS3 proteins also resulted in a rapid IκBα degradation. In addition to activation of inflammatory responses, NF-κB activation has recently been proposed as a marker of DC maturation (27).

**HCV core and NS3 proteins inhibit myeloid DC allostimulatory capacity and modulate cytokine production**

Pathogen-derived signals in the tissue environment trigger blood monocytes to differentiate into myeloid DCs with high T cell stimulatory capacity (28). In HCV-infected livers, increased numbers of DCs are found concentrated around newly formed lymphatic vessels (29). Based on the observation that HCV core and NS3 proteins activate monocytes, the precursors for myeloid DCs, we tested the hypothesis that HCV core and NS3 proteins mediate some of the myeloid DC defects found in patients with chronic hepatitis C infection. DCs were generated in the presence or absence of HCV core, NS3, or E2 proteins, and allostimulatory capacity was tested in immature DCs. DCs exposed to HCV core or NS3, but not to E2 proteins showed significantly reduced allostimulatory capacity compared with untreated immature DCs (Fig. 4a). This was similar to the decreased allostimulatory capacity of day 7 monocyte-derived immature DCs from HCV-infected patients compared with control DCs (Fig. 4b). The reduction in DC

**FIGURE 1.** Recombinant HCV proteins induce monocyte activation. Adherence-isolated normal monocytes (10⁶/ml) were stimulated with LPS (0.1 μg/ml) or HCV core or NS3 (concentrations as indicated, micrograms per milliliter), HCV E2 (1 μg/ml) proteins, or β-galactosidase (1 μg/ml) for 16 h. Supernatants were analyzed for IL-10, TNF-α, and IL-12 by ELISA. Mean ± SE of three experiments are shown.

**FIGURE 2.** Monocytes from HCV patients produce more IL-10 in response to stimulation with rHCV core and NS3, not E2, proteins than normal monocytes. Monocytes (10⁶/ml) from controls and from chronic HCV-infected patients were stimulated with LPS (0.1 μg/ml), and HCV core, NS3, or E2 proteins (1 μg/ml) for 16 h, and supernatants were analyzed for TNF-α and IL-10 by ELISA. Mean ± SE of four controls and five HCV patients are shown.
allostimulatory capacity was greater in the HCV-infected patients (65.2 ± 6.15%) compared with HCV core (39.39 ± 4.15%) or with NS3 (42.52 ± 3.54%)-treated normal DCs. Combination of core and NS3 proteins resulted in no additive inhibition of DC allostimulatory capacity (data not shown).

T cell proliferation is regulated by the composition of the cytokine environment and by expression of costimulatory molecules on DCs (30). DC-derived IL-12 is critical for supporting Ag-specific T cell activation, while IL-10, an immunoinhibitory cytokine, inhibits both APC IL-12 production and T cell proliferation (31, 32).

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During the alloantigen-induced T cell proliferation with HCV core- and NS3-treated DCs, we found significantly elevated IL-10 (p < 0.009 and 0.008, respectively) and reduced IL-2 levels (p < 0.009 and 0.006, respectively) (Fig. 5a). A similar pattern of cytokine abnormalities was found during T cell proliferation in the presence of DCs from HCV-infected patients, including increased IL-10 and reduced IL-2 production (Fig. 5b). However, the significant decrease in total IL-12 levels (p40 + p70; p < 0.0033) found in the presence of DCs from HCV-infected patients was not seen with HCV core- or NS3-treated DCs, suggesting that DC abnormalities after in vivo HCV infection are more complex than those induced by the individual HCV proteins.

To dissect whether abnormal cytokine production seen during MLR was due to T cell or DC abnormalities, cytokine production was tested in day 7 immature DCs before addition of T cells. HCV core- and NS3 protein-treated DCs had increased levels of IL-10 and reduced production of IL-12 at the levels of both protein and mRNA (Fig. 6, a and b). The same pattern of increased IL-10 and reduced IL-12 production was seen in DCs of HCV-infected patients (Fig. 6, c and d), suggesting that cytokine abnormalities play a role in the altered allostimulatory capacity of DCs. IL-12 and IL-10 both have critical regulatory effects on T cell activation and proliferation (31–33). Thus, the role of reduced IL-12 and/or increased IL-10 in mediation of decreased allo-Ag-induced T cell proliferation with HCV core- and NS3-treated DCs was further evaluated. Addition of anti-IL-10-neutralizing Ab during the MLR, but not of the isotype-matched control Ab, partially restored the defect in allostimulatory capacity of HCV core- or NS3-treated DCs (Fig. 7). Interestingly, IL-12 alone failed to augment the reduced allostimulatory defect in the same system, and there was no synergy by the combination of IL-12 and anti-IL-10 Ab treatment. These results suggested that IL-10 is a mediator of reduced T cell activation in the presence of HCV core- or NS3-treated DCs.

Next, we investigated expression of DC phenotypic markers, costimulatory molecule expression, and apoptosis. Although the alloantigen-induced T cell proliferation was significantly reduced, there was no reduction in the expression of costimulatory molecules CD80 (75.7 ± 9%), CD86 (86.2 ± 13%), or HLA-DR (83.9 ± 9%), on DCs from HCV-infected patients or core- or NS3-treated DCs compared with controls. Expression of DC surface markers, including CD1a (88.1 ± 6%), CD11c (91.5 ± 6%), and HLA-ABC (96.0 ± 4%), was also comparable between DCs from HCV patients and controls with or without HCV protein treatment. We found no differences in the expression of CD40 (36.1 ± 8%), CD83 (19.3 ± 6%), or CD81 (94.1 ± 2%) between DCs from HCV-infected patients or core- or NS3-treated DCs or controls. There was no increase in the percentage of apoptotic cells between controls and DC generated in the presence of HCV core or NS3 or from HCV-infected patients (data not shown).

HCV core and NS3 proteins inhibit differentiation, not maturation, of myeloid DCs

Interaction with pathogen-derived Ags and migration to regional lymph nodes results in maturation of immature DCs in vivo, which process can be induced in vitro with cytokines or LPS stimulation (34). Thus, we investigated whether induction of DC maturation could restore the reduced allostimulatory capacity of immature DCs seen after HCV core and NS3 treatment and in HCV-infected patients. LPS-induced maturation of normal immature DCs significantly increased allostimulatory capacity (Fig. 8a) and expression of CD83 (Fig. 8C). In DCs differentiated in the presence of HCV

FIGURE 5. Increased IL-10 and decreased IL-2 during allogeneic MLR with DC generated in the presence of rHCV proteins (A) and DCs from patients infected with HCV (B). Immature DCs from controls (A) or HCV patients (B) generated in the absence or presence of HCV proteins (core and NS3, 10 μg/ml) were used as stimulatory cells in allogeneic MLR; cell-free supernatants collected on day 4 were analyzed for IL-10, IL-12 (p40 + p70), and IL-2. No HCV proteins were added during the MLR. Results from eight experiments are shown as mean ± SE.
core and NS3, LPS maturation augmented DC allostimulatory capacity to the level of normal controls (p < 0.001). In fact, DC maturation completely ameliorated the reduced allostimulatory function of core- and NS3-treated immature DCs (Fig. 8a). In contrast, in DCs of HCV-infected patients, maturation only partially improved DC allostimulatory capacity to the level of normal immature, but not to the level of normal mature DCs (Fig. 8b). These data suggested that HCV core- and NS3-induced signals only partially mediate the complex defects of DC allostimulatory function in chronically HCV-infected patients.

Finally, we wished to dissect the effects of HCV core and NS3 proteins between DC differentiation and maturation. Data in Fig. 8 showed that LPS maturation could overcome the inhibitory effects of HCV core and NS3 proteins on DC differentiation. Consistent with this, HCV core- and NS3 proteins alone failed to induce maturation of naive immature DCs (Fig. 9). Furthermore, HCV core and NS3 added with LPS on day 5 to immature DCs failed to inhibit functional maturation of normal DCs (Fig. 9). These results altogether suggested that HCV core and NS3 proteins reversibly inhibit differentiation, but not maturation of DCs, whereas DCs of HCV-infected patients have defects in allostimulatory capacity that cannot be fully restored by induction of DC maturation.

**Discussion**

Defective antiviral immunity, including reduced Ag presentation and allostimulatory capacity of DCs, low efficiency of viral-specific T cell responses, production of Abs that lack neutralizing capacity, and dysbalance of pro- and anti-inflammatory cytokines, contributes to the chronic hepatitis C infection (3, 4, 7–9, 34–37). Some of these abnormalities could be the result of direct interactions between the HCV virus and cells, while others may occur due to nonspecific immune activation. In this study, we report that monocytes and DCs are activated directly by HCV core or NS3 proteins, indicating that some HCV proteins can trigger innate immune responses in uninfected cells. Our data revealed complex interactions between HCV core and NS3 proteins and the innate immune system that included induction of both pro- and anti-
inflammatory pathways in monocytes as well as inhibition of differentiation and allostimulatory capacity of myeloid DCs. We found that the effects of core and NS3 proteins on normal monocytes and DCs were similar to the cellular abnormalities found in HCV-infected individuals. However, HCV core and NS3 proteins only partially reproduced the spectrum of defects observed in DCs of HCV-infected patients. Unlike in DCs of HCV-infected patients, LPS-induced DC maturation restored the reduced allostimulatory capacity in HCV core- and NS3-treated DCs, suggesting that HCV core and NS3 proteins mediate some, but not all, of the inhibitory effects of the HCV virus on myeloid DC functions.

Our results demonstrated that proinflammatory pathways, including TNF-α production and IL-1α mRNA induction, were triggered by HCV core and NS3 proteins in monocytes. TNF-α induction by core and NS3 proteins was even more profound in monocytes of HCV-infected patients, suggesting that patients’ monocytes may be primed in vivo. Such increase in the nonspecific inflammation in the liver and elevated serum levels of inflammatory cytokines in HCV-infected patients has been reported to correlate with the extent of liver injury (38). Induction of many of the inflammation-related genes is regulated by activation of the NF-κB site in their promoter region (39), which is commonly induced by pathogens, including certain viruses (40–42). We found that recognition of HCV core- or NS3 protein-induced signals resulted in a rapid decrease in cytoplasmic IκBα protein levels, which is an initial step in activation of the NF-κB intracellular signaling pathway in monocytes (43). Upon inflammatory cell activation, IκBα is phosphorylated, then degraded via the proteosome pathway, resulting in NF-κB nuclear translocation and subsequent activation of NF-κB-driven inflammatory genes (39). NF-κB activation by HCV core protein has been previously reported in transfected HepG2 cells that expressed the HCV core protein (44–46). In HCV core protein-expressing hepatocyte systems, NF-κB activation is likely to occur as a result of signaling events between the endogenously expressed core protein and the intracellular portion of the NF-κB signaling pathway. In our experiments, HCV core and NS3 proteins were signals derived from the extracellular environment. Thus, our results suggest that the danger signals triggered by core and NS3 proteins in innate immune cells result in activation of classical proinflammatory cascades that contribute to the overall immune activation by the HCV virus.

HCV core protein has been shown to interact with the TNFR family (TNFR1), Fas, lymphotixin β receptor, and the complement receptor, C1qR, which can all be present on monocytes and DCs (45, 47–50). Although core and NS3 proteins activated inflammatory pathways in monocytes and inhibited DC differentiation, the E2 protein failed to induce similar effects. E2 is localized on the outer surface of the fully assembled HCV virus and has been proposed to interact with various receptors, including CD81, low density lipoprotein receptor, and most recently, the scavenger receptor B (9, 13, 14, 18). The expression of the putative HCV receptor, CD81, on DCs was not changed in HCV-infected patients or in core- or NS3-treated cells compared with normal controls (data not shown), and it is yet to be determined whether CD81 binds viral proteins or the intact virus and whether this binding mediates viral internalization and/or cellular activation. The biological significance of the lack of E2-mediated monocyte and DC activation remains to be investigated. However, it is notable that,
based on our data, the intact HCV virus may not activate cells of the innate immune system via E2, and only core and NS3 proteins, products of defective viral replication or dying infected hepatocytes, would trigger danger signals in innate immune cells.

A consistent effect of HCV core and NS3 proteins both in monocytes and DCs was the induction of the immunoregulatory cytokine, IL-10. Furthermore, elevated IL-10 production was the characteristic of both monocytes and DCs of HCV-infected patients, suggesting that increased IL-10 production, whether triggered by HCV proteins or by other factors related to HCV infection, is a fundamental feature of the HCV-induced immune defects in vivo and in vitro. Indeed, neutralization of IL-10 in the MLR resulted in a partial restoration of the reduced allostimulatory capacity of HHCV core- and NS3-treated DCs. The increased IL-10 production by HCV-stimulated monocytes could contribute to several aspects of the defective immune activation in HCV infection. First, the concomitant high levels of TNF-α and IL-10 induction by HCV core and NS3 proteins and observed in monocytes of HCV-infected patients suggest that TNF-α production in these patients may be resistant to the inhibitory effects of IL-10 (51). Second, IL-10 is a potent inhibitor of T cell activation and Ag-specific T cell proliferation, whose functions are reduced in chronic HCV (33, 52). IL-10 has been shown to induce T cell anergy by specifically altering the CD28 costimulation pathway (53). Third, IL-10 produced by monocytes and DCs have autocrine inhibitory effects on DC differentiation and maturation and, particularly, on IL-12 production by DCs, which is a pivotal element of Ag-specific T cell activation (31). Increasing evidence suggests that DCs,
Results are expressed as [methyl-3H]thymidine incorporation by activated 24 h (mature M-DC). Day 7 DCs were used as stimulatory cells in MLR. (I-DC) for 24 h. DC maturation was induced with LPS (0.1 ng/mL) with increased expression of CD1a, HLA-DR, and the costimulatory molecule we identified with IL-10 have been shown to induce tolerance (54). They can induce Ag-specific T cell proliferation or tolerance. DCs treated with IL-10 have been shown to induce tolerance (54–56). Thus, the myeloid DC phenotype we identified in HCV patients and after core or NS3 treatment closely resembles tolerogenic DCs that are characterized by increased IL-10, decreased IL-12, and IL-2 induction during MLR, with no changes in costimulatory molecule expression.

The unique ability of DCs to activate or tolerize T lymphocytes depends on the stage of maturation, expression of MHC and costimulatory molecules, and cytokine production profile (57). Viral and bacterial products, inflammatory cytokines, and Ag uptake can initiate DC maturation (34, 58–60) to induce increased expression of MHC and costimulatory molecules and superior capacity to trigger T lymphocytes compared with immature DCs (61). Although immature DCs strategically locate in tissues that represent pathogen-entry sites, uptake, and present Ags to lymphocytes, mature DCs reduce their endocytic capacity, enhance chemokine and inflammatory cytokine production, and become mobile to deliver pathogen-derived Ags for T cell activation. During HCV infection, intact virions as well as excess HCV proteins circulate at detectable levels in the peripheral blood, and even higher protein levels may occur locally in the liver from apoptotic and dying hepatocytes (15, 62, 63). As monocytes and DCs are capable of uptake of apoptotic and necrotic cells, both DCs in the liver and circulating DC precursors can be affected by the viral proteins to result in reduced Ag presentation, as seen in the chronic HCV-infected patients. Indeed, DCs were found in hepatitis C-infected livers in close contact with lymphatic vessels, providing evidence for the importance of DCs in the liver in HCV infection (29, 64).

Our data show that the presence of HCV and/or its products interferes with DC differentiation and LPS-induced maturation. Previous studies on myeloid DC in HCV-infected patients reported impaired TNF-α-induced maturation that was associated with decreased expression of CD1a, HLA-DR, and the costimulatory molecules CD83 and CD86 (9). Others described decreased CD1a expression, but otherwise normal DC phenotype, with a slight increase of CD83 expression (10). We found no significant reduction in the expression of the costimulatory molecules or CD83 on DCs from HCV-infected individuals or after core and NS3 protein treatment. These differences in DC phenotypes could be due to differences in DC maturation triggered by TNF-α and LPS. Although both stimuli induce DC maturation, LPS, but not TNF-α, was found to induce transcription of genes responsible for DC growth arrest and control of immune responses (34). LPS is also more powerful in activating the expression of the genes involved in Ag processing and T cell stimulation, while TNF-α is an ineffective stimulus for terminal DC differentiation (34). Increased circulating TNF-α was reported in serum of HCV-infected patients, and significant role was attributed to LPS in the pathogenesis of HCV infection, suggesting that both of these maturation stimuli have in vivo physiological importance (36, 65).

Our data demonstrating that DCs from HCV-infected patients have limited potential to mature based on their reduced allostimulatory capacity, despite an up-regulation of costimulatory molecules during LPS maturation, are similar to observations by others with TNF-induced DC maturation (8, 9). The greater reduction in DC allostimulatory capacity in chronic HCV-infected patients compared with core- or NS3 protein-treated DCs and the fact that maturation normalized DC allostimulatory capacity in core- and NS3-treated, but not in HCV-infected patients’ DCs suggest that the intact HCV may mediate more complex immunoregulatory effects than its isolated protein components. However, we found no additive effects of core and NS3 on inhibition of DC allostimulatory functions, suggesting that these proteins may inhibit DC allostimulatory functions via the same pathway. Further investigations should determine the molecular basis for the core- and NS3-mediated immunosuppression of DCs.

### Acknowledgments

We are thankful to Drs. H. Bonkovsky, S. Mehta, R. Koff, and L. Shick for their assistance with recruitment of the patients with HCV infection.

### References


