Extracellular Hepatitis C Virus Core Protein Activates STAT3 in Human Monocytes/Macrophages/Dendritic Cells via an IL-6 Autocrine Pathway*§

Hepatitis C virus (HCV) infection is highly efficient in the establishment of persistent infection, which leads to the development of chronic liver disease and hepatocellular carcinoma. Impaired T cell responses with reduced IFN-γ production have been reported to be associated with persistent HCV infection. Extracellular HCV core is a viral factor known to cause HCV-induced T cell impairment via its suppressive effect on the activation and induction of pro-inflammatory responses by antigen-presenting cells (APCs). The activation of STAT proteins has been reported to regulate the inflammatory responses and differentiation of APCs. To further characterize the molecular basis for the regulation of APC function by extracellular HCV core, we examined the ability of extracellular HCV core to activate STAT family members (STAT1, -2, -3, -5, and -6). In this study, we report the activation of STAT3 on human monocytes, macrophages, and dendritic cells following treatment with extracellular HCV core as well as treatment with a gC1qR agonistic monoclonal antibody. Importantly, HCV core-induced STAT3 activation is dependent on the activation of the PI3K/Akt pathway. In addition, the production of multifunctional cytokine IL-6 is essential for HCV core-induced STAT3 activation. These results suggest that HCV core-induced STAT3 activation plays a critical role in the alteration of inflammatory responses by APCs, leading to impaired anti-viral T cell responses during HCV infection.

HCV² is remarkably efficient in the establishment of persistent infection. It is estimated that over 70% of infected people become persistently infected (1). A number of factors contribute to the high efficiency of persistence, including impairment of the host immune response (2). Several studies have reported that immune responses to HCV infection are significantly diminished in the peripheral blood (5), and the appearance of an HCV-specific antibody response is delayed and detectable only at 3–4 months after viral infection (6). At present, the mechanism(s) by which chronic HCV infection impairs the immune response remain(s) unresolved. One likely possibility is that HCV employs active immunosuppressive strategies to interfere with antigen-presenting (APCs, i.e. DC, macrophages), thus diminishing the subsequent magnitude and function of adaptive effector cells.

Resolution of HCV infection has been shown to be associated with a robust CD8⁺ and CD4⁺ T cell immunity against the virus (7). Pro-inflammatory cytokines (such as IL-12, TNF-α, and IL-1β) produced by APCs are crucial for the generation of effective anti-viral T cell immunity by lowering the activation threshold of T cells. Data from our lab (8, 9) as well as others (10) demonstrate that in addition to its role as an RNA-binding viral capsid protein, extracellular HCV core protein exerts immunomodulatory functions. Intriguingly, HCV core protein is secreted from HCV-infected hepatocytes, and extracellular core protein is detectable in the circulating bloodstream of HCV-infected patients (11, 12). This circulating/extracellular core has been reported to inhibit the production of IL-12 in human macrophage/DC and IFN-α in plasmacytoid DC (13). Importantly, DCs exposed to extracellular HCV core abrogated the generation of CD4⁺ Th1 responses (14). In addition, the suppressive activity of HCV core on IL-12 production in APCs requires ligation with gC1qR (15). Complement protein C1q is a ligand for gC1qR and plays a pivotal role in clearing apoptotic cells and limiting inflammatory responses (16–18). Thus, HCV may subvert the immune system by hijacking the C1q/gC1qR pathway and inhibit the host anti-viral response. However, the molecular mechanism of extracellular HCV core or gC1qR signaling in inhibiting the inflammatory response is yet to be determined.

Signal transducer and activator of transcription (STAT) proteins play an important role in the regulation of inflammatory responses by APCs. In monocytes, STAT3 is a particularly critical transcription factor in limiting excessive inflammatory responses (19). It is commonly activated via canonical JAK-STAT signaling where a cytokine or extracellular factor binds its cognate receptor, resulting in the auto-phosphorylation of JAK and subsequent tyrosine phosphorylation of STAT3. Upon phosphorylation at tyrosine 705, STAT3 homodimerizes via its SH2 domain, translocates into the nucleus, and induces the
transcription of a number of genes (20). Conditional knockout of STAT3 in macrophages dysregulates inflammatory responses and leads to severe colitis (21). In addition, macrophages and dendritic cells within the tumor microenvironment constitutively phosphorylate STAT3 and suppress the generation of potent anti-tumor T cell responses (19). Importantly, treatment with pharmacologic STAT3 inhibitors reverses the suppression (19). Recently, the activation of STAT3 has been shown to be responsible for inducing genes involved in the differentiation of myeloid-derived suppressor cells (22). Taken together, these results strongly implicate STAT3 activation as an inhibitor of classical APC activation.

In this study, we report the phosphorylation and nuclear translocation of STAT3 on APCs following exposure to extracellular HCV core or gC1qR agonist mAb. Phosphorylation of STAT3 is dependent upon activation of the PI3K/Akt pathway. In addition, treatment of APCs with extracellular core or gC1qR mAb significantly induced multifunctional cytokine IL-6 gene synthesis 9.84-fold. Neutralizing antibody to IL-6 abrogated gC1qR-mediated STAT3 activation on APCs, suggesting that STAT3 activation following exposure to HCV core or gC1qR mAb is IL-6-dependent. These data suggest that HCV core protein alters the activation and function of APCs via STAT3 activation and is involved in the inhibition of anti-viral T cell responses.

**MATERIALS AND METHODS**

*Reagents*—Recombinant β-gal and β-gal-fused HCV core protein were obtained from Virogen (Watertown, MA). Mouse mAb specific for gC1qR (5F4) was generated as described previously (15). Recombinant human IFN-α, IFN-γ, GM-CSF, IL-4, and IL-6 were obtained from R&D Systems (Minneapolis, MN). PI3K-specific inhibitor LY294002 was purchased from Calbiochem. The transcription and translation inhibitors actinomycin D and cycloheximide, respectively, were also purchased from Calbiochem.

*Cells and Cell Culture*—Human peripheral blood mononuclear cells (PBMCs) were isolated from healthy blood donors (Virginia Blood Services, Richmond, VA) by lymphocyte gradient centrifugation (Cedarlane Laboratories, Burlington, NC), and monocytes were purified by adherence to polystyrene as described previously (15). Adherent cells, typically and monocytes were purified by adhesion to polystyrene as previously described (Cedarlane Laboratories, Burlington, NC), and monocytes were cultured as described above. THP-1 cells (1 × 10^6 per treatment) were cultured in RPMI 1640 medium supplemented with 10% (v/v) FBS (HyClone, Logan, UT) and penicillin/streptomycin (100 U/ml recombinant human GM-CSF and 800 IU/ml recombinant human IL-4 at 37 °C with 7% CO₂ in a humidified atmosphere. Monocyte-derived macrophages and dendritic cells were induced first by positive selection with CD14 microbeads (STEMCELL Technologies, Inc., Vancouver, BC, Canada). Then, purified monocytes were differentiated into DCs by culture in complete media with 800 IU/ml recombinant human GM-CSF and 800 IU/ml recombinant human IL-4 at 37 °C with 7% CO₂ in a humidified atmosphere for a total of 6 days. Fresh media and cytokines were added at days 2 and 4 of culture. Monocytes were differentiated into macrophages through the addition of 800 IU/ml of M-CSF and were cultured as described above. THP-1 cells (ATCC) were cultured in RPMI 1640 medium supplemented as recommended by the ATCC.

Western Blotting—THP-1 cells (1 × 10^6 per treatment) were serum-starved overnight and then cultured in 400 μl of media in a 24-well plate. The cells were then treated as described in the figure legend. The samples were washed in cold “stop buffer” (50 mM HEPES, 15 mM NaCl, 10 mM EDTA, 1 mM vanadate), and the cell pellets were flash-frozen in liquid nitrogen. The cell pellets were resuspended in lysis buffer (20 mM Tris-HCl (pH7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM pyruvic acid, 1 mM sodium orthovandenate, 1 mM NaF, protease inhibitor mixture set V (Calbiochem)). The lysates were cleared of debris by centrifugation and suspended in 5 × sample buffer (125 mM Tris-HCl (pH6.8), 10% glycerol, 2% SDS, 0.00125% bromphenol blue (Sigma), 20% β-Me). Samples were then boiled for 5 min and centrifuged for 1 min at 10,000 × g. Equal amounts of cell lysate were subjected to SDS-PAGE analysis and transferred to 0.2 μm of nitrocellulose paper. Western blot analysis was then performed for the indicated proteins. All primary antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA) and used at a 1:1000 dilution except goat anti-actin HRP (Santa Cruz Biotechnology, Santa Cruz, CA, 1:10,000) and goat anti-rabbit Ig HRP (Jackson ImmunoResearch, West Grove, PA: 1:10,000). Supersignal West Pico chemiluminescent substrate (Thermo Scientific, Rockford, IL) was used for chemiluminescent detection. Blots were stripped using the Blot Restore membrane rejuvenation kit (Chemicon, Billerica, MA) according to the manufacturer’s instructions. For the experiment of involvement of PI3K/Akt on STAT3 activation, THP-1 cells (1 × 10^6 cells per treatment) were pretreated with 10 μM LY294002 (Sigma) or vehicle control DMSO for 10 min and then treated with 2 μg/ml anti-gC1qR or isotype control (IgM) for 4 h. STAT3 and Akt phosphorylation were then assessed by Western blotting as described above.

Flow Cytometry and Confocal Microscopy—Primary human monocytes (0.5 × 10^6 cells per treatment) were cultured as previously described and treated as described in the figure legend. The cells were then washed in cold stop buffer and fixed with 2% paraformaldehyde. Following fixation, the cells were permeabilized in 90% cold methanol. The cells were then blocked with 10% goat serum and stained with Alexa Fluor 647 conjugated anti-pSTAT3 Y705 (Cell Signaling Technology, Inc.). As a control for negative pSTAT3 staining by flow cytometry, monocytes were treated with HCV core protein for 4 h in the presence of JAK inhibitor 1 (Calbiochem). Data were collected on BD FACSCanto (BD Biosciences) and analyzed using FlowJo 8.8.6. For the confocal microscopy studies, THP-1 cells (0.5 × 10^6 per treatment) were treated as described in the figure legend. The cells were then washed once in PBS (0.1% BSA, 0.1% NaN₃) and then fixed and permeabilized in a 96-well plate in 100% methanol for 10 min at 4 °C. Following fixation, the cells were washed in PBS and blocked with 10% goat serum. pSTAT3 Tyr-705 (Cell Signaling Technology, Inc.) primary antibody was then added to the appropriate samples at a 1:100 dilution for 1 h at 4 °C. After two washes, Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody (Invitrogen) was added for 1 h. Nuclei were visualized using DAPI (Invitrogen). The cells were then cytoplasm onto slides with mounting media (Prolong, Invitrogen), and images were acquired using a
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Zeiss 510-UV laser scanning confocal microscope (Carl Zeiss MicroImaging, Inc., Germany) at 100×.

Gene Array and Real-time Quantitative PCR—For the analysis of the gene array, THP-1 cells (1 × 10⁶ per treatment) were treated as described in the figure legend, and mRNA from the samples was isolated using the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Using the isolated mRNA, a common cytokines PCR array was performed by SABiosciences (Frederick, MD). To verify the increased IL-6 gene induction, RNA was isolated by TRIzol reagent (Invitrogen) and reverse-transcribed (Invitrogen) according to the manufacturer’s instructions. The isolated RNA was then DNase-treated (Invitrogen) and re-suspended (Invitrogen) according to the manufacturer’s instructions. The resultant cDNA was amplified using Fast SYBR Green master mix (Applied Biosystems, Carlsbad, CA) in duplicate for each treatment. Specific primers for IL-6 (QT00083720), and hypoxanthine-guanine phosphoribosyltransferase (QT00059066) were acquired from Qiagen. The samples were assessed using the Applied Biosystems Step One Plus real-time PCR system. No template control, in which molecular-grade water was used instead of template, was included in each assay. Relative units were calculated by a comparative Ct method.

IL-6 and IL-10 Neutralization—THP-1 cells (1 × 10⁶ per treatment) were cultured as described above. The cells were then treated with 1 μg/ml or 10 μg/ml anti-IL-6 or anti-IL-10 neutralizing antibody or IgG isotype control (R&D Systems) for 30 min, and then treated with anti-gC1qR as described previously. THP-1 cells were also treated with 50 ng/ml IL-6 or IL-10 (R&D Systems Inc.) for 10 min in the presence of IL-6 or IL-10 neutralizing antibody or isotype control as a control. STAT3 phosphorylation was then assessed by Western blotting as described above.

Statistical Analysis—Unpaired Student’s t tests were used to determine the statistical significance of the data. Statistical analysis was performed using Prism 4 version 4.0c.

RESULTS

STAT3 Is Activated in Human Monocytes/Macrophages/DCs following Exposure to Extracellular HCV Core or gC1qR Agonist mAb—We have previously demonstrated that human APCs (i.e. monocytes/macrophages/DCs) became dysfunctional upon exposure to extracellular HCV core. In addition, these APCs failed to generate CD4⁺ Th1 responses (14). To identify the molecular mechanism(s) responsible for HCV core-induced APC dysfunction, we examined the activation status of important transcription factors involved in influencing APC activation and function following exposure to HCV core. The STAT family has been reported to act as key transcription factors in the regulation of APC function (23). To this end, we determined whether extracellular core affects the activation of STAT proteins (STAT1, -2, -3, -5, and -6) on human antigen-presenting cells by assessing the phosphorylation of individual STAT proteins in a human monocytic cell line (THP-1). These studies revealed that HCV core/gC1qR ligation did not affect the activation of STAT1, -2, -5, and -6 (data not shown).

Importantly, STAT3 phosphorylation levels were increased following extracellular core treatment but not by control protein β-gal treatment (Fig. 1A). In addition, when various doses of extracellular core (0, 1, 2, and 4 μg/ml) were tested for the ability to induce STAT3 activation, lower doses of extracellular core (at least 2 μg/ml) were sufficient for STAT3 phosphorylation (Fig. 1B). These results suggest that extracellular HCV core specifically activates STAT3 but not other STAT proteins. Because extracellular core has been shown to bind to the complement receptor, gC1qR, on the surface of monocytes and inhibit their activation (8), we next examined the effect of gC1qR agonist mAb on inducing STAT3 activation. STAT3 phosphorylation levels were increased by treatment with gC1qR agonist mAb (5F4) with similar kinetics to extracellular core but not isotype control (IgM) (Fig. 1C).

We next examined whether extracellular core could induce STAT3 phosphorylation in primary human monocytes, macrophages, and DCs. PBMC-derived monocytes were treated either with extracellular core, recombinant protein control β-gal, anti-gC1qR agonistic mAb (5F4), or isotype control (IgM). After 4 h, the samples were stained for pSTAT3 and examined by flow cytometry. As shown in Fig. 2, A and B, treatment with either extracellular core or gC1qR agonist mAb increased the intensity of pSTAT3-staining on primary human monocytes. To further assess the ability of extracellular core to induce STAT3 activation on human macrophages and DCs, CD14⁺ cells were isolated from PBMCs and differentiated into macrophages and DCs. These monocyte-derived macrophages and DCs were then treated with extracellular core or control β-gal for 4 h, and pSTAT3 levels were assessed by Western blot analysis. Similar to STAT3 activation on HCV core-treated THP-1 cells as shown in Fig. 1, A and B, extracellular core protein is able to induce STAT3 activation in primary macrophages and DCs (Fig. 2, C and D).

Extracellular Core or gC1qR Agonist mAb Leads to the Nuclear Translocation of pSTAT3—Upon STAT3 phosphorylation, STAT3 translocates into the nucleus and functions as a transcription factor for a number of pro-/anti-inflammatory genes (24). We sought to ensure that pSTAT3 translocated into the nucleus following exposure of extracellular HCV core on antigen-presenting cells. THP-1 cells were treated with extracellular core or gC1qR agonist mAb for 6 h, or with IL-6 for 10 min as a positive control (Fig. 3A). Nuclear localization of pSTAT3 was then assessed by confocal microscopy. Quantification of at least 70 cells per treatment per experiment shows a statistically significant increase in nuclear pSTAT3 localization in cells treated with extracellular core and anti-gC1qR for 6 h versus their respective controls (Fig. 3B). These data suggest that STAT3 activation induced by extracellular core or gC1qR mAb results in the nuclear translocation of STAT3 and subsequent induction of a number of genes linked with the impairment of classic APC activation.

STAT3 Phosphorylation by gC1qR Ligation Is Dependent on Activation of the PI3K Pathway and Requires de Novo Gene/Protein Synthesis—We have previously reported that the engagement of HCV core with the gC1qR on APCs activates the PI3K pathway and inhibits TLR4 (Toll-like receptor 4)-induced inflammatory responses (15). Therefore, we assessed whether STAT3 phosphorylation observed following treatment with extracellular HCV core or gC1qR cross-linking also requires...
PI3K/Akt activation. THP-1 cells were pretreated for 30 min with LY294002, a pharmacological inhibitor of PI3K, and then treated with anti-gC1qR antibodies or isotype control for 4 h (Fig. 4A). Phosphorylation of Akt was reduced by an average of 10.3-fold in three independent experiments. These results clearly indicate that gC1qR-mediated signaling requires the activation of PI3K/Akt for STAT3 phosphorylation.

We next sought to determine whether signaling through the gC1qR resulted in direct STAT3 phosphorylation or indirectly activated STAT3 via a secondary effect. To test this possibility, THP-1 cells were pretreated for 10 min with a transcriptional inhibitor (actinomycin D) or translational inhibitor (cycloheximide) and then treated with anti-gC1qR mAbs for 4 h. Either actinomycin D or cycloheximide treatment abolished STAT3 phosphorylation (Fig. 4, B and C). These results suggest that gC1qR-mediated STAT3 phosphorylation requires new gene synthesis and protein expression, and therefore a newly synthesized gene(s) is likely involved in activating STAT3 following HCV core/gC1qR ligation.

**IL-6 Synthesis Is Increased by Extracellular Core or gC1qR Agonist mAb and Induces STAT3 Activation**—STAT3 can be activated by a number of cytokines and growth factors (19). To identify the STAT3 activating factor following gC1qR ligation in an unbiased fashion, we assessed the gene induction of over 80 cytokines and growth factors using a PCR-based array. THP-1 cells were treated with anti-gC1qR (5F4) or isotype control (IgM) for 3 h, and the resultant RNA was analyzed. Interestingly, IL-6 and, to a lesser extent, IL-10 were both induced following anti-gC1qR mAb treatment by a 9.84- and 2.59-fold increase over the untreated control, respectively (supplemental Table 1). Real time RT-PCR analysis of THP-1 cells following treatment with extracellular core and gC1qR agonist mAb verified the induction of IL-6 mRNA expression (Fig. 5, A and B).

Because IL-6 is a strong activator of STAT3, we tested whether increased IL-6 expression by extracellular core or gC1qR ligation is involved in STAT3 activation. To test this possibility, we examined the effect of IL-6 neutralizing antibody on abrogating gC1qR-mediated STAT3 activation (Fig. 5C). THP-1 cells were pretreated with neutralizing antibodies to IL-6 or isotype control for 10 min, and cells were then treated with anti-gC1qR mAb or isotype control IgM for 4 h. To ensure that the neutralizing antibody was functioning properly, recombinant IL-6 was added to cells pretreated with neutralizing antibody (lanes 4 and 5) or control antibody (lanes 2 and 3), and STAT3 phosphorylation was then assessed by Western blotting. Consistent with previous results, the gC1qR agonist mAb is able to activate STAT3 (lane 7), but not by IgM control antibody (lane 6). Notably, neutralization of IL-6 completely inhibits gC1qR-mediated STAT3 phosphorylation (lanes 4 and 15), and IgG IL-6 control antibody did not affect STAT3 activation (lanes 10 and 11). These results suggest that IL-6 synthesis induced by extracellular...
core or gC1qR agonist mAb is responsible for activating STAT3 in an IL-6 autocrine manner. However, treatment of neutralizing IL-10 antibody did not affect STAT3 activated induced by HCV core/gC1qR ligation (data not shown).

**DISCUSSION**

In this report, we demonstrate an important role for extracellular HCV core or gC1qR signaling in the activation of STAT3 in human monocytes, macrophages, and DC via an IL-6 autocrine pathway. STAT3 activation of these cells by extracellular HCV core or an agonistic monoclonal antibody to gC1qR is dependent upon the PI3K/Akt pathway and requires newly synthesized production of IL-6. As STAT3 activation by APC plays a critical role in dampening Th1/Tc1 inflammatory responses, these data provide a novel molecular mechanism for HCV core-mediated alteration of APC activation and differentiation. Further, HCV core-induced activation of STAT3 in APCs likely contributes to
the observed impaired T cell responses in chronic HCV patients.

Interestingly, our previous report demonstrates that the activation of the PI3K/Akt pathway is responsible for the inhibition of TLR-mediated IL-12 production by HCV core/gC1qR engagement on APCs (15). Therefore the activation of PI3K/AKT appears to be central to HCV core-mediated alteration of APC function through STAT3 activation and inhibition of IL-12 production. However, it is not known whether STAT3 activation and IL-12 inhibition share common or distinct downstream signaling mediators triggered by PI3K/AKT activation following HCV core/gC1qR ligation. It has been reported that Akt, a downstream target of PI3K, regulates TLR-signaling by inhibiting the activation of MAP-KKK protein Ask1 and thereby inhibiting the activation of MAPK proteins crucial for IL-12 synthesis (25). Conversely, Akt can indirectly mediate STAT3 phosphorylation through Rac-1 dependent IL-6 production (26). These studies suggest that two distinct signaling mediators are involved in the induction of STAT3 activation and the inhibition of IL-12 production following the activation of PI3K. We are currently investigating the possibility of the involvement of Rac-1 on HCV core-mediated STAT3 activation.

Recently, it has been reported that STAT3 activation plays a pivotal role in the alteration of APC differentiation and function (21, 27) and inhibits CD4+ Th1 cell polarization (28). Importantly, STAT3 activation is crucial for inducing myeloid-derived suppressor cells (29). These regulatory myeloid cells are not a defined subset of cells but rather consist of heterogeneous populations (30). During chronic viral infection, alternatively activated macrophage and DC populations appear to be increased (31), and myeloid-derived suppressor cell (MDSC) and DC production of immunosuppressive factors restrains cell-mediated immune responses at the local inflammatory site (i.e. viral infection or tissue damage) (32). Because of our findings that extracellular core activates a crucial anti-inflammatory transcription factor, STAT3, it is likely that HCV core secreted from HCV-infected hepatocytes alters the activation and function of APCs in the local liver microenvironment and inhibits their inflammatory responses. We are currently testing this possibility by assessing STAT3 activation upon co-culture of THP-1 with HCV-infected hepatocytes (Huh7.5-JFH1). Based on the report that secreted core protein is detectable in HCV-infected hepatocyte (Huh7.5-JFH1), we expect to see STAT3 activation by THP-1 cells. Nevertheless, the altered function of macrophages and DC by secreted core could affect the differentiation and proliferation of activated T cells that have migrated to the site of HCV infection and replication. As a consequence of inhibition of T cell responsiveness by HCV core-mediated APC dysfunction, HCV infection fails to clear and establishes the persistent infection (32). Furthermore, intracellular expression of HCV core in hepatocytes has been reported to be associated with STAT3 activation (33), and this STAT3 activation enhances hepatocarcinogenesis (34). Thus, HCV core-induced STAT3 activation by both hepatocytes and APC play an important role in the development of severe chronic liver disease.

Our studies also demonstrate that a profound induction of IL-6 mRNA is found on APCs following exposure to extracellular HCV core or gC1qR agonist antibody. Abrogation of HCV core/gC1qR-mediated STAT3 activation by IL-6 neutralization antibody provides compelling evidence that extracellular HCV core or gC1qR signaling results in the activation of STAT3 via an IL-6 autocrine pathway. IL-6 is a multifunctional cytokine (35) and is involved in influencing DC differentiation (36, 37). Forced expression of IL-6 in DC results in the development of immunosuppressive DCs (36),

![Image: HCV core/gC1qR ligation results in STAT3 nuclear translocation.](image-url)

**FIGURE 3.** HCV core/gC1qR ligation results in STAT3 nuclear translocation. A, THP-1 cells were treated with 10 μg/ml HCV core protein or protein control β-gal or 2 μg/ml anti-gC1qR antibody or isotype control for 6 h. As a positive control, cells were treated with 50 ng/ml IL-6 for 10 min. The cells were then analyzed by confocal microscopy following staining with DAPI (blue) and pSTAT3 (phospho-Tyr-705, green). Data is representative of three independent experiments. B, nuclear pSTAT3-positive cells were counted from a number of fields of view of the indicated treatments. Percent positivity was calculated by dividing the number of cells with pSTAT3 staining in the nucleus by the total number of cells. Unpaired Student’s t tests were used to determine statistical significance. *, p < 0.05.
whereas IL-6 knockout mice exhibit an increased number of mature DCs (36). In addition, IL-6-dependent STAT3 activation plays a pivotal role in inhibiting APC maturation and function, leading to the suppression of CD4 Th1 responses (38). In humans, treatment of PBMCs with IL-6 results in the accumulation of MDSCs with potent immunosuppressive capabilities (39). Importantly, the suppressive capacity of melanoma-derived MDSCs is reversed in the presence of STAT3 inhibitor (40). Taken together, these studies demonstrate the importance of IL6/STAT3 signaling in regulating the immune response. Additionally, human genetic studies have recently reported that polymorphisms in the IL-6 pro-
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In summary, we report that HCV core protein transmits a signal through the gC1qR resulting in the phosphorylation of STAT3 in human monocytes, macrophages, and dendritic cells. Phosphorylation requires the activation of PI3K/Akt as well as transcription and translation. Further, we show that gC1qR-mediated STAT3 phosphorylation is dependent on an IL-6 autocrine pathway. These data suggest that HCV core/gC1qR ligation may result in altered differentiation of the monocyte/macrophage population, resulting in an impaired anti-viral immune response. This phenomenon may have important implications in HCV pathogenesis and may provide a novel immune escape strategy utilized by the virus. Further, this study highlights both STAT3 and IL-6 as attractive molecular targets for HCV therapies. Interestingly, STAT3-specific small molecule inhibitors are currently being tested in a number of cancer therapy studies (43).

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FIGURE 5. Extracellular HCV core or gC1qR agonist mAb induces IL-6 mRNA synthesis, resulting in IL-6-mediated STAT3 phosphorylation. A and B, THP-1 human monocytes were treated with 2.5 μg/ml HCV core protein or protein control β-gal or 2 μg/ml anti-gC1qR antibody or isotype control for 3 h. The samples were then assessed for production of IL-6 mRNA by real-time RT-PCR. Expression levels were normalized to hypoxanthine-guanine phosphoribosyltransferase, and fold induction was assessed relative to untreated control. Unpaired Student’s t tests were used to determine statistical significance between isotype- and anti-gC1qR-treated samples and β-gal- and HCV core-treated samples. *, p < 0.05. C, THP-1 cells were pretreated with the indicated concentration of isotype control or neutralizing IL-6 antibodies for 10 min. The cells were then treated with recombinant IL-6 (5 ng/ml) for 10 min or with 2 μg/ml anti-gC1qR antibodies or isotype control for 4 h. The cells were then immunoblotted for pSTAT3 and actin, and STAT3 phosphorylation levels were assessed with densitometry as described in the legend for Fig. 1.
