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Induction of interferon- γ contributes to Toll-like receptor-3 activated hepatic stellate cell-mediated hepatitis C virus inhibition in hepatocytes

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SUMMARY

There is limited information about the role of hepatic stellate cells (HSC) in liver innate immunity against hepatitis C virus (HCV). We thus examined whether HSC can produce antiviral factors that inhibit HCV replication in human hepatocytes. HSC expressed functional Toll-like receptor 3 (TLR-3), which could be activated by its ligand, polyinosine-polycytidylic acid (poly I:C), leading to the induction of interferon- γ (IFN- γ) at both mRNA and protein levels. TLR-3 signaling of HSC also induced the expression of IFN regulatory factor 7 (IRF-7), a key regulator of IFN signaling pathway. When HCV JFH-1-infected Huh7 cells were co-cultured with HSC activated with poly I:C or incubated in media conditioned with supernatant (SN) from poly I:C-activated HSC, HCV replication was significantly suppressed. This HSC SN action on HCV inhibition was mediated through IFN- γ , which was evidenced by the observation that antibody to IFN- γ receptors could neutralize the HSC-mediated anti-HCV effect. The role of IFN- γ in HSC-mediated anti-HCV activity is further supported by the observation that HSC SN treatment induced the expression of IRF-7 and IFN stimulated genes (ISGs), OAS-1 and MxA in HCV-infected Huh7 cells. These observations indicate that HSC may be a key regulatory bystander, participating in liver innate immunity against HCV infection using an IFN- γ -dependent mechanism.

Keywords

Hepatic stellate cells; hepatitis C virus; interferon stimulated genes; interferon- γ ; toll like receptor-3

INTRODUCTION

Hepatic stellate cells (HSC), also known as perisinusoidal cells or Ito cells, are liver pericytes that reside in the space between sinusoidal endothelial cells and parenchymal cells of the human liver. In normal liver, HSC are in a quiescent state and represent 5–8% of the total number of human liver cells (1). Quiescent HSC are rich in vitamin A and store nearly

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

80% of the retinoids of the whole body in lipid droplets in the cytoplasm (1, 2). HSC activation plays an important role in hepatic fibrogenesis. Following liver injury, HSC become activated, and activated HSC enhance migration and deposition of extracellular matrix components, resulting in liver fibrosis (3, 4). Beyond this well-known role, recent evidence indicated that HSC play a role in liver immunity. It has been reported that HSC could function as liver resident antigen-presenting cells (APC) that present lipid antigens to natural killer T (NKT) cells (5). HSC can enhance differentiation and accumulation of regulatory T cells (Tregs), which may lie at the basis of the tolerogenic nature of the liver (6). More importantly, HSC also express Toll-like receptors (TLRs). HSC express TLR-4 and can be activated by LPS, promoting liver fibrosis (7). HSC also possess a functional TLR-9 signaling (8).

The interaction between hepatitis C virus (HCV) and host innate immunity plays a key role in the immunopathogenesis of HCV disease. The host innate immune system recognizes pathogens and responds to their stimuli mainly through TLRs. TLRs are key sensors of innate immunity to pathogens. Several TLR members play a critical role in recognition of viral nucleic acids (9). TLR-3 has a crucial role in virus-mediated innate immune responses (10–12), as it recognizes dsRNA (13) that either constitutes the genome of one class of viruses or is generated during the life cycle of many viruses, including HCV (10–12, 14). Sensing through TLR-3 activates the IFN signaling pathway and induces the production of type I IFNs (IFN- α/β). IFN- α/β have been recognized as the first line of the TLR-3 activation-mediated antiviral response (15). In addition, TLR-3 signaling also induces type III IFN expression (16–18). Therefore, activation of TLR-3 by poly I:C in viral target cells could inhibit virus infections, such as herpes simplex virus-1 (HSV-1) (16), HIV (19, 20) and HCV (14).

Because the majority of HCV-infected subjects develop chronic infection, it is likely that HCV uses complex and unique mechanisms to evade or subvert the host innate immunity to establish persistent infection. In order to counteract the host cell innate immunity HCV uses mechanisms to block recognition and signaling of TLRs. Studies of HCV-host interactions have revealed that the HCV NS3/4A serine protease ablates TLR-3 signaling by cleaving the TLR-3 adaptor protein, Toll-IL-1 receptor domain-containing adaptor inducing IFN- β (TRIF) (21). Thus, to activate TLRs by their ligands represents a promising approach for the treatment of viral infections. While most studies have focused on the interactions between HCV and its target cells, hepatocytes, we know little about whether other residential cells in the liver participate in innate immune responses to HCV infection. Also there is limited information about whether liver HSC possess functional innate defense mechanism(s) responsible for enhancing liver immune responses and restricting HCV replication in hepatocytes. Thus, this study examined whether TLR-3 signaling of HSC can mount an effective innate immunity that can control HCV infection of and replication in human hepatocytes.

MATERIALS AND METHODS

Reagents

Mouse antibody against HCV core antigen was purchased from ABR Affinity BioReagents, Thermo Scientific (Rockford, IL). Mouse anti-IL-10R β antibody was purchased from R&D Systems Inc. (Minneapolis, MN) and Mouse IgG from Molecular Probes (Eugene, OR). Hoechst 33342 was also purchased from Molecular Probes (Carlsbad, CA). LyoVec transfection reagent and poly I:C (Low Molecular Weight) were purchased from InvivoGen (San Diego, CA). Bafilomycin A1 was purchased from EMD Chemical, Inc (Gibbstown, NJ). The ELISA kit for IFN- γ 1 was from eBioscience Inc. (San Diego, CA), and that for IFN- γ 2/3 was purchased from Biolegend (San Diego, CA).

Cell culture

LX-2, an immortalized human hepatic stellate cell line, was kindly provided by Dr. Scott L. Friedman (Mount Sinai School of Medicine, New York, NY). LX-2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), penicillin (100U/mL), and streptomycin (100 μ g/mL) as described (22). Huh7 cells (generously provided by Dr. Charles Rice, The Rockefeller University, New York, NY) were maintained in DMEM with 10% FBS, penicillin (100U/mL), and streptomycin (100 μ g/mL).

TLR-3 ligand stimulation

LX-2 cells were seeded at a density of 10^5 per well in a 24 well-plate. After 24 h, the cells were stimulated with TLR-3 ligand (poly I:C) using LyoVec transfection reagent. The cell culture medium was replaced with fresh medium 16 h posttransfection. Cells were collected for mRNA extraction and culture SN was collected 48 h posttransfection for HCV inhibition experiments in JFH-1-infected Huh7 cells. As a negative control of the transfection experiment, cells were incubated with the LyoVec only. For the blocking experiments using Bafilomycin A1, a vacuolar H⁺-ATPase inhibitor that inhibits the acidification of endosomes (23), LX-2 cells were treated with 100 nM of Bafilomycin A1 for 1 h prior to poly I:C stimulation.

HCV JFH-1 infection

The generation of infectious HCV JFH-1 and infection of Huh7 cells (MOI of 0.01) were carried out as previously described (24, 25). HCV JFH-1 infection of Huh7 cells was monitored by immunostaining with the mouse anti-HCV core antibody or by the real-time RT-PCR for HCV RNA.

Co-culture of Huh7 cells with LX-2 cells and SN from LX-2 cell cultures

For the co-culture experiments, LX-2 cells were first stimulated with different doses (0.25, 1 and 4 μ g/mL) of poly I:C by LyoVec for 16 h, and then co-cultured with HCV JFH-1-infected Huh7 cells in 0.4 μ m-pore-transwell tissue culture plates (Costar, Cambridge, MA). LX-2 cells were placed in the lower compartment, and Huh7 cells were cultured in the upper compartment. Huh7 cells were then collected for RNA extraction and real-time RT-PCR at 48 h after co-culture. For the experiments using LX-2 SN, HCV JFH-1-infected Huh7 cells were cultured in media with or without SN from LX-2 cells stimulated with poly I:C (5%, 10% and 20%, vol/vol) for 48 h. LX-2 SN was added to Huh7 cells infected with JFH-1 at day 3 post infection. LX-2 cells SN from incubated with LyoVec only was used as a negative control for SN treatment experiment.

RNA extraction and real-time RT-PCR

Total RNA from cultured cells was extracted with Tri-Reagent (Molecular Research Center, Cincinnati, OH) as previously described (26). Total RNA (1 μ g) was subjected to RT using the RT system (Promega, Madison, WI) with random primers for 1 h at 42°C. The reaction was terminated by incubating the reaction mixture at 99°C for 5 min, and the mixture was kept at 4°C. The resulting cDNA was used as a template for the real-time PCR quantification. The real-time PCR was performed with 1/10 of the cDNA with the iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) as previously described (27). The amplified products were visualized and analyzed using the software MyiQ provided with the thermocycler (iCycler iQ real time PCR detection system; Bio-Rad Laboratories). The oligonucleotide primers were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA) and sequences are available upon request. The cDNA was amplified by PCR and the products were measured using SYBR green I (Bio-Rad Laboratories, Inc., Hercules, CA).

The data were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and presented as the change in induction relative to that of untreated control cells.

Immunofluorescence assay

HCV JFH-1-infected Huh7 cells were cultured at a density of 10^5 /well in 24-well plates. Huh7 cells were washed with cold $1 \times$ PBS (with Ca^{2+} and Mg^{2+}) twice. Cells were fixed at 4°C in 4% paraformaldehyde-4% sucrose in PBS for 20 min followed by 0.2% Triton X-100 for an additional 10 min. Cells were blocked in Block Solution (Pierce, Rockford, IL) for 1 h at room temperature. To examine the expression of HCV core protein, HCV-infected cells were incubated with antibody to HCV core protein (1:500) for 2 h at room temperature. After washing five times with $1 \times$ PBS, the cells were incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG antibody (green, 1:100) for 1 h. After washing five times with $1 \times$ PBS, the cells were then mounted on glass coverslips in mounting media (Biomedex, Foster City, CA) and viewed with a fluorescence microscope (Zeiss, Jena, Germany). Hoechst 33342 was used for nuclei staining.

Enzyme-linked immunosorbent assay

SN collected from poly I:C-stimulated LX-2 cultures was examined for protein levels of IFN- γ 1 and IFN- γ 2/3 by ELISA, which was performed according to the manufacturer's instructions.

Statistical analysis

Student's t-test was used to evaluate the significance of difference between groups, and multiple comparisons were performed by regression analysis and one-way analysis of variance. P values of less than 0.05 were considered significant. All data are presented as mean $\pm$ SD. Statistical analyses were performed with SPSS 11.5 for Windows. Statistical significance was defined as $P < 0.05$.

RESULTS

TLR-3 signaling of LX-2 cells inhibits HCV replication in hepatocytes

We first examined whether LX-2 cells or LX-2 SN have a cytotoxic effect on Huh7 cells. Little cytotoxic effect was observed in Huh7 cells either cocultured with LX-2 cells stimulated with or without poly I:C or treated with LX-2 SN (data not shown). We then determined whether LX-2 cells stimulated with poly I:C release soluble antiviral factor(s) that suppresses HCV replication in Huh7 cells. We demonstrated that HCV JFH-1 replication was not affected in Huh7 cells stimulated with poly I:C (Fig. 1a). In contrast, HCV replication was significantly inhibited in Huh7 cells co-cultured with LX-2 cells stimulated with poly I:C (Fig. 1b). The degree of HCV suppression in Huh7 cells was correlated with the doses of poly I:C used for LX-2 cell stimulation (Fig. 1b). We then determined whether SN from poly I:C-stimulated LX-2 cell cultures have anti-HCV activity. When added to HCV JFH-1-infected Huh7 cells, SN from poly I:C-stimulated LX-2 cell cultures inhibited viral RNA expression in a concentration-dependent manner (Fig. 1c). We further examined the anti-HCV activity of LX-2 cells under three different conditions: Huh7 cells were incubated with LX-2 SN either 24 h before HCV infection, or simultaneously with HCV infection, or 8 h after infection. Cells pretreated for 24 h with 10% (v/v) of LX-2 SN and then infected had lower levels (about 90% decrease) of HCV RNA than untreated and infected cells (Fig. 1d). Similarly, cells treated with LX-2 SN and infected simultaneously or after 8 h HCV JFH-1 infection also had significantly lower levels of HCV RNA than the control cells (Fig. 1d). LX-2 SN-mediated inhibition of HCV replication was

also confirmed by diminished percentage of HCV core antigen positive cells in JFH-1-infected Huh7 cells treated with LX-2 SN (Fig. 1e).

TLR-3 activation induces IFN- γ , TLR-3 and IRF expression

TLR-3, a sensor of dsRNA, plays an essential role in initiating intracellular IFN-mediated innate immunity against virus infections. Thus, we examined whether HSC express functional TLR-3, activation of which induces IFN- γ production. We showed that poly I:C, in a dose dependent fashion, significantly induced IFN- γ 1 and IFN- γ 2/3 expression at both mRNA (Fig. 2a and b) and protein levels (Fig. 2c and d). This induction of IFN- γ in poly I:C-stimulated LX-2 cells was not affected by the exposure of the cells to HCV (Fig. 2e and f). To investigate the mechanism(s) of poly I:C-mediated induction of IFN- γ , we found that poly I:C significantly increased TLR-3 expression in LX-2 cells (Fig. 3a). Although poly I:C had little effect on IRF-3 expression (Fig. 3b), it significantly induced IRF-7 expression in LX-2 cells (Fig. 3c).

Bafilomycin A1 blocks TLR-3 activation-mediated IFN- γ expression

To further determine whether the TLR-3/IFN signaling pathway is critical in the LX-2 SN-mediated anti-HCV effect, we examined whether Bafilomycin A1, an inhibitor of the TLR-3 signaling pathway, could block the poly I:C action. As shown in Fig. 4a, TLR-3 activation-mediated IRF-7 expression was compromised by Bafilomycin A1 treatment. In addition, poly I:C-mediated induction of IFN- γ expression was inhibited by Bafilomycin A1 pretreatment (Fig. 4b and c). Bafilomycin A1 alone had little effect on the expression of IRF-7 and IFN- γ (Fig. 4).

IFN- γ is associated with anti-HCV activity of HSC

To investigate whether the induced IFN- γ is responsible for LX-2 SN-mediated anti-HCV activity, we incubated HCV JFH-1-infected Huh7 cells with antibody to the extracellular domain of IL-10R β (IFN- γ receptor) prior to LX-2 SN treatment for 1 h. As shown in Fig. 5, antibody to IL-10R β partially compromised the ability of LX-2 SN to inhibit HCV replication in Huh7 cells.

LX-2 SN induces ISG expression in HCV-infected Huh7 cells

The action of IFN on virus-infected cells elicits an antiviral state, which is characterized by the induction of IFN-stimulated genes (ISGs) (28). It is unclear, however, whether HCV-mediated suppression of intracellular immunity in infected hepatocytes can be restored by extracellular antiviral factors. We thus examined the expression of several key IFN inducible genes in HCV JFH-1-infected Huh7 cells incubated with SN from LX-2 cells stimulated with poly I:C. As shown in Fig. 6, although ISG56 and PKR were not affected, SN from TLR-3-activated LX-2 cells concentration-dependently induced OAS-1 and MxA gene expression in HCV-infected hepatocytes.

DISCUSSION

Beyond the well-known role of HSC in liver fibrosis, recent studies (5, 6, 29) indicated that HSC also play a role in liver immunity. HSC were identified as professional liver-resident antigen presenting cells (5) and regulatory bystanders, promoting Tregs and suppressing Th17 cell differentiation (6). HSC also express TLRs, including TLR-3, TLR-4 and TLR-9 (7, 8, 30). When activated by the TLR-3 ligand, HSC could produce the antiviral factors that inhibit HCV replicon expression (30). In this study, we further examined the anti-HCV activity of HSC in the HCV JFH-1 system that recapitulates viral entry, replication, and production of infectious virus. We showed that uninfected Huh7 cells pretreated with

activated LX-2 SN became less susceptible to HCV JFH-1 infection, expressing less HCV RNA than untreated cells (Fig. 1d). The protective effect on Huh7 cells by LX-2 SN was also observed even after HCV infection had taken place in hepatocytes. The induction of endogenous IFN- γ by TLR-3 signaling appears to in part contribute to anti-HCV activity of LX-2 cells, as the antibody to IFN- γ receptor could partially compromise the effect of LX-2 SN on HCV JFH-1 replication in Huh7 cells. In addition to TLR-3, poly(I:C) is also recognized by the cytosolic RNA helicases retinoic acid-inducible I (RIG-I) and melanoma differentiation-associate gene 5 (MDA-5) (31). In order to determine which pathway plays a major role in poly I:C-mediated IFN- γ induction in LX-2 cells, we used Bafilomycin A1, an inhibitor of the TLR-3 signaling pathway, to treat LX-2 cells prior to poly I:C stimulation. The observation that Bafilomycin A1 treatment could largely block the action of poly I:C on IFN- γ and IRF-7 (Fig. 4) indicates that TLR-3 activation is the key in IFN signaling of LX-2 cells.

IFN- γ has been shown to have antiviral activities against a number of viruses (32–35), including HCV (36–39). IFN- γ exhibits potent antiviral action on HCV replication in both replicon and JFH-1 infectious cell systems (33, 36, 37, 40, 41). A recent study showed that HCV infection of primary liver cells stimulates expression of IL-29 (IFN- γ 1), but not IFN- α or IFN- β (39). This production of IL-29 was sufficient to inhibit HCV infection of primary hepatocytes (39). The important role of IFN- γ in control of HCV infection is also evidenced by several recent studies showing that both spontaneous HCV clearance and a sustained viral response (SVR) after pegylated (PEG) IFN- α and ribavirin combination treatment correlated with single nucleotide polymorphisms (SNPs) in the IL28B gene locus, which encodes IFN- γ 3 (42–45). IFN- γ -based therapy for HCV genotype 1 chronic infection has been investigated in clinical trials (46, 47).

Although the mechanisms of IFN- γ -mediated antiviral activity remain to be determined, it has been proposed that similar to IFN- α/β , IFN- γ elicits an antiviral state through the induction of ISGs. IFN- γ 3 inhibited HCV replication through the activation of the JAK-STAT pathway, inducing the expression of ISGs (38). Our data showed that LX-2 SN treatment specifically induced the expression of OAS-1 and MxA in HCV-infected Huh7 cells, which provides a sound mechanism for IFN- γ -mediated HCV inhibition in Huh7 cells.

It is reported that HSC express HCV receptors (CD81, LDL receptor, and C1q) (48, 49), suggesting that HSC may be a potential target for HCV. However, there have been no reports showing that HCV can productively infect HSC. We did not observe HCV JFH-1 infection of LX-2 cells (data not shown). Nevertheless, recent studies (5, 6, 29) have suggested that HSC are involved in liver innate immunity. Our data that HSC possess a functional TLR-3 signaling system and produce IFNs that inhibit HCV replication in hepatocytes provide additional evidence to support the notion that the activation of TLR-3 signaling in bystander cells can help with the control of HCV infection/replication in the liver. This notion, however, requires future *ex vivo* and *in vivo* studies to further define the role of HSC in liver innate immunity against HCV infection.

Taken together, our finding that TLR-3 signaling of LX-2 cells induced IFN- γ expression that contributes to HCV inhibition in infected hepatocytes has clinical relevance and significance, as TLR-3 signaling of HSC may represent a novel strategy for treatment of people infected with HCV. This approach is likely to be effective as it activates the liver stellate cells to produce sufficient amount of IFN- γ , which has the ability to induce ISG expression in infected cells where the intracellular IFN signaling pathway is compromised by HCV (Fig. 7). Currently, therapeutic TLR-3 agonists have been developed for treatment of viral infections, including HCV (50). It is hopeful that future *in vivo* studies will confirm the role of HSC in liver innate immunity against HCV infection.

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Abbreviations

HSC	hepatic stellate cells
HCV	hepatitis C virus
TLR	Toll-like receptor
IFN	interferon
IRF-7	IFN regulatory factor 7
ISGs	IFN stimulated genes

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Fig. 1.

Poly I:C-stimulated LX-2 cells or supernatant (SN) from cell culture suppresses HCV replication in Huh7 cells. (a) Effect of poly I:C on HCV JFH-1 replication in Huh7 cells. Huh7 cells were infected with HCV JFH-1 for 48 h, and then stimulated with poly I:C (1 µg/mL) and total cellular RNA was extracted from cells for the HCV real-time RT-PCR. The data are expressed as HCV RNA levels relative (%) to control (without poly I:C stimulation). (b) Co-culture of HCV JFH-1-infected Huh7 cells with poly I:C-stimulated LX-2 cells. LX-2 cells were plated in the low compartment of a 24-well plate and stimulated with different doses of poly I:C (0.25, 1 and 4 µg/mL) for 16 h, while HCV JFH-1-infected Huh7 cells (day 3 postinfection) were plated in the upper compartment for co-culture. After

48 h co-culture, total cellular RNA extracted from Huh7 cells was subjected to real-time RT-PCR for HCV and GAPDH RNA quantification. (c) Effect of SN of LX-2 cell culture stimulated with poly I:C (1 μ g/mL) on HCV replication in Huh7 cells. HCV JFH-1-infected Huh7 cells (day 3 postinfection) were cultured in the presence or absence of SN of LX-2 stimulated with poly I:C by transfection at the indicated concentration for 48 h. Total cellular RNA extracted from Huh7 cells was subjected to a real-time RT-PCR for HCV and GAPDH RNA quantification. (d) Suppression of HCV RNA expression by LX-2 SN under different conditions. Huh7 cells were cultured in media conditioned with or without LX-2 SN for either 24 h prior to HCV infection, or simultaneously or 8 h postinfection. The cells were then washed five times to remove input HCV after 6 h incubation with HCV JFH1 and then cultured in the presence or absence of LX-2 SN for 72 h. Intracellular RNA extracted from hepatocytes at 72 h postinfection was subjected to real-time RT-PCR for HCV and GAPDH RNA quantification. The data are expressed as HCV RNA levels relative (%) to control (without poly I:C stimulation or without LX-2 SN treatment, which is defined as 100%). The results are mean $\pm$ SD of triplicate cultures, representative of three experiments (* $P < 0.05$, ** $P < 0.01$). (e) Effect of LX-2 SN on HCV core protein expression in Huh7 cells. HCV JFH-1-infected Huh7 cells (day 3 postinfection) were cultured in the presence or absence of poly I:C stimulated LX-2 cell culture SN at the indicated concentrations for 48 h. HCV core protein expression was determined by immunofluorescence staining with antibody against HCV core protein (green). The nuclei were stained with Hoechst 33342 (blue). One representative experiment is shown (original magnification 200 X, scale bar: 100 μ m).

Fig. 2.

Effect of TLR-3 activation on IFN-γ expression in LX-2 cells. LX-2 cells were stimulated with different concentrations of poly I:C (0.25, 1 and 4 μg/mL) for 48 h. Total RNA extracted from cells was subjected to real-time RT-PCR for the mRNA levels IFN-γ1 (a) and γ2/3 (b). SN was collected for ELISA to measure the protein levels of IFN-γ1 (c) and γ2/3 (d). (e and f) Effect of HCV on poly I:C-induced IFN-γ expression in LX-2 cells. LX-2 cells were plated in the low compartment of a 24-well plate and stimulated with poly I:C (1 μg/mL), while HCV JFH-1-infected Huh7 cells (day 3 postinfection) were plated in the upper compartment for co-culture. After 48 h co-culture, total cellular RNA extracted from LX-2 cells was subjected to the real-time RT-PCR for IFN-γ RNA quantification. The data (a, b, e and f) are expressed as mRNA levels for relative (fold) to the control (without stimulation, which is defined as 1). The results are mean ± SD of three different experiments (*P < 0.05, **P < 0.01).

Fig. 3.

Effect of poly I:C on TLR-3 and IRF expression. LX-2 cells were stimulated with poly I:C (1 μ g/mL) by transfection for 48 h. Total RNA extracted from cells was subjected to the real-time RT-PCR for the mRNA levels of TLR-3 (a), IRF-3 (b) and IRF-7 (c). The data are expressed as mRNA levels relative (fold) to the control (without poly I:C stimulation, which is defined as 1). The results are mean $\pm$ SD of three different experiments (**P < 0.01, poly I:C vs control).

Fig. 4.

Blocking TLR-3 signaling pathway counteracts poly I:C effect. LX-2 cells were pretreated with or without Bafilomycin A1 (100nM) for 1 h prior to poly I:C transfection. Total RNA extracted from cells was subjected to real-time RT-PCR for mRNA levels of IRF-7 (a), IFN- γ 1 (b) and γ 2/3 (c). The data are expressed as mRNA levels stimulated relative (fold) to the control (without stimulation, which is defined as 1). The results are mean $\pm$ SD of three different experiments (** $P < 0.01$, poly I:C vs control, or poly I:C vs poly I:C with Bafilomycin A1).

Fig. 5.

Effect of antibody to IL-10R β on LX-2 supernatant (SN)-mediated anti-HCV activity. HCV JFH-1-infected Huh7 cells (day 3 post infection) were pretreated with antibody to IL-10R β or control IgG at the concentration of 5 μ g/mL for 1 h followed with SN from poly I:C-stimulated (10%, v/v) LX-2 cells for 48 h. Recombinant human IFN- γ 3 (10ng/mL) was used as a positive control. HCV JFH-1 replication was measured by real-time RT-PCR for HCV RNA 48 h post treatment. Value is mean $\pm$ SD of three different cultures (*P < 0.05).

Fig. 6. Effect of LX2 SN on the expression of ISG56, OAS-1, PKR and MxA in HCV JFH-1-infected Huh7 cells. HCV JFH-1-infected Huh7 cells (day 3 postinfection) were cultured in the presence or absence of SN from LX-2 cells stimulated with poly I:C (1 μ g/mL) for 48 h at indicated concentrations for 48 h. Total cellular RNA extracted from Huh7 cells was subjected to real-time RT-PCR for ISG56, OAS-1, PKR and MxA mRNA quantification. The data are expressed as RNA levels relative (fold) to control (without SN treatment, which is defined as 1). The results are mean $\pm$ SD of three different experiments (* P < 0.05; ** P < 0.01).

Fig. 7.

Schematic diagram of the mechanisms involved in HSC-mediated suppression of HCV replication in hepatocytes. HCV disrupts TLR-3 signaling by cleaving the TRIF adaptor protein and suppresses IFN-β expression in hepatocytes. HSC possess functional TLR-3 signaling and can be activated, leading to production of IFN-γ, which binds to IFN-γ receptors expressed in HCV-infected hepatocytes, and induces ISGs expression that can suppress HCV replication.