

Host Factors and Failure of Interferon- α Treatment in Hepatitis C Virus

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Failure of interferon- α (IFN- α) treatment in patients with chronic hepatitis C virus (HCV) infection is a challenging obstacle for clinical and experimental hepatology. Both viral and host factors have been implicated in reducing responsiveness to IFN- α therapy. The role of viral factors has been studied extensively and has been summarized in several review articles; however, much less attention has been paid to host factors. In this paper, we review evidence of host factor involvement in IFN- α treatment failure. We discuss possible underlying mechanisms responsible for these effects. Potential therapeutic strategies to enhance the effectiveness of IFN- α therapy for HCV are also proposed. (HEPATOLOGY 2004;39:880–890.)

Chronic HCV infection affects approximately 4 million Americans and more than 170 million people worldwide. Of those infected, 20% to 50% will develop cirrhosis and hepatocellular carcinoma.^{1,2} Currently, the standard pharmacological treatment for HCV infection is IFN- α in combination with ribavirin. Unfortunately, more than 60% of patients with chronic HCV infection either will experience no control of viral replication by therapy (nonresponders) or will experience a relapse when therapy is stopped (relapsers).^{1,3} The molecular mechanisms underlying failure of IFN- α treatment are not well understood, but evidence indicates that both viral and host factors are involved. Viral factors,

such as viral genotype and viral load, are known elements associated with a poor response to IFN- α therapy.^{1,4} Individuals infected with the HCV genotype 1, those having a high viral load at baseline, or both, tend to be more resistant to IFN- α therapy compared with individuals infected with other HCV genotypes or those having low viral loads.^{1,4} The molecular mechanisms responsible for virally mediated resistance to IFN- α therapy have been studied extensively and have been summarized in several reviews.^{4–8} In addition to viral factors, several host factors also are implicated in modulating the effectiveness of IFN- α therapy for the treatment of HCV infection.⁹ Likely, these host factors play an equally important role in modulating the efficacy of IFN- α treatment. Therefore, an understanding of how these factors influence IFN- α therapy may provide therapeutic targets to improve the efficacy of IFN- α treatment. In this paper, we review the evidence of host factors that may antagonize the effectiveness of IFN- α therapy and discuss the underlying mechanisms. Several potential therapeutic strategies targeting these host factors are also proposed.

Signaling and Antiviral Activity of IFN- α in the Liver

IFN- α was initially discovered because of its ability to interfere with viral replication. Now, IFN- α is known to exert a wide range of biological effects, including antiviral, antiproliferative, and immunomodulatory activities.¹⁰ The action of human IFN- α is mediated through interactions with a multisubunit cell surface receptor consisting of two distinct receptor subunits, IFN- α receptor 1 (IFNAR1) and IFNAR2.¹⁰ Currently, only one form of the human IFNAR1 chain in existence has been identified. In contrast, there are three forms of the human IFNAR2 subunit: the full-length IFNAR2c, and the two truncated IFNAR2b and IFNAR2a isoforms. The IFNAR2c variant is involved in ligand binding and signal transduction, whereas both the IFNAR2b and IFNAR2a truncated forms lacking intracellular domains inhibit IFN- α signaling through competition with IFNAR2c for binding the IFN- α ligand.¹¹ In human hepatocytes, high levels of IFNAR1 and the functional IFNAR2c forms are expressed, which provides a molecular rationale for IFN- α treatment in chronic HCV infection.¹²

The IFN- α signaling cascade is initiated when IFN- α binds the receptor (Fig. 1). Binding of IFN- α to the receptor leads to activation of IFNAR-associated tyrosine kinases (Janus kinase 1 [JAK1] and tyrosine kinase 2 [Tyk2]), which phosphorylate both IFNAR1 and IFNAR2 subunits. The phosphorylated IFNAR1 provides a docking site for the Src-homology 2 domain-containing signal transducer and activator of transcription factor 2

Abbreviations: IFN- α , interferon- α ; HCV, hepatitis C virus; IFNAR, IFN- α receptor; JAK, Janus kinase; STAT, signal transducers and activators of transcription factor; PKR, protein kinase R; HBV, hepatitis B virus; SOCS, suppressor of cytokine signaling; CIS, cytokine-inducible Src-homology 2-containing protein; IL, interleukin; TNF, tumor necrosis factor; PTP, protein tyrosine phosphatase; SHP, tyrosine phosphatase; PIAS, protein inhibitor of activated STAT; MAP, mitogen-activated protein; HLA, human leukocyte antigen.

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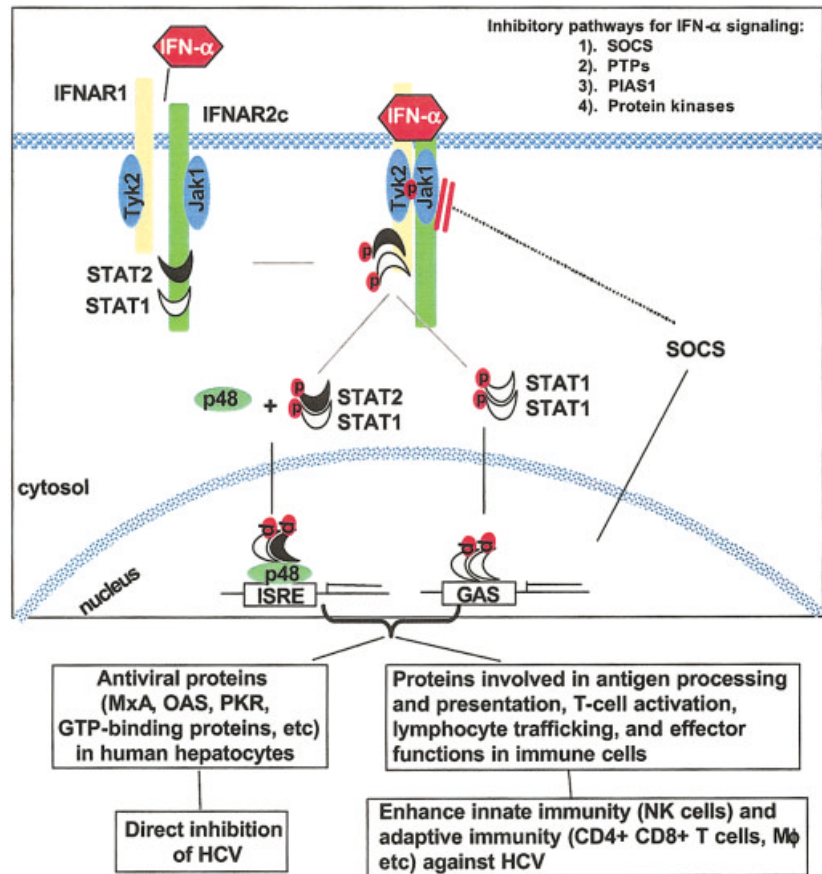


Fig. 1. IFN- α activates signaling pathways and antiviral mechanisms against HCV. The binding of IFN- α to its receptor causes IFNAR1 and IFNAR2c dimerization, followed by phosphorylation of JAK1, Tyk2, STAT1, and STAT2. Phosphorylated STAT1 and STAT2 and the Interferon regulatory factor 9/p48 protein form an active transcription factor complex, which translocates into the nucleus and binds to IFN-stimulated response element and initiates transcription of many target genes. IFN- α also induces formation of other STAT complexes such as STAT1:STAT1, STAT1:STAT3, and STAT3:STAT3, which bind to γ -activated sequences on the promoters of different genes. The antiviral activity of IFN- α against HCV is likely to be mediated via induction of antiviral proteins in hepatocytes that directly inhibit HCV replication and induction of immunoregulatory proteins in immune cells that enhance both innate and adaptive immunity against HCV. Several inhibitory proteins are likely involved in terminating IFN- α signaling. These include SOCS, PTPs, PIAS, and protein kinases.

(STAT2), which then becomes phosphorylated by Tyk2 or JAK1 on tyrosine residue 690. The other STATs, including STAT1, STAT3, and STAT5, subsequently are recruited to the membrane for phosphorylation and activation. The activated STAT1 and STAT2 monomers then are released back into the cytosol, where they form heterodimers and are joined by an Interferon regulatory factor 9/p48 protein to form an active transcription factor complex known as IFN-stimulated gene factor 3. The complex translocates into the nucleus and binds to IFN-stimulated response element to initiate transcription of target genes, including several antiviral and immunoregulatory proteins.¹⁰ IFN- α also induces formation of other STAT complexes, including STAT1:STAT1, STAT1:STAT3, and STAT3:STAT3, which bind to the γ -activated sequence in the promoter regions of IFN-inducible genes. In primary human hepatocytes, IFN- α activates STAT1, STAT2, STAT3, and STAT5, followed by induction of a wide variety of antiviral and proapoptotic genes that may contribute to the antiviral and antitumor activities of IFN- α in human livers.¹²

IFN- α in combination with ribavirin is the only currently available treatment choice for chronic HCV infection. IFN- α exerts antiviral activity against HCV by both inducing antiviral gene products responsible for interrupting viral

replication in hepatocytes^{12,13} and by modulating the immune system (Fig. 1).^{14,15} Using microarray techniques, we have demonstrated that IFN- α treatment induces expression of a variety of genes in primary human hepatocytes, including three major antiviral proteins: MxA, 2'-5' oligoadenylate synthetase, and protein kinase R (PKR).^{12,13} These antiviral proteins inhibit many viruses,¹⁶ but do not appear to be involved in the anti-hepatitis B virus (HBV) effects induced by IFNs as demonstrated in a transgenic mouse model of HBV infection.¹⁷ The antiviral effects of these antiviral proteins on HCV currently remain obscure. IFN- α treatment also induces expression of many other hepatocellular genes that either have direct antiviral effects or have immunoregulatory effects, which may play a more prominent role in IFN- α -mediated anti-HBV effect.¹⁸ These genes encode proteins including immunoproteasome and ubiquitin-like proteins, guanosine 5'-triphosphate-binding proteins, chemokines, signaling molecules, and antigen processing-related proteins.¹⁸ Additionally, IFN- α stimulates both innate and acquired immunity against HCV infection through induction of genes involved in antigen processing and presentation, T-cell activation, lymphocyte trafficking, and effector functions.^{14,15} Hence, the antiviral activity of IFN- α against HCV is most likely mediated through induction of antiviral genes

in the liver^{12,13} and enhancement of both innate and acquired immunity.^{14,15}

Negative Regulation of IFN- α Signaling

Several inhibitory pathways are identified that negatively regulate IFN- α -activated signal cascades. Because activation of these inhibitory processes can cause suppression of IFN- α therapy, understanding the molecular basis of these pathways could provide strategic advances for improving the efficacy of IFN- α therapy. Among the known inhibitory pathways, the suppressor of cytokine signaling (SOCS) family has been studied the most extensively and also is the most notably important negative factor for IFN- α signaling.¹⁹ To date, eight SOCS proteins have been identified: SOCS1–7 and the cytokine-inducible Src-homology 2-containing protein (CIS). Each factor contains a central Src-homology 2 domain and a C-terminal SOCS box. Responding to cytokine stimulation, SOCS1–3 and CIS proteins are induced rapidly and negatively regulate the initial cytokine signal responsible for its own induction in a classic negative feedback loop. The functions of the remaining SOCS 4–7 are as yet unknown. Many cytokines, including interleukin (IL)-6, IL-1, IL-10, tumor necrosis factor- α (TNF- α), growth hormone, IFN- α , and IFN- γ ,^{20–22} are known to induce SOCS1–3 and CIS expression in cultured hepatocytes and the liver. SOCS also are expressed in various models of liver injury induced by administration of CCl₄ (carbon tetrachloride)²² or Concanavalin A,²³ or by partial hepatectomy.²⁴ Experimental evidence demonstrates that the overexpression of SOCS1 and SOCS3 inhibits IFN- α -induced signaling and antiviral activity.²⁵ Because SOCS1–3 and CIS are inducible by various factors, it stands to reason that these inhibitory proteins may be an important primary mediator in reducing treatment efficacy by negatively regulating IFN- α signaling in chronic HCV infection.

In addition to SOCS, a large family of proteins that dephosphorylate activated tyrosine residues known as protein tyrosine phosphatases (PTPs) also plays important roles in downregulating IFN- α signaling. The nuclear isoform of the T-cell PTP recently has been identified as a major phosphatase responsible for dephosphorylation of activated STAT1 in the nucleus.²⁶ Other PTPs, such as tyrosine phosphatase-1 (SHP1) and SHP2, also have been shown to inhibit IFN- α signaling.²⁷ Currently, it is estimated that as many as 2,000 PTPs may be encoded in the mammalian genome, and most of them have not yet been identified. Thus, PTPs could be an important family of proteins that suppresses IFN- α signaling and thereby attenuates therapy in chronic HCV infection. Additional studies are required to identify these

PTPs. By specifically interacting with tyrosine-phosphorylated STAT1 dimers, protein inhibitor of activated STAT1 (PIAS1) has been shown to attenuate IFN- γ -activated gene expression.²⁸ Thus, it is likely that PIAS1 also inhibits IFN- α -activated STAT1 signals and antiviral activity and may be involved in the suppression of IFN- α therapy.

The JAK-STAT signaling pathway also can be inhibited through activation of multiple protein kinases. In primary hepatocytes (Hong F. and Gao B., unpublished data, 2002) and in U266 cells,²⁹ forskolin-induced protein kinase A attenuates IFN- α/β activation of STAT1. This suggests that agonists, such as glucagon and β 2-adrenergic receptor agonists, which elevate cyclic adenosine 5'-monophosphate (cAMP) levels and activate protein kinase A, negatively can regulate IFN- α signaling and can reduce antiviral ability. Additionally, activation of the p42/44 mitogen-activated protein kinase (MAP kinase) has been shown to attenuate IL-6-activated STAT3 signaling³⁰ and to downregulate IFN- α signaling.³¹ Activation of the p42/44 MAP kinase may be involved in attenuation of IFN- α therapy in patients with chronic HCV infection because hepatic activation of this kinase was reported from these patients.³²

Because effective and successful IFN- α therapy is dependent on the integrity of the signaling pathway for production of antiviral proteins, any host factor affecting the functional components of the JAK-STAT signaling cascade can negatively modulate IFN- α treatment efficacy (see Table 1). Evidence supporting this concept is discussed at length in subsequent sections.

Potential Roles of Cytokines and Chemokines in Negative Regulation of IFN- α Therapy in Chronic Hepatitis C Infection

IFN- α Receptor. IFN- α activity is mediated by interaction with the IFNAR1 and IFNAR2c subunits, triggering the JAK-STAT signaling cascade, and inducing transcription of antiviral proteins.¹⁰ Thus, expression of IFNAR1 and IFNAR2c is key to the antiviral activity of IFN- α for the treatment of viral hepatitis. Downregulation of receptor expression could cause IFN- α treatment failure. Indeed, numerous clinical reports showed that high levels of IFNAR1 mRNA and IFNAR2c mRNA in the liver correlated with a sustained response to IFN- α therapy for chronic HCV infection.^{33,34} Although the overlap between the expression of IFNAR mRNA in responders versus nonresponders was extensive, the difference between the two groups was significant ($P < .01$), indicating that treatment efficacy is dependent on IFN- α receptor expression levels. The poor responses observed in

Table 1. Involvement and the Underlying Mechanisms of Host Factors in Host Resistance and Inhibition of IFN- α Therapy

Host Factors	Modulation of IFN- α Therapy	Possible Underlying Mechanisms
Cytokines		
IFNAR	High levels of IFNAR1 and IFNAR2c in the liver correlate with a sustained response to IFN- α therapy. ^{33,34} High levels of soluble IFNAR2a correlate with a poor response to IFN- α therapy. ³⁵	IFNAR2a, a truncated form of IFNAR2c, inhibits IFN- α signaling via competition with IFN- α for binding to IFNAR2c. ¹¹
TNF- α	High TNF- α levels are associated with a poor response to IFN- α therapy. ^{41,42}	TNF- α inhibits IFN- α signaling via the induction of SOCS and SHP2. ²²
IL-10	High serum IL-10 levels correlate with a poor response to IFN- α therapy. ^{43,45,47} Inheritance of the -592A* and -819*T alleles of IL-10 promoters, producing low levels of IL-10, is associated with a sustained response to IFN- α treatment, ^{49,50} which was not confirmed by other studies. ^{51,52}	IL-10 inhibits IFN- α signaling via the induction of SOCS ²¹ ; IL-10 decreases HCV-specific IFN- γ -producing T cells and increases Th2 T-cell response ⁴⁷ ; IL-10 inhibits T-cell proliferation, MHC expression, and IL-2R expression. ⁴⁸
IL-1 β	High serum IL-1 β levels are implicated in a poor response to IFN- α therapy. ⁵³	IL-1 β attenuates IFN- α signaling by a proteasome-dependent pathway. ⁵⁶
IFN- γ	IFN- γ has been implicated in HCV clearance, ^{57,58} but has been shown to be ineffective in treatment of HCV infection. ⁶¹ IFN- γ has been shown to potentiate IFN- α therapy, ⁶³ but others reported that IFN- γ may be involved in a poor response to IFN- α therapy. ⁶⁴⁻⁶⁶	IFN- γ modulates immune response and inhibits IFN- α signaling.
IL-8	High serum levels of IL-8 correlate with a poor response to IFN- α therapy. ⁶⁸	IL-8 inhibits the antiviral activity and signaling of IFN- α in vitro and in vivo. ^{69,70}
IL-6	Low levels of IL-6 correspond with a sustained response to IFN- α therapy. ⁷²	IL-6 inhibits IFN- α signaling likely via the induction of SOCS3.
CCR5	CCR5 promoter 59029-A allele are associated with a sustained response to IFN- α therapy. ⁷³	CCR5 modulates host immune response to enhance the antiviral activity of IFN- α . ⁷³
Genetics, race, gender, age, and obesity		
HLA	HLA DR7, DRB1*0404, DRB1*0701, DRB1*11, and DQA1*0201-DQB1*02 have been associated with a sustained response to IFN- α treatment, ⁷⁴⁻⁷⁶ but others failed to confirm these associations. ⁷⁷	HLAs affect IFN- α therapy response via modulating host immune response.
Race	African American HCV patients respond poorly to IFN- α therapy. ⁷⁸⁻⁸⁰	The mechanisms are not clear and may be related to predominant infection of genotype 1 HCV in African American patients in the USA ^{79,81} and an impaired ability of IFN- α to inhibit HCV production in these patients. ⁸⁰
Age	Older individuals have a lower response to IFN- α treatment than younger individuals. ^{82,83}	More advanced liver disease and an impairment of acquired and innate immunity in the elderly may be responsible for a poor response in older individuals.
Gender	Being female and younger (≤ 39 yrs) are favorable markers for a sustained response to IFN- α therapy. ⁸²	Estrogen may enhance the efficacy of IFN- α treatment. ⁸²
Obesity	Body fat mass and hepatic steatosis have been recognized as independent risk factors for a poor response to IFN- α therapy. ^{86,87}	A decrease in IFN- α bioavailability, an impairment of immune response to HCV, and an increase in progression of fibrosis in obese patients. ⁸⁶
Exogenous factors		
Alcohol	IFN- α therapy is ineffective in patients who drink large amounts of alcohol. ⁹²⁻⁹⁴	Alcohol inhibits IFN- α signaling via PKC- and MAP kinase-dependent mechanisms. ³¹ Chronic alcohol consumption decreases STAT2 and PKR ⁹⁵ and inhibits innate and acquired immunity. ^{96,97}
Disease characteristics		
Fibrosis, cirrhosis	Fibrosis and cirrhosis are associated with a poor response to IFN- α therapy. ^{98,99}	Changes in intrahepatic inflammatory response during fibrosis progression may affect IFN- α response.
Hepatic iron load	High hepatic iron load is associated with a poor response to IFN- α therapy, ^{102,103} but others reported that iron load did not modulate responses to IFN- α therapy. ¹⁰⁴	Elevation of iron may promote liver injury and inflammation in chronic hepatitis C infection, which decreases response to IFN- α therapy. ¹⁰⁵
Viral factors via host inhibitory mechanisms		
Genotype, viral load, the duration and status of HCV infection	HCV-1a/b is the least sensitive to IFN- α treatment. Patients with high pretreatment viral titer and long disease duration have a poor response to IFN- α therapy. ⁴⁻⁸	HCV E2, NS5A, and core proteins inhibit IFN- α activation of signals and antiviral proteins (such as PKR). ⁴⁻⁸ HCV-mediated cellular immune responses may also modulate IFN- α therapy.

some patients despite high levels of IFNAR mRNA may be attributable to other factors (such as viral or host factors) that inhibit IFN- α signaling and therapy. In contrast, high serum levels of soluble IFNAR2a proteins correlated with a poor response to IFN- α treatment.³⁵ This may neglect the ability of soluble IFNAR2a to act as a dominant negative factor and to interfere with IFN- α signaling.¹¹ Although clinical evidence suggests that the levels of IFNAR1 mRNA and IFNAR2c mRNA are good predictors of therapeutic responses to IFN- α , little is known about regulation of IFNAR1 and IFNAR2c mRNA and protein expression. For example, levels of IFNAR mRNA are reportedly downregulated in fibrotic livers,³⁶ but the reason for this downregulation remains unknown. Further studies exploring how IFNAR expression is regulated in chronic HCV infection may assist in understanding IFN- α response rates and may provide therapeutic targets to improve IFN- α treatment in chronic HCV infection.

TNF- α . High levels of TNF- α are observed in liver diseases, including alcoholic hepatitis,^{37,38} fibrosis, and cirrhosis.³⁹ In viral hepatitis, elevated levels of TNF- α were found in the serum,⁴⁰ mononuclear cells, and the liver.⁴¹ Evidence suggests that elevated TNF- α plays an important role in the pathogenesis of chronic HCV infection and contributes to poor treatment responses. Reportedly, much higher levels of TNF- α in the liver and peripheral mononuclear cells were detected in nonresponders compared with responders after IFN- α therapy.^{41,42} Our laboratory demonstrated that injections of synthetic TNF- α markedly inhibited IFN- α -induced signals in the liver, and that TNF α was responsible for suppression of IFN- α signaling in the liver injury model induced by CCl₄.²² Further studies suggested that induction of both SOCS3 and SHP2 contributes to the inhibitory effect of TNF- α on IFN- α signaling in the liver.²²

IL-10. IL-10 is a pleiotropic cytokine with anti-inflammatory and immunosuppressive activities. IL-10 has no apparent antiviral activity, but may modulate IFN- α treatment outcomes in patients with chronic HCV infection. Several lines of evidence suggest that IL-10 attenuates the efficacy of IFN- α treatment. It was reported that high serum levels of IL-10 correlated with poor responses to IFN- α therapy⁴³ and recurrence of hepatitis C infection after liver transplantation.⁴⁴ Downregulation of IL-10 by ribavirin has been suggested as one mechanism contributing to the greater efficacy of combination therapy with IFN- α .⁴⁵ Two recent reports^{46,47} showed that for nonresponders, IL-10 treatment increased HCV viral burden, although such treatment normalized serum ALT levels, improved liver histologic results, and reduced liver fibrosis, suggesting that IL-10 treatment may suppress

antiviral defense mechanisms. The anti-inflammatory and proviral effect of IL-10 in patients with viral hepatitis may result from a decrease in the number of HCV-specific IFN- γ producing T cells and an increase in T helper 2 T cell response.⁴⁷ The negative effect of IL-10 on the antiviral therapy of IFN- α in HCV infection also may be the result of inhibition of IFN- α signaling via induction of SOCS²¹ and suppression of T-cell proliferation, major histocompatibility complex expression, and IL-2 receptor expression.⁴⁸ Data from several studies examining the association between IL-10 promoter polymorphisms and IFN- α treatment efficacy for chronic HCV infection provide additional support for the theory that high levels of IL-10 inhibit IFN- α therapy^{49,50}; however, other investigators have not been able to confirm these associations.^{51,52} For example, two reports^{49,50} showed that inheritance of the -592*A and -819*T alleles, which resulted in lowered expression of IL-10, corresponded significantly to a sustained response to IFN- α therapy, whereas inheritance of the haplotype GCC, which expressed high levels of IL-10, was significantly associated with nonresponsiveness to IFN- α therapy. In contrast, two recent studies^{51,52} found no correlation between genotypes of IL-10 polymorphisms and responses to IFN- α therapy. The controversial findings between these studies may be the result of different patient populations, numbers of patients, and definitions of partial and full response. Thus, the association of IL-10 promoter polymorphisms with the response to IFN therapy remains controversial and inconclusive. Further detailed analyses are required to clarify the association between IL-10 promoter polymorphisms and responses to IFN- α therapy.

IL-1 β . Levels of IL-1 β , which were significantly elevated in the serum and liver of hepatitis C patients,⁵³ were shown to inhibit IFN- α/β -activated signals and antiviral activity in monkey hepatic parenchymal cells,⁵⁴ human fibroblasts,⁵⁵ hepatocellular carcinoma G2 cells,⁵⁶ and in primary human hepatocytes (Jaruga R. and Gao B., unpublished data, 2002). Furthermore, Kishihara et al.⁵³ reported that in patients with chronic HCV infection, spontaneous production of IL-1 β by peripheral mononuclear cells was significantly decreased only in complete responders after the administration of IFN- α . This suggests that high levels of IL-1 β may contribute to the reduced therapeutic responses to IFN- α in patients with chronic HCV infection, which is likely mediated via suppression of STAT1 activity in a proteasome-dependent manner.⁵⁶

IFN- γ . IFN- γ is a type II IFN and possesses similar IFN- α activities, including antiviral, antiproliferative, and immunomodulatory activities. However, IFN- γ functions through distinct but related signaling pathways.

Binding to its receptor (IFNGR1 and IFNGR2), IFN- γ activates JAK1, JAK2, STAT1, and STAT3.¹⁰ Results obtained by Thimme et al.^{57,58} suggest that hepatic entry and accumulation of HCV-specific IFN- γ -producing T cells plays an important role in viral clearance in acute HCV infection. Moreover, IFN- γ exhibits similar antiviral activity against HCV replication in cultured Huh-7 cells^{59,60} and inhibition of HBV replication in transgenic mice,¹⁷ as IFN- α does. However, IFN- γ treatment has been shown to be ineffective for chronic HCV and chronic HBV infection,^{61,62} and is not recommended for the treatment of chronic viral hepatitis. Several studies have attempted to determine whether IFN- γ modifies IFN- α therapy, but the findings are controversial. Katayama et al.⁶³ reported that priming with IFN- γ before initiation of IFN- α treatment produced a beneficial effect in a very small percentage of chronic HCV patients through changing the balance of cytokines in the host to a Th1-type response. Carreno et al.⁶² showed that IFN- γ did not improve the efficacy of IFN- α treatment and resulted in even further decompensation of liver disease. Reports about the effects of endogenous IFN- γ on IFN- α therapy in patients with chronic HCV infection also are controversial. For example, it was reported that an intrahepatic increase in mRNA expression of Th1-like cytokines, IL-2 and IFN- γ , in patients with chronic HCV infection was strongly correlated with the severity of liver injury and poor responses to IFN- α therapy.^{64,65} Bergamini et al.⁶⁶ reported that combination therapy with IFN- α plus ribavirin significantly downregulated serum levels of IFN- γ and expression of IFN- γ in T cells relative to IFN- α monotherapy in HCV patients, which was associated with increased IFN- α responses in these patients. In contrast, different studies suggested that IFN- γ upregulation is one mechanism involved in potentiating IFN- α therapy by ribavirin.⁶⁷ Taken together, the effects of IFN- γ on IFN- α therapy in chronic HCV infection remain controversial and inconclusive. Our unpublished data show that long-term, but not short-term, treatment with IFN- γ inhibited IFN- α signaling in cultured hepatocytes. More detailed clinical studies are required to clarify the effect of IFN- γ on IFN- α therapy in chronic HCV infection.

IL-8. Polyak et al.⁶⁸ reported that levels of IL-8 were significantly higher in nonresponders relative to responders and normal healthy subjects. Because IL-8 is able to inhibit directly the antiviral activity of IFN- α *in vitro*⁶⁸ and *in vivo*,⁶⁹ higher levels of IL-8 in nonresponders may contribute in part to poor responses to IFN- α therapy. Furthermore, the nonstructural 5A protein of HCV has been shown to induce expression of IL-8 protein, which could inhibit IFN- α therapy.^{70,71}

Other Cytokines and Chemokines. In addition to the cytokines previously discussed, many other different cytokines and chemokines also are implicated in modulating IFN- α therapy. It was reported that lower levels of IL-6 corresponded with sustained responses to IFN- α ,⁷² suggesting that IL-6 may attenuate IFN- α therapy. Such inhibition likely is mediated through IL-6 induction of SOCS3.²³ The transforming growth factor- β 1 +29 C/C genotype also was implicated in enhancing patient susceptibility to chronic hepatitis C infection and reduced IFN- α treatment responses.⁵¹ A recent paper by Promrat et al.⁷³ showed that HCV patients with the CCR5 promoter 59029-A allele were more likely to exhibit a sustained virologic response to IFN- α therapy than those without the allele. The effect probably is mediated through modulation of host immune responses to enhance the antiviral activity of IFN- α .⁷³

Host Factors

Genetics, Gender, Race, Age, and Obesity. Many genetic and biological factors can modulate IFN- α therapy. These include human leukocyte antigens (HLAs), cytokine polymorphisms, ethnicity, and gender. The association between HLA and HCV treatment outcomes has been extensively studied, but the findings are variable and inconclusive. A sustained response to IFN- α monotherapy or IFN- α plus ribavirin therapy has been associated with DR7, DRB1*0404, DRB1*0701, DRB1*11, DQA1*0201-DQB1*02,^{74–76} but other studies failed to confirm these associations.⁷⁷ HLAs, which are controlled by the major histocompatibility complex genes, modulate immune response by controlling antigen presentation to T lymphocytes. Therefore, HLAs most likely modulate the outcome of HCV infection and IFN- α treatment response by influencing host immune responses. However, the link between HLA gene polymorphisms and cellular immune responses during HCV infection and IFN- α treatment has not been satisfactorily studied.

As mentioned above, the relationship between cytokine polymorphisms and response to IFN- α treatment has been investigated widely, but the data remain controversial and inconclusive. This is probably because promoter polymorphisms are far less relevant than protein expression, and how these gene promoter polymorphisms control gene transcription has not been carefully studied. Additional studies of a large population of patients are required to clarify the relationship between cytokine promoter polymorphisms and response to IFN- α treatment.

Several clinical studies reported that African American HCV patients had poor responses to IFN- α monotherapy (less 5% patients were responders).^{78–80} Such impaired

responses could be overcome partially through combination treatment with ribavirin,⁷⁹ but other studies failed to confirm such improvement.⁸⁰ There is no clear explanation as to why African American HCV patients responded poorly to IFN- α therapy. The African American HCV patients in the United States are predominantly infected by HCV genotype 1, which may explain in part their overall lower response rate to IFN- α therapy.^{78,81} However, African American HCV patients still had lower response rates to IFN- α therapy even after genotypes were corrected.⁷⁸ A recent study showed that African American HCV patients infected with genotype 1 virus had a significantly impaired first-phase viral RNA decline after IFN- α treatment compared with white subjects, suggesting that inhibition of HCV production by IFN- α is impaired in African Americans.⁸⁰ Further studies are required to clarify why IFN- α treatment fails to inhibit HCV in African Americans.

Numerous studies documented that females responded better to IFN- α therapy than did males, but some studies showed that the overall rate of successful responses to IFN- α treatment was similar in both genders. Hayashi et al.⁸² reported that the rate of complete responses to IFN- α therapy was 33.3% in men aged 39 years and younger, 25.0% in men aged 40 years and older, 75.0% in women aged 39 years and younger, and 15.6% in women aged 40 years and older, although the overall response rate to IFN- α treatment was similar in males (27.1%) and females (24.1%). This study indicated that younger women (39 years of age and younger) attain better responses to IFN- α treatment. These data strongly suggest that estrogen may enhance the efficacy of IFN- α therapy. Further studies are required to clarify the effect of estrogen in IFN- α therapy and whether estrogen replacement therapy may improve treatment efficacy.

Patient age is another factor that is associated with responsiveness to IFN- α therapy in chronic HCV infection.^{82,83} Generally, it is believed that younger individuals respond better to IFN- α treatment than older individuals. Martinot-Peignoux et al.⁸³ reported that the average age of complete responders was 35 years, which was 5 years younger than the average age of nonresponders, indicating that aging imparts a negative influence on the treatment efficacy of IFN- α . The obvious explanation is that older HCV patients are likely to have more advanced liver disease, such as fibrosis and cirrhosis (themselves predictors of poor virologic responses). Impairments of cellular, humoral, and innate immunity in the elderly may be another important factor responsible for decreasing successful responses to IFN- α treatment in older patients.⁸⁴

Body fat mass and hepatic steatosis are now recognized as important cofactors in fibrotic progression in chronic

HCV infection^{85,86} and as independent risk factors for poor responses to IFN- α therapy.^{86,87} Several mechanisms underlying the reduced treatment responses caused by obesity and hepatic steatosis have been recently proposed.⁸⁶ These include a decrease in IFN- α bioavailability, an impairment of immune responses to HCV, and an increase in fibrosis progression in obese patients.⁸⁶ Weight reduction in obese subjects has been shown to improve liver histologic results,⁸⁸ which also may improve the efficacy of IFN- α therapy in these patients.

Exogenous Factors Such as Alcohol. Alcohol consumption is considered one of the most important exogenous factors contributing to poor responses to IFN- α therapy. Alcoholics appear predisposed to hepatitis C viral infection,⁸⁹⁻⁹¹ and IFN- α therapy was reported to be ineffective in patients who consumed large amounts of alcohol.⁹²⁻⁹⁴ Alcohol consumption may also impact on adherence to IFN- α therapy. Patients are advised to cease all drinking while they receive IFN- α . Although the molecular mechanisms responsible for alcohol-mediated inhibition of IFN- α therapy are not yet fully understood, direct inhibition of IFN- α/β - and IFN- γ -activated signals in hepatocytes by alcohol is one important mechanism.³¹ This inhibition is mediated via activation of p42/44 MAP kinase and protein kinase C.³¹ Upregulated levels of p42/44 MAP kinases, and downregulated STAT2 and antiviral PKR proteins, were reported in alcoholic cirrhosis, which itself may contribute in part to the poor response.⁹⁵ Additionally, the broad immunosuppressive effects of alcohol consumption may attenuate further the immunoregulatory effect of IFN- α in HCV patients. Specifically, chronic ethanol consumption has been shown to reduce induction of viral-specific cytotoxic T-lymphocyte and natural killer (NK) cell activity, thereby reducing overall immunity to infection.^{96,97} Furthermore, alcohol consumption is associated with elevations in a variety of cytokines, all of which are implicated in inhibiting IFN- α signaling and IFN- α therapy (see the discussion above). Collectively, alcohol consumption has a deleterious effect on IFN- α therapy, and patients should avoid alcohol before, during, and after IFN- α treatment.

Disease Characteristics. Both the fibrosis score and its rate of progression have been suggested as independent predictors of response to IFN- α treatment.^{98,99} Patients with established cirrhosis are more resistant to IFN- α therapy than those who have fibrosis, whereas patients with fibrosis are less responsive to IFN- α therapy than those without fibrosis. Patients with slowly progressing fibrosis had better responses to IFN- α treatment than those with rapidly progressive fibrosis.⁹⁹ Interestingly, several recent studies showed that pegylated IFN- α plus ribavirin treatment significantly reduced the rate of fibro-

sis progression in patients with chronic HCV.^{100,101} The reason why patients with fibrosis and cirrhosis respond poorly to treatment is unclear. It may be due to changes in intrahepatic inflammatory responses during fibrosis progression, which could affect IFN- α response.

Several studies showed that high hepatic iron loads in patients with chronic HCV infection also were associated with poor responses to IFN- α ,^{102,103} but other studies reported that iron load did not modulate such responses.¹⁰⁴ The decreased response to IFN- α therapy in patients with high hepatic iron loads likely is the result of promotion of liver injury and inflammation in chronic hepatitis C infection by elevated hepatic iron.¹⁰⁵

Viral Factors via Host Inhibitory Mechanisms.

Several viral factors can predict responses to IFN- α therapy. Among them, viral genotype is the strongest predictive parameter for IFN- α response, followed by pretreatment viral titers and the duration and status of HCV infection at the time of treatment.⁴⁻⁸ High quasi-species genetic complexity at the beginning of treatment is an additionally important predictor of poor responses to IFN- α therapy.¹⁰⁶⁻¹⁰⁸ These viral factors cause resistance to IFN- α therapy through multiple complex mechanisms, including the interaction between several genomic or viral proteins and numerous host cell functions. Currently, it is believed that HCV strains are not intrinsically resistant to IFN- α treatment, but can escape antiviral responses via changes in HCV quasi-species or host inhibitory mechanisms.⁵ For example, several HCV viral proteins (E2, NS5A, and core protein) have been shown to inhibit IFN- α activation of signals and antiviral proteins (*e.g.*, PKR).⁴⁻⁸ The details about these viral factors involved directly or indirectly in resistance to IFN- α therapy have been summarized in several excellent reviews.⁴⁻⁸ The HCV-mediated cellular immune response also may modulate IFN- α therapy. However, how these immune responses affect IFN- α treatment has not been studied. HCV infection is associated with elevations in a wide variety of cytokines,¹⁰⁹ which could inhibit IFN- α signaling (see discussion in previous paragraph). For example, HCV NS5A protein has been shown to inhibit IFN- α therapy via induction of IL-8 protein expression.^{70,71}

Conclusions

Understanding the mechanisms underlying HCV- and host-mediated resistance to IFN- α therapy as well as improving the efficacy of IFN- α for chronic HCV infection continue to challenge clinical and experimental hepatology. Suppression of the pathways that negatively regulate IFN- α signaling and therapy is an obvious way to im-

prove the efficacy of IFN- α therapy. For example, blocking SOCS and PTPs are expected to enhance the antiviral activity of IFN- α . IFN- α treatment rapidly induces a variety of antiviral proteins and also stimulates expression of SOCS proteins that will in turn inactivate IFN- α signaling.¹⁹ SOCS are induced in the liver by many cytokines,²⁰⁻²² and the HCV core protein,¹¹⁰ and in several models of liver injury,²²⁻²⁴ so it is plausible to speculate that SOCS protein expression is likely elevated in the livers of chronic HCV patients. Therefore, blocking SOCS expression using antisense oligonucleotides, small interference RNA (siRNA), or dominant-negative SOCS DNA may be a practical way to enhance IFN- α signaling and to improve the effectiveness of IFN- α therapy. The T-cell PTP is a major phosphatase responsible for dephosphorylation of activated STAT1 in the nucleus.²⁶ Thus, inhibition of this phosphatase may enhance IFN- α treatment response in HCV patients. As mentioned above, multiple cytokines are implicated in attenuating IFN- α therapy, which pose as viable therapeutic targets for improving IFN- α therapy. For example, the most extensively studied cytokine is TNF- α , which has been implicated in the progression of liver injury and inhibition of IFN- α therapy in patients with chronic HCV infection⁴¹ and in blocking IFN- α signaling in mice.²² Therefore, anti-TNF- α therapy will confer beneficial effects by protecting against liver injury and improving the efficacy of IFN- α therapy in chronic HCV infection. The operational status of host immune response may be an important factor contributing to the outcome of IFN- α therapy, but this has not yet been determined unequivocally. Obviously, further studies are required to clarify the effect of host immune responses on IFN- α therapy treatment outcomes, which may provide therapeutic targets for improving the efficacy of IFN- α therapy.

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