Ribavirin Regulates Hepatitis C Virus Replication Through Enhancing Interferon-Stimulated Genes and Interleukin 8

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Background. The manner in which ribavirin (RBV) enhances the antiviral effects of interferon (IFN) against hepatitis C virus (HCV) remains unknown. We investigated whether RBV modifies IFN-stimulated genes (ISGs) in vivo and in vitro.

Methods. We measured the messenger RNA (mRNA) levels of ISGs in T lymphocytes from patients with HCV infection who were receiving IFN-α therapy with or without RBV. We added RBV and/or IFN-α to a plasmid-based HCV replication system containing a full-length HCV genotype 1a sequence in HepG2 and Huh7 cell lines and the JFH-1 HCV genotype 2a sequence in Huh7 cell lines and measured levels of ISGs and autocrine IFN-β.

Results. The expression of protein kinase R and myxovirus resistance A mRNA was enhanced more with IFN-α and RBV than by IFN-α alone in assays in vivo and in vitro. Such enhancement depended on autocrine IFN-β being enhanced by RBV. RBV upregulated interleukin 8 (IL-8) in the absence of IFN-α. The IL-8 upregulation induced by RBV was responsible for the activation of activator protein 1 (AP-1).

Conclusions. Ribavirin augments the anti-HCV effects of IFN-α induced by ISGs through enhancing autocrine IFN-β. Moreover, RBV can enhance IL-8 through activating AP-1. Improved understanding of ISG modulation by RBV would help to establish a means of eliminating HCV.

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease, infecting about 170 million individuals worldwide [1]. Although ribavirin (RBV) plus pegylated interferon (IFN)-α has become a standard treatment for patients with chronic hepatitis C, this therapy eliminates HCV in only up to 60% of infected individuals [2, 3].

RBV (1-β-d-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a guanosine analogue with a broad range of antiviral activity against several RNA and DNA viruses [4]. This effect has transferred over into clinical applications against respiratory syncytial virus infection [5]. Of note, RBV together with IFN-α is used to treat HCV infection [3]. Since the discovery of RBV 30 years ago, various proposed mechanisms of action have included the inhibition of HCV RNA-dependent RNA polymerase [6], promotion of the Th1 immune response [7], inhibition of inosine-5-monophosphate dehydrogenase [8, 9], and mutagenesis leading to error catastrophe [10, 11]. However, the precise mechanism of how RBV acts against HCV remains undefined. Although some evidence supports these anti-HCV actions of RBV in vitro, RBV monotherapy only minimally impacts chronic hepatitis C infection in the clinical setting [12, 13]. That is, RBV can only eliminate HCV when combined with IFN.

IFN stimulates a large number of genes called IFN-stimulated genes (ISGs) that mediate its anti-HCV effects. Moreover, host liver cells can produce autocrine IFN-β through the pathways of Toll-like receptors induced by pathogens as a defense strategy [14]. The production of IFN is thought to be essential for protection against viral infections. Some ISGs, such as...
as protein kinase R (PKR), myxovirus resistance protein A (MxA), and 2′-5′-oligoadenylate synthase (OAS) have powerful antiviral effects against HCV [15]. In particular, PKR activated (phosphorylated) by IFN phosphorylates eukaryotic initiation factor-2 subunit alpha (eIF-2α), which in turn stops protein synthesis and exerts antiviral effects [16].

Because the additive anti-HCV effects of RBV are clinically obvious [3], we postulated that RBV would enhance the antiviral effects of IFNs through ISGs. We thus investigated the mechanism of how RBV enhances ISGs.

**MATERIALS AND METHODS**

**Patients and Blood Samples**

Peripheral blood T lymphocytes were isolated from patients with chronic hepatitis C with use of the Pan T cell isolation kit II (Miltenyi Biotec) according to the manufacturer’s protocol. The patients were treated with either peg-IFN-α combined with RBV (n = 53) or peg-IFN-α alone (n = 12). Supplementary Table 1 shows the clinical features of the patients. All enrolled participants provided written informed consent to research testing under protocols approved by the institutional review boards of Ehime University (approval number 0710004). The study protocol conformed to the ethical guidelines of the Declaration of Helsinki.

**Cell Lines, Infection, and Transfection**

The human hepatoma cell lines Huh7, HepG2 (American Type Culture Collection) were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (Invitrogen).

We used the plasmid-based binary HCV replication system [17, 18], in which the plasmid harbored an infectious full-length genotype 1a cDNA sequence corresponding to the H77 prototype strain [19], to yield pT7-flHCV-Rz (pH77; provided by Dr Raymond T. Chung). We transfected Huh7 and HepG2 using 3 µg of pH77 and Lipofectamine (Invitrogen) as described elsewhere [17, 18]. The cells were infected with a replication-defective adenovirus vector containing T7 polymerase (Ad-T7pol) at a multiplicity of infection of 10 at a replication-defective adenovirus vector containing T7 polymerase (Ad-T7pol) at a multiplicity of infection of 10 at 24 hours after transfection.

We used the pJFH1-full (provided by Dr Takaji Wakita) HCV replication system encoding the HCV genotype 2a sequence in vitro [20]. Huh7 cells were resuspended in Opti-MEM I (Invitrogen) containing 10 µg of HCV RNA and electrically pulsed at 960 microfarads and 260 volts using a Gene Pulser II (Bio-Rad) for RNA transfection.

We added 100 IU/mL IFN-α (Schering-Plough) and/or 50 µM RBV (Sigma-Aldrich) as described elsewhere [10, 16, 17]. The expression of T7 polymerase was not remarkably altered by IFN-α and/or RBV (Supplementary Figure 1).

**RNA Interference and Antibody Neutralization of IFN-β**

Cells were transfected with 20 nM of a small interfering RNA (siRNA) targeting IFN-β or with a scrambled control siRNA (Invitrogen) using Lipofectamine RNAiMax (Invitrogen) at 1 day before transfection with pH77, as described elsewhere [18]. We neutralized IFN-β by adding 1.0 µg/mL of anti-human IFN-β antibody or isotype control antibody (R&D Systems) to the culture medium at 3 hours after infection and then incubated the cells for 48 hours as described elsewhere [18].

**Quantitative Real-time Reverse-Transcription Polymerase Chain Reaction**

Cellular RNA was extracted using TRIzol (Invitrogen) and digested with DNase I using a DNA free-kit (Ambion) according to the manufacturer’s protocol. Cellular messenger RNA (mRNA) was quantified using the reverse transcription of 1 µg of isolated RNA using rTth or an oligo d(T)16 (Invitrogen) primer under standard conditions [10]. Real-time polymerase chain reaction (PCR) amplification proceeded using LightCycler technology (Roche Diagnostics) and SYBR green I dye, as described elsewhere [16]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), IFN-β, OAS, MxA, and interleukin 8 (IL-8) (Search LC) were detected using primer sets under the recommended conditions. Real-time PCR amplification of HCV RNA and PKR proceeded as described elsewhere [16]. Data are expressed as copy numbers of HCV RNA or cellular mRNA per molecule of GAPDH mRNA.

**Western Blotting**

Protein extracted from cells was lysed with 100 µL of RIPA buffer [16]. Concentrations of extracted proteins in lysates were measured using the DC protein assay kit (Bio-Rad), and 30 µg of lysate was loaded in 4%–12% Bis-Tris gradient gels (Invitrogen). Proteins of interest were detected using antibodies to human PKR, eIF-2α (Santa Cruz Biotechnology), phosphorylated PKR (Invitrogen), phosphorylated eIF-2α (Cell Signaling Technology), T7 RNA polymerase (Novagen), and actin (Chemicon International), as well as species-specific conjugated secondary antibodies derived from kits (GE Healthcare).

**Enzyme-Linked Immunosorbent Assay of IL-8 and HCV Core Protein**

Concentrations of IL-8 were measured in cultured cell lysates (0.2 mg/mL) in RIPA buffer using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems) according to the manufacturer’s instructions. HCV core antigen in cell lysates (0.2 mg/mL) were quantified using HCV core antigen ELISA kits (Ortho-Clinical Diagnostics) [16]. The lower limits of detection for IL-8 and for HCV were 31.2 pg/mL and 44.4 fmol/L, respectively. All assays proceeded in duplicate.

**PCR Array for Genes Related to IL-8 Transcription**

We isolated and confirmed the quality of RNA and then performed PCR array analyses using Transcription PCR Array kits (Qiagen) according to the manufacturer’s instructions. All
individual experiments were performed 3 times, and data were analyzed using the recommended programs. Significant changes were identified in DCt (threshold cycle) data using Student t test.

Luciferase Assays
Cells were transfected with a wild type IL-8 promoter conjugated to firefly luciferase reporter constructs (pIL-8) or a mutant type (mutation of activator protein 1 [AP-1], nuclear factor κ-light-chain-enhancer of activated B cells [NF-κB], or the CCAAT/enhancer binding protein β [CEBPB] binding site), provided by Dr Charalabos Pothoulakis [21, 22], together with a control reporter plasmid conjugated with Renilla luciferase reporter constructs (phRL-TK Int-; Promega). Luciferase activities in the samples were measured 48 hours later using the Dual-Luciferase Reporter Assay System (Promega) and a luminometer (Micro-Tec). The level of transcription was evaluated as the ratio of firefly luciferase to renilla luciferase.

Statistical Analysis
All values are expressed as means and standard error (SE). Data were statistically analyzed using JMP, version 8.0 (SAS Institute). Differences in mean values were analyzed using the Wilcoxon test. P values of <.05 were considered statistically significant.

RESULTS

IFN-α Enhanced ISGs More With Than Without RBV in Patients Infected With HCV
We examined the effect of RBV combined with peg-IFN-α by measuring the time course of ISG mRNA levels in T lymphocytes isolated from peripheral blood mononuclear cells (PBMCs) collected from patients before and after therapy with IFN-α and/or RBV. Levels of PKR mRNA were significantly enhanced by the combination compared with IFN-α alone (mean ± SE at 4 hours, 668.5 ± 149.9 vs 132.5 ± 51.5; P < .05) (Figure 1). Moreover, MxA mRNA was upregulated at 4 hours after the combination was administered (mean ± SE, 0.76 ± 0.15 vs 0.33 ± 0.18; P < .05). However, OAS mRNA was not significantly changed. Mean levels of IL-8 mRNA were higher in patients treated with IFN-α and RBV than in those given IFN-α alone. However, the difference did not reach significance. The combination RBV and IFN-α rapidly
Figure 2. Interferon (IFN)–stimulated genes (ISGs) are modified by ribavirin (RBV) combined with IFN-α. A, Hepatitis C virus (HCV) RNA and messenger RNA of ISGs (protein kinase R [PKR], myxovirus resistance protein A [MxA], 2′-5′-oligoadenylate synthase [OAS], and interleukin 8 [IL-8]) were evaluated.

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upregulated both PKR and MxA mRNA, compared with IFN-α alone, in patients with HCV infection.

**IFN-α and RBV Suppress HCV Replication**

We assayed the effects of RBV combined with IFN-α in vitro. The response to autocrine IFN-β is somewhat incomplete in Huh7 cells that are used with major HCV replication systems [23]. Therefore, we investigated the anti-HCV mechanisms of RBV using a plasmid-based HCV replication system in the hepatocyte-derived cell lines HepG2 (pH77-HepG2) and Huh7 (pH77-Huh7), as well as in JFH-1 cells derived from Huh7 cells that are generally used to study HCV replication. We added IFN-α and/or RBV to these cells. IFN-α, RBV, and a combination of both each diminished HCV RNA levels (Figure 2A) and diminished the expression of HCV core proteins (Figure 2B).

**RBV Additively Upregulates ISGs With IFN-α in HepG2 but Not in Huh7 Cells**

IFN-α significantly induced PKR, MxA, and OAS mRNA during days 1–5 in all cell lines (pH77-HepG2, pH77-Huh7, and JFH-1) in vitro (Figure 2A), whereas RBV alone could not induce these ISG mRNAs without IFN-α. Note, RBV plus IFN-α additively upregulated PKR and MxA mRNA more than did IFN-α alone during days 2–5 in pH77-HepG2 cells (PKR: 3.4 ± 0.1 vs 2.3 ± 0.1 on day 2 [P < .05]; MxA: 3.5 × 10^{-3} ± 0.1 × 10^{-3} vs 2.4 × 10^{-3} ± 0.2 × 10^{-3} on day 2 [P < .05]). However, neither PKR nor MxA, both of which were additively upregulated by RBV, was identified in either pH77-Huh7 or JFH-1 cells.

We also assayed PKR and its related proteins to determine how RBV and IFN-α modulate their activities (Figure 2C). RBV and IFN-α alone and in combination elicited PKR overexpression in the 3 cell lines. The expression of PKR and of phosphorylated PKR (activated form of PKR) was most upregulated in pH77-HepG2 cells by RBV plus IFN-α rather than by either alone. Moreover, phosphorylated eIF-2α, which indicates activated PKR function, was most upregulated by RBV plus IFN-α in pH77-HepG2 cells. These data indicate that PKR and its activation are additively upregulated by RBV when combined with IFN-α rather than by IFN-α alone in pH77-HepG2 cells.

On the other hand, RBV upregulated IL-8 mRNA (Figure 2A), and unlike other ISGs, RBV further upregulated IL-8 mRNA when combined with IFN-α. We measured the amount of IL-8 protein expressed in cell lysates using ELISA (Figure 2D). We found that RBV significantly upregulated IL-8 expression with or without IFN-α in all 3 cell lines and that IFN-α additively upregulated IL-8 in pH77-HepG2 and JFH-1 cells.

The RBV-induced upregulation of ISGs was more evident in pH77-HepG2 than in either pH77-Huh7 cells or in JFH-1 cells derived from Huh7 cells. We therefore investigated the effect of autocrine IFN-β in pH77-HepG2 cells, because the response of Huh7 cell lines to this cytokine is impaired.

**IFN-α and RBV Enhanced Autocrine IFN-β in HepG2 Cells**

Autocrine IFN-β was significantly upregulated by RBV plus IFN-α, compared with IFN-α alone in pH77-HepG2 cells (4.3 × 10^{-3} ± 0.3 × 10^{-3} vs 2.7 × 10^{-3} ± 0.2 × 10^{-3} on day 2 [P < .05] and 2.1 × 10^{-5} ± 0.3 × 10^{-5} vs 0.6 × 10^{-5} ± 1.0 × 10^{-6} on day 5 [P < .05]) (Figure 2E), but not in pH77-Huh7 or JFH-1 cells. The effect of the additive upregulation of autocrine IFN-β by RBV in pH77-HepG2 cells was similar to that of PKR and MxA (Figure 2A). These results suggest that PKR and MxA are upregulated via the RBV-induced upregulation of autocrine IFN-β.

**Knockdown of IFN-β Diminished the Additive Effects of RBV Plus IFN-α**

We examined the effects of IFN-β inhibition to determine whether the upregulation of ISGs by RBV depends on an increase in autocrine IFN-β. We used pH77-HepG2 cells at day 2 after adding reagents, because ISGs were significantly upregulated with autocrine IFN-β enhancement under these conditions. Autocrine IFN-β was downregulated by >90% by IFN-β siRNA in the presence of IFN-α, RBV, or both (Figure 3A). We further assessed the modulation of ISG mRNAs via IFN-β mRNA downregulation.

IFN-α continued to upregulate PKR and MxA after IFN-β siRNA downregulation. The additive upregulation of PKR and MxA by RBV plus IFN-α was diminished (Figure 3B). These results indicated that the upregulation induced by RBV depends on its ability to increase autocrine IFN-β.

The additive upregulation of IL-8 induced by RBV plus IFN-α was diminished, compared with that induced by IFN-α alone. However, RBV continued to obviously upregulate IL-8, compared with the absence of RBV (Figure 3B). This behavior differed from that of other ISGs and suggested that RBV-induced IL-8 upregulation partly depends on autocrine IFN-β. However, the main mechanism of RBV-induced IL-8 upregulation does not depend on autocrine IFN-β.

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*Figure 2 continued.* in plasmid-based HCV replication systems with IFN-α and/or RBV in HepG2 or Huh7 cells transfected with pH77 and infected with Ad-T7 and in JFH-1 cells derived from Huh7 cells. B. HCV core protein expression was evaluated in these 3 cell lines with IFN-α and/or RBV. C. Western blots of PKR-related proteins from all 3 cell lines. D. Levels of IL-8 protein evaluated by enzyme-linked immunosorbent assay (ELISA) from total cellular extracts treated with IFN-α and RBV. E. Ribavirin plus IFN-α upregulated IFN-β more than IFN-α alone only in Ad-T7-infected HepG2 cells transfected with pH77. Data are shown as means ± standard error (SE) of 6 replicates. *P < .05, compared with absence of IFN-α and RBV; E. *P < .05 compared with RBV. *P < .05 compared with IFN-α at each time point; Wilcoxon test.
Figure 3. Silencing interferon (IFN)-β diminishes ribavirin (RBV) plus IFN-α-induced upregulation of protein kinase R (PKR) and myxovirus resistance protein A (MxA) but not of interleukin 8 (IL-8). A, Transfection with IFN-β-specific small interfering RNA (siRNA) reduced IFN-β mRNA levels by 90% with
Neutralization of IFN-β Attenuates ISG Upregulation by RBV Plus IFN-α

We neutralized secreted IFN-β by including an anti–IFN-β antibody in the culture medium and then added IFN-α and/or RBV to the cell cultures to further assay IFN-β inhibition. The upregulation of PKR and MxA induced by RBV plus IFN-α was diminished, compared with that by IFN-α alone, in cells cultured with anti–IFN-β antibody (Figure 3C). The amount of the reduction was quite similar to that induced by IFN-β siRNA. These results indicate that RBV plus IFN-α induced these ISGs through an increase in the amount of secreted IFN-β. However, RBV still upregulated IL-8 after neutralization with anti–IFN-β. These results suggest that the main pathway of RBV-induced IL-8 upregulation is not via autocrine IFN-β. These findings corresponded to those of the assay with IFN-β siRNA and confirmed that autocrine IFN-β is responsible for the RBV-induced enhancement of PKR and MxA, but not of IL-8.

IL-8 Is Enhanced by RBV Through AP-1 Signaling

The ELISA results showed that RBV increased IL-8 expression in both pH77-HepG2 and pH77-Huh7 cells (Figure 4A), but to a greater extent in the former. The results of luciferase assays using a reporter construct of the promoter region of the IL-8 gene confirmed that RBV dose-dependently upregulated IL-8 transcription, thus confirming a direct effect of RBV (Figure 4B).

Figure 4. Ribavirin (RBV) dose-dependently upregulates interleukin 8 (IL-8) expression and IL-8 transcription through AP-1 signaling. A, RBV dose dependently increased IL-8 expression in both HepG2 and Huh7 cells. B, Results of luciferase assays show that RBV dose-dependently upregulates IL-8 transcription. C, Polymerase chain reaction (PCR) array analysis of IL-8 promoter-related genes shows that RBV upregulates AP-1 related genes. D, Data from reporter constructs with or without mutations in activator protein 1 [AP-1], CCAAT/enhancer binding protein β (CEBPB), and nuclear factor κ- light-chain-enhancer (NF-κB) sites show that RBV-induced IL-8 transcriptional upregulation is responsible for AP-1. Data are shown as means ± standard error (SE) of 6 replicates. **P < .01, compared with absence of RBV; Wilcoxon test.

Neutralization of IFN-β Attenuates ISG Upregulation by RBV Plus IFN-α

We neutralized secreted IFN-β by including an anti–IFN-β antibody in the culture medium and then added IFN-α and/or RBV to the cell cultures to further assay IFN-β inhibition. The upregulation of PKR and MxA induced by RBV plus IFN-α was diminished, compared with that by IFN-α alone, in cells cultured with anti–IFN-β antibody (Figure 3C). The amount of the reduction was quite similar to that induced by IFN-β siRNA. These results indicate that RBV plus IFN-α induced these ISGs through an increase in the amount of secreted IFN-β. However, RBV still upregulated IL-8 after neutralization with anti–IFN-β. These results suggest that the main pathway of RBV-induced IL-8 upregulation is not via autocrine IFN-β. These findings corresponded to those of the assay with IFN-β siRNA and confirmed that autocrine IFN-β is responsible for the RBV-induced enhancement of PKR and MxA, but not of IL-8.

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Figure 3 continued. or without IFN-α and RBV in HepG2 cells expressing hepatitis C virus (HCV). B, Transfection with IFN-β siRNA diminished additive upregulation of PKR, MxA by RBV plus IFN-α compared with IFN-α alone, indicating that enhanced IFN-β is associated with additive upregulation of IFN stimulating genes by RBV. C, Neutralization of secreted IFN-β using anti–IFN-β antibody diminished additive upregulation of PKR and MxA by RBV, indicating that secreted IFN-β is responsible for their upregulation. However, silencing and neutralizing IFN-β did not modify RBV-induced IL-8 upregulation (B, C). Samples prepared from HepG2 cells transfected with pH77 and infected with Ad-T7 at day 2 after adding reagents. Data are shown as means ± standard error (SE) of 6 replicates. *P < .05.
We examined the mechanism of RBV-induced IL-8 upregulation using a PCR array for transcription factors (Supplementary Table 2) and identified genes associated with the IL-8 promoter (Figure 4C). RBV upregulated the AP-1-related genes c-Jun, c-Fos, JunB, JunD, and ATF3, but not CEBPB or NF-κB.

We further analyzed the mechanism of RBV-induced IL-8 upregulation. The results of the luciferase assays using reporter constructs with or without mutations in the AP-1, CEBPB, and NF-κB sites confirmed that AP-1 is responsible for RBV-induced IL-8 transcriptional upregulation (Figure 4D).

DISCUSSION

IFN is considered to be essential for eliminating HCV, and RBV has additive anti-HCV effects only when combined with IFN-α. Here, we investigated modifications of the ISGs (PKR, MxA, OAS, and IL-8) that are reportedly associated with HCV replication to determine how RBV plus IFN-α affects HCV. We found that RBV upregulates PKR activity as well as MxA and IL-8 expression through enhancing autocrine IFN-β. Moreover, RBV significantly upregulated IL-8 without IFN-α, which is responsible for AP-1 upregulation. The benefits and disadvantages of ISG modification by RBV must be understood before HCV can be eliminated with or without IFN. Moreover, such understanding might lead to a discovery of novel and more-powerful RBV-like compounds against HCV.

Our findings in vivo showed that RBV upregulates PKR and MxA mRNA in peripheral T lymphocytes from HCV-infected patients treated with IFN-α. These findings are compatible with a report describing that RBV induces ISGs in the human liver and in PBMCs [24]. The ISGs in PBMCs could be predictive factors for evaluating the effectiveness of anti-HCV therapy [24, 25]. However, this strategy is somewhat limited, because ISG expression and/or mechanisms regulating ISG expression might differ between PBMCs and liver cells [26]. We were also concerned that the PBMC population might be altered during IFN-based therapy. We therefore isolated T lymphocytes for this analysis. However, the significantly different distribution of the HCV genotype in patients with or without RBV still imposed limitations. We confirmed the alterations of ISGs by IFN-α and/or RBV using assays of hepatocytes in vitro because of limited dependence on the enhancement of autocrine IFN-α. However, RBV only affected PKR, MxA, and autocrine IFN-β, when combined with IFN-α. This phenomenon would be in accord with clinical evidence showing that RBV can only eliminate HCV when combined with IFN-α [30], because the upregulation of autocrine IFN-β by RBV alone is insufficient. Moreover, the less dramatic induction of autocrine IFN-β by RBV would be comparable to the clinical significance of the anti-HCV effects elicited by RBV.

The upregulation of PKR and MxA through IFN-β enhancement will be important for the anti-HCV effects of RBV, but RBV also dose-dependently elevated IL-8 in a process that partially depends on the enhancement of autocrine IFN-β. However, the main mechanism of IL-8 enhancement was independent of autocrine IFN-β. IL-8 is a chemokine that serves as a chemical signal to attract neutrophils at sites of inflammation, and therefore, it is also known as neutrophil chemotactic factor [31]. One possible benefit of RBV-induced IL-8 upregulation is that neutrophils recruited by IL-8 would phagocytose target antigens, which would trigger Toll-like receptors and result in increased autocrine IFN-β and ISG production in vivo. On the other hand, the negative regulation of anti-HCV activity by IL-8 [32] suggests that the upregulation of IL-8 induced by RBV would not benefit HCV elimination [33, 34]. Moreover, IL-8 is related to inflammation including viral hepatitis and would increase oxidant stress [35], which might weaken the effects of anti-HCV drugs. Whether the IL-8 upregulation by RBV would positively or negatively affect the anti-HCV activity of IFN in the body should be evaluated in a future study in vivo that would include the immune systems.
Autocrine IFN-β partially affected RBV-induced IL-8 enhancement, but it mostly depended on the activation of transcription by AP-1, and not by NF-κB or CEBPB. Activator protein-1 is a family of transcription factors comprising homodimers or heterodimers of Jun and Fos or activating transcription factor proteins [36]. Jun-Jun and Jun-Fos dimers preferentially bind to the phorbol 12-O-tetradecanoate-13-acetate–responsive element located in the IL-8 promoter. The results of the PCR array showed that RBV upregulates the Fos and Jun families. These AP-1 proteins are regulated by mitogen-activated protein kinases, which in turn are partially regulated by Toll-like receptor signaling pathways [36]. To clarify the role of RBV-induced AP-1 activation in the context of HCV infection would be a target of further investigation.

In summary, we showed that RBV additively upregulates PKR and MxA through enhancing autocrine IFN-β and IL-8 through AP-1 activation. These properties of RBV could both positively and negatively affect HCV elimination. Understanding the precise effects of RBV in the liver infected with HCV would be essential to developing a suitable therapeutic strategy with which to eliminate HCV in the clinical setting.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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