Interferons α and λ Inhibit Hepatitis C Virus Replication With Distinct Signal Transduction and Gene Regulation Kinetics

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Background & Aims: Hepatitis C virus (HCV) is a major cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma. Current therapy with pegylated interferon α (IFN-α) in combination with ribavirin is associated with adverse effects and often fails to induce a sustained response. IFN-λs, recently discovered IFN gene family members, exhibit antiviral and cell stimulatory activities similar to IFN-α. We aimed to determine whether IFN-λ exhibits antiviral activity toward HCV and to compare the signal transduction and effector gene pathways with those of IFN-α. **Methods:** Using the HCV replicon system and cell culture infectious reporter virus, we compared IFN-α and IFN-λ effects on HCV RNA replication and protein expression, as measured by quantitative reverse-transcriptase polymerase chain reaction, luciferase expression, and Western blot. Receptor expression and signaling pathways were explored using flow cytometry and Western blot. IFN-α- and IFN-λ-mediated gene expression changes were compared using microarray analyses. **Results:** IFN-λ exhibited dose- and time-dependent HCV inhibition, independent of types I and II IFN receptors. The kinetics of IFN-λ-mediated signal transducers and activators of transcription (STAT) activation and induction of potential effector genes were distinct from those of IFN-α. IFN-λ induced steady increases in levels of known interferon stimulated genes (ISGs), whereas IFN-α ISGs peaked early and declined rapidly. IFN-λ inhibited replication of HCV genotypes 1 and 2 and enhanced the antiviral efficacy of subsaturating levels of IFN-α. **Conclusions:** These results demonstrate distinct differences in IFN-λ- and IFN-α-induced antiviral states. Understanding these differences may prove useful for developing new HCV treatment strategies.

Hepatitis C virus (HCV) infection is a growing public health problem affecting 170 million people worldwide (approximately 3 million in the United States). Acute HCV infection is largely asymptomatic with 60%–70% having no apparent symptoms.[1] Only a minority of those infected is able to clear HCV after several months.[2,3] Instead, 70%–80% of patients become chronic carriers who, in addition to being the source for most new infections, can progress to chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[3] These clinical sequelae now comprise the leading indication for liver transplantation in the United States and account for significant morbidity and mortality each year.[4]

Despite its potentially grave clinical consequences, the only licensed therapy for chronic HCV infection is pegylated interferon (IFN)-α, either alone or in combination with the nucleoside analog ribavirin. This therapy is expensive, associated with poor response rates, and laden with significant adverse effects.[5] The treatment is capable of inducing sustained virologic responses in only approximately 50% of HCV genotype 1-infected patients.[6,7]

Until recently, the study of HCV was hampered by the lack of a suitable tissue culture system. With advent of the replicon system (see Lindenbach and Rice[8] for review; Figure 1A), it became possible to study the effects of immunomodulatory cytokines such as IFNs, interleukin (IL)-1β, and tumor necrosis factor (TNF) family members on HCV replication in vitro.[9–16] The further development of a cell culture system allowing the complete replication of HCV[17] opens up the possibility to study IFN effects on the complete HCV life cycle. IFNs exhibit a broad range of antiviral mechanisms in vivo, both, directly, by inducing an antiviral state through expression of various intracellular genes[18,19] and, indirectly, by stimulating immune effector functions including activation of natural killer and macrophage cells and up-regulation of major histocompatibility complex expression on and activation of professional antigen-presenting cells.[20,21] In the HCV replicon system, addition of IFN-α and IFN-γ has been shown to inhibit HCV RNA replication in a dose-dependent manner.[9,11,12] Recent findings indicate that, when used in combination, IFN-α and IFN-γ act synergistically to down-regulate HCV RNA levels in replicon-containing cells.[22] Although less efficient than IFN-α and IFN-γ, the addition of IL-1β to HCV replicon-harboring Huh-7 cells has also been shown to reduce the level of HCV RNA.[16] Interestingly, although TNF-α shares overlapping sets of antiviral defense mechanisms with the cytokines mentioned above and contributes to the clearance of other viral infections,[23–25] it does not have any effect on HCV replication in Huh-7 hepatoma cells.[10,14]

Type I IFNs are a homologous family of cytokines clustered on chromosome 9 that include 13 IFN-α isotypes, IFN-β,

Abbreviations used in this paper: DCs, dendritic cells; EMCV, encephalomyocarditis virus; HCVcc, HCV cell culture virus; IFIT, interferon-induced protein with tetratricopeptide repeats; IFN, interferon; IRES, internal ribosome entry site; ISGs, interferon-stimulated genes; ISGF3, interferon-stimulated gene factor 3; ISRE, interferon-stimulated response element; neo, neomycin phosphotransferase; RT-PCR, reverse transcription-polymerase chain reaction; STATs, signal transducers and activators of transcription; TCID, tissue culture infectious dose; VSV, vesicular stomatitis virus.

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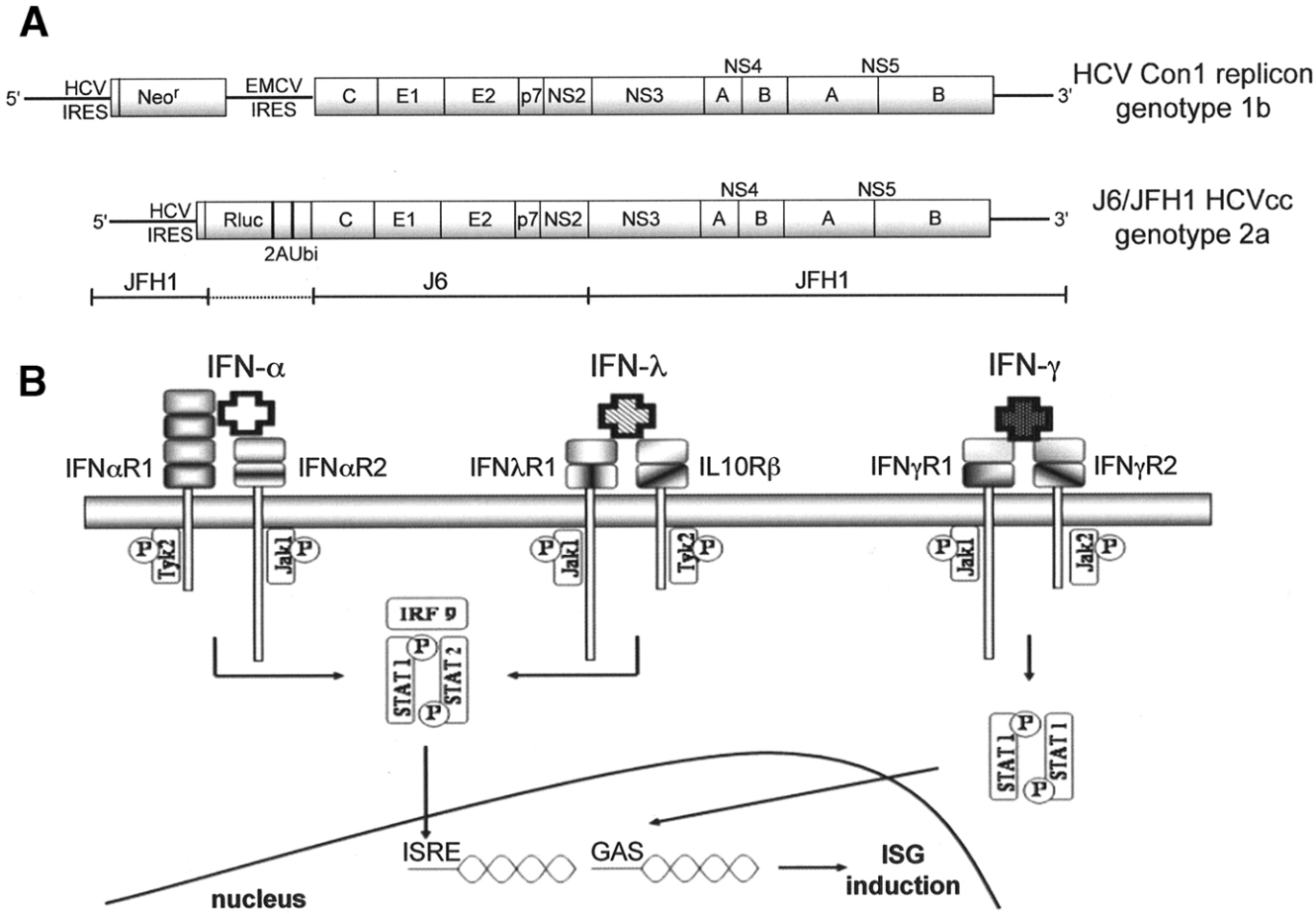

Figure 1. Schematic of the HCV full-length replicon, HCVcc genome and IFN pathways. (*A*) HCV-Con1 (*top*) is a full-length genotype 1b bicistronic replicon containing the HCV 5′nontranslated region plus nucleotides 342 to 389 of the core coding region, a neomycin resistance gene, the EMCV-IRES, the entire HCV polyprotein coding sequence, and the HCV 3′nontranslated region. The FL-J6/JFH-5'C19Rluc2AUbi HCVcc genotype 2a genome (J6/JFH1 HCVcc, *bottom*) contains J6-derived structural genes and JFH1-derived nonstructural and nontranslated regions as indicated. The amino terminus of the polyprotein contains a ubiquitin- and self-cleavable (2AUbi) Renilla luciferase cassette (Rluc). (*B*) Binding of IFN-α, IFN-γ, and IFN-λ to their distinct heterodimeric receptors leads to activation and translocation of STAT molecules that induce the expression of interferon-inducible genes (ISG) through the interferon-stimulated response element (ISRE) or γ-activated sequence (GAS).

IFN-ω, IFN-κ, and IFN-ε.[26,27] All of these transduce their signals through the same IFN-α/β receptor that is formed by 2 subchains: IFN-αR1 and IFN-αR2. IFN-γ, located on chromosome 12, is the only member of the type II IFN group and binds to the IFN-γ receptor that is composed of the IFN-γR1 and IFN-γR2 subchains. A newly discovered family of cytokines, termed either IL-28A, IL-28B, and IL-29[28] or IFN-λ1, -λ2, and -λ3,[29] utilize a heterodimeric receptor consisting of the newly identified IFN-λR1 and a second subunit, IL-10R2 (Figure 1B). These cytokines are functionally similar to type I IFNs; they have been shown to render cells resistant to encephalomyocarditis virus (EMCV) or vesicular stomatitis virus (VSV) infections, and their expression is induced by viral infections in various cell lines suggesting similar regulatory and functional effector elements to type I IFNs. Recent studies show some specificity to their antiviral effects and differences in efficacy in vitro vs in vivo. Although IFN-λ was not noted to have antiviral activity against HSV-2 in vitro, it demonstrated substantial antiviral effects in vivo, highlighting the possibility that the majority of IFN-λ's antiviral effects may involve immune modulation.[30] Similar to type I IFN signaling, IFN-λ receptor engagement leads to the formation of the IFN-stimulated gene factor (ISGF) 3 and subsequent transcription of IFN-stimulated response element (ISRE) controlled genes encoding 2′5′OAS or MxA protein.[29] Despite the functional similarities with type I IFNs, clear evidence exists that IFN-λs represent a distinct family.[31] In a recent paper, Robek et al showed that, in an immortalized murine hepatocyte cell line (HBV-Met), IFN-λ inhibits hepatitis B virus replication and leads to the induction of interferon-stimulated gene (ISG)15 and interferon-induced protein with tetratricopeptide repeats (IFIT)3.[32] The induction of the observed genes appeared to be prolonged relative to those induced by IFN-β, which peaked at 6 hours and declined thereafter. In addition, treatment of Huh-7 cells harboring the subgenomic or full-length HCV replicons with 100 ng/mL of IFN-λ resulted in induced MxA expression and a >90% reduction in HCV RNA levels.[32] However, the role of the IFN-α and IFN-γ receptor molecules in IFN-λ-mediated effects on HCV replication and the involved signal transduction pathways that lead to IFN-λ-specific gene expression were not studied.