Impaired HCV Clearance in HIV/HCV Coinfected Subjects Treated with PegIFN and RBV Due to Interference of IFN Signaling by IFN α R2a

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Enhanced endogenous interferon (IFN) stimulated gene (ISG) signature has been associated with nonresponsiveness to hepatitis C treatment using pegylated-IFN α (pegIFN α) and ribavirin (RBV) in human immunodeficiency virus/hepatitis C virus (HIV/HCV) coinfecting patients. Using a proteomic approach, we identified high levels of IFN α receptor 2a (IFN α R2a) in the serum of null responders to pegIFN α /RBV. IFN α R2a inhibited antiviral activity of all formulations of IFN α in JFH/Huh7.5 cells. Furthermore, serum from null responders, but not from those who achieved sustained virologic response, suppressed IFN-signaling and ISG expression in IFN α -stimulated PBMCs of healthy donors in an IFN α R2a specific fashion. An IFN α R2a transgenic mice model (C57BL/6) was generated that had significantly higher levels of IFN α R2a in the serum than the controls ($P=0.001$). Total ISG expression in the lymph nodes was significantly higher compared to wild-type mice (P value=0.0016). In addition, *IFITM1* and *SP110* had significantly increased expression in the liver, *IFITM1* and *ISG15* in the lymph node, and *ISG15* and *PLSCR1* in the spleen (P value<0.05). The underlying mechanism of resistance to hepatitis C treatment may involve transsignaling of the JAK/STAT pathway by the sIFN α R2a-IFN α / β complex and result in the enhanced ISG signature observed in null responders. In this regard, the transgenic mice model simulated nonresponders to IFN α therapy and provides valuable insights into the role of sIFN α R2a-IFN α interactions *in vivo*.

Introduction

HEPATITIS C VIRUS (HCV) is a single-stranded, enveloped, positive sense RNA virus that causes both acute and chronic hepatitis. Chronic HCV infection occurs in 60%–80% of patients with acute infection and can lead to many complications of the liver, including cirrhosis, hepatocellular carcinoma, and hepatic failure (Fields and others 2007). Coinfection of human immunodeficiency virus (HIV) with HCV leads to higher HCV viral load, rapid progression of liver fibrosis, liver-related mortality, and poor response to interferon (IFN)-based treatment (Alter and others 1999).

The current standard of treatment for HCV infection involves pegylated IFN α (pegIFN α) and ribavirin (RBV). This regimen may achieve viral clearance or sustained virologic response (SVR) in up to 30% of patients infected with HCV genotype 1, but is significantly lower for those of African descent or coinfecting with HIV (Fried and others 2002; Chung and others 2004; Muir and others 2004).

IFNs have remained the backbone of HCV treatment since the 1990s (Poordad and Dieterich 2012). IFNs and their receptors (IFN α R1 and 2 heterodimer) are important in antiviral and antiproliferative activities through the JAK-STAT signaling paradigm (Schindler and others 2007). Modulated by the IFN α receptor 1 (IFN α R1) and IFN α receptor 2 (IFN α R2), the type I IFN regulates transcription of IFN stimulated genes (ISGs) (Liu and others 2011). Several mechanisms have been suggested as reasons for poor HCV clearance rates among HIV/HCV coinfecting subjects. Recently we showed an enhanced endogenous ISG signature that was associated with lack of response to pegIFN α and RBV treatment in HIV/HCV infected subjects (Lempicki and others 2006). However, only limited data are available regarding the host and viral factors that result in this enhanced ISG response in some but not all HIV/HCV infected subjects. Furthermore, why these patients do not respond to exogenous IFN is unclear.

In this study, we performed a proteomic approach to identify soluble factors associated with the lack of response

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to IFN-based HCV treatment. We further tested the ability of candidate proteins to interfere with IFN signaling *in vitro* and *in vivo* using a transgenic mice model.

Materials and Methods

Study subjects

HIV/HCV coinfecting study subjects were selected based on previous treatment with pegIFN α 2a (180 mg/week) or pegIFN α 2b (1.5 mg/kg/week) with weight based RBV (1.0–1.2 g/day) for 48 weeks. Subjected belonged to 2 groups: (1) null responder (NR) those who did not have more than 2 log HCV RNA decline from baseline by 2 weeks of treatment and (2) SVR: those who had less than detectable levels of HCV RNA 24 weeks after stopping treatment. All patients signed informed consent, which was approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board.

Proteomic profiling of pooled serum by antibody array

Pooled serum was used to detect differentially expressed proteins using Cytokine 507 array (RayBiotech, Inc.) where 507 cytokine antibodies were printed on a microscope glass slide. Pooled serum at day 0 and 3 was prepared by mixing equal portions of 14 NR or 14 SVR. The samples were first labeled with Cy3 and applied to the arrays in duplicate per manufacturer's protocol. The arrays were then scanned by Agilent microarray scanner with 10 μ m resolution and their spot intensities were analyzed by Agilent Feature Extraction 9.0.

ELISA to quantify serum IFN α R2 levels

Blood was collected by venipuncture longitudinally when subjects were undergoing treatment. Serum was separated from whole blood, spun and stored at -80°C for further use. Serum was thawed and levels of soluble IFN α R2 in subjects were determined using the EIA kit (Mybiosource, Inc.) per the manufacturers' instructions. The range of IFN α R2a detected by EIA was 25–1,600 pg/mL. Serum samples were diluted 1/10 and 1/100 and tested in duplicate.

In vitro assay for biological activity of IFN α R2

Ability of IFN α R2a (R&D Biosystems) to block anti-HCV activity of various formulations of IFN α was determined using JFH/Huh7.5 cells in continuous culture system as previously described (Zhang and others 2009). Freshly isolated PBMCs from healthy donors were incubated with human sera from HIV/HCV coinfecting (NR or SVR) and IFN α 2b in the presence or absence of IFN α R2a antibody (R&D Biosystems). Total RNA was isolated and ISG expression was determined using the Applied Biosystems Custom TaqMan Expression Assay, a multiplex PCR platform.

Transgenic mice construction

Four founder clones were produced: (1) *Mus musculus* IFN α R2a (Mmu.IFN α R2a) with aminoterminal Kozak translation initiation sequence, (2) CAG2 promoter with the chicken β -actin promoter with CMV enhancer, with the se-

quence based on the pCCALL2 series vectors, and containing Gateway attL4 and attR1 sequences for multisite recombination, (3) albumin promoter with the sequence derived from literature sources and cloned from a DNA already at the NCI, Frederick Protein Expression Laboratory with Gateway attL4 and attR1 sequences, and (4) Pol2 promoter with the murine Pol2 promoter. These clones were completely sequenced to verify that they were correct and matched the expected database sequences. The clones were spectinomycin-resistant, enabling growth in any *Escherichia coli* strain using 50 μ g/mL spectinomycin to maintain the plasmids.

Expression clones were produced by using Multisite Gateway recombination to merge promoters with the IFN α R2a gene into constructs utilized to generate transgenic mice. Three expression clones were created, including the CAG-Mmu.IFN α R2a, Alb-Mmu.IFN α R2a, and Pol2-Mmu.IFN α R2a. DNA was prepared using the Sigma GenElute maxiprep kit and verified by agarose gel electrophoresis.

The construct DNA from 3 expression clones were microinjected onto fertilized eggs of C57BL/6 mice to create the first generation of IFN α R2a transgenic mice in the NIH animal facility in Frederick, MD and housed under the NIH animal welfare rules and regulations. All 3 transgenic mice strains were developed and confirmed to have overexpression of sIFN α R2a. The presence of the transgene was confirmed using the Sigma-Aldrich Extract-N-Amp Tissue PCR kit and 2% agarose gel electrophoresis from mice tails. The overexpression of sIFN α R2a was verified by Western blot analysis using mice serum. Two microliters of serum was added to 24 μ L water, 10 μ L 4 $\times$ LDS, and 4 μ L β -Me and heated for 10 min at 70°C . Samples were run on -12% BT gels with 5% milk in TBS-T (0.05% T in TBS) as blocker. The transgene was detected using 1 μ g/mL rat anti-IFN α R2 in 5% milk TBS-T and 1:10,000 anti-rat HRP in 5% milk TBS-T.

The CAG-IFN α R2a transgenic mice with the chicken β -actin promoter were backcrossed to produce the stable second generation of transgenic mice ($n=9$). C57BL/6 mice were used as wild-type controls ($n=4$).

ISG expression

RNA was extracted from lymph node, spleen, and liver of 6–12-week-old transgenic and control mice using the Qiagen RNeasy Mini Kit and cDNA was generated using the Applied Biosystems High-Capacity cDNA Archive Kit. Real-time PCR was performed using the 96-well Applied Biosystems Custom TaqMan Expression Assay to analyze ISG expression in each tissue type. The following ISGs were analyzed: *IFI27L1*, *IFITM1*, *IRF3*, *ISG15*, *ISG20*, *OAS1b*, *PLSCR1*, *SP110*, and *STAT1*. Each ISG expression was normalized to that of *GAPDH*.

Statistical analysis

The *F*-test to compare variances and unpaired *t*-test were used to determine statistical significance of high levels of IFN α R2a and mean ISG expression in NR versus SVR. Paired *t*-test was used to determine statistical significance of inhibitory effects of IFN α R2a on IFN α . A Student's *t*-test was used to determine statistical significance of ISG expression in transgenic mice compared to control mice.

FIG. 1. (a) Differential expression of serum proteins before and 3 days after treatment with pegylated interferon $\alpha 2$ (pegIFN $\alpha 2$) and ribavirin (RBV) among null responders (NR) and sustained virologic responders (SVR). Serum proteins from 10 NR and 10 SVR human immunodeficiency virus/hepatitis C virus (HIV/HCV) coinfecting subjects were identified using a Ray Biotech protein array as described in the materials and methods. The differentially expressed proteins were selected using a rigorous statistical analysis with a P value < 0.01 and fold change > 2 . The color red indicates high expression and the green color indicates low expression of the respective protein. Overall, there were no statistically significant differences in the expression of proteins between day 0 and 3 in both groups. As shown in figure, 15 serum proteins were expressed at lower levels on day 0 and 3 among NRs when compared to the levels of expression in SVR. Similarly, 10 serum proteins were expressed at higher levels on day 0 and 3 among NR compared to that observed in SVR. (b) Elevated levels of soluble IFN α/β receptor 2 in the serum of null responders, but not sustained responders who were treated with pegIFN $\alpha 2$ and RBV. Levels of IFN α receptor 2a (IFN α R2a) in serum were quantitated using an EIA as described in the Materials and Methods section. As shown in Fig. 2, null responders had significantly higher levels of soluble IFN α R2a in their serum compared to SVR (P value < 0.0001).

Results

Null responders to PegIFN and RBV have increased IFN α R2a in serum

To identify the soluble factors differentially expressed in the serum of NR and SVR subjects, we performed proteomic analysis using an antibody array (Raybiotech). Twenty-five proteins were selected based on criteria of P value < 0.05 and fold change greater than 2 as shown in Fig. 1a. There were no differences in proteomic levels between day 0 and 3 in both NR and SVR subjects. Fifteen proteins were upregulated in SVR compared to NR, and 10 proteins were upregulated in NR compared to SVR. Among the 10 upregulated proteins in NR, high levels of sIFNAR2a in the serum of NR were validated using a confirmatory ELISA as shown in Fig. 1b by the unpaired t -test (P value < 0.0001) and F -test to compare variances (P value = 0.005). Due to the potential biological significance of the molecule, IFN α R2a was selected for further validation by EIA.

IFN α R2a inhibits antiviral activity of all formulations of IFN α

We then tested whether the presence of high levels of IFN α R2a could inhibit the biological activity of IFN α . Figure 2 illustrates the inhibitory effect of IFN α R2a on different formulations of IFN α in JFH/Huh7.5 cells. The addition of IFN α R2a reduced suppression of HCV replication by IFN α -2b from $64.7\% \pm 13.0\%$ to $20.7\% \pm 5.8\%$ (P value = 0.0128). In PegIFN α 2b, suppression of HCV replication was reduced

FIG. 2. Soluble IFN α/β receptor 2 can inhibit the anti-HCV effects of various IFN α formulations *in vitro*. Using an *in vitro* HCV continuous culture system (J6/JFH-1/Huh7.5), copy numbers of J6/JFH-1 were quantitated by real-time PCR in culture supernatants with or without addition of equimolar IFN α formulations (IFN α 2b, pegIFN α 2a, pegIFN α 2b, AlbIFN α 2b) in the presence or absence of IFN α/β receptor 2 (5 μ g/mL) as described in the Materials and Methods section. As shown in Fig. 3, all IFN formulations exhibited potent suppression of HCV replication and addition of sIFN α R2 abrogated this effect significantly (all P values < 0.05).

from $57.3\% \pm 11.4\%$ to $9.7\% \pm 3.5\%$ (P value = 0.0284). In PegIFN α 2a, suppression of HCV replication was reduced from $59.7\% \pm 10.3\%$ to $7.0\% \pm 2.6\%$ (P value = 0.0095). In Alb-IFN α , suppression of HCV replication was reduced from $75.3\% \pm 4.6\%$ to $6.0\% \pm 2.0\%$ (P value = 0.0018). Suppression of HCV replication was dramatically reduced with the addition of IFN α R2a in each of the formulations of IFN α .

Serum from NR suppress IFN α -mediated ISG expression

We then determined whether serum from NR, which has high levels of IFN α R2a, would have an effect on the biological activity of IFN α . Freshly isolated PBMCs were stimulated with recombinant IFN α with serum from NR and SVR in the presence or absence of anti-IFN α R2a antibodies. As shown in Fig. 3, serum from NR specifically inhibited ISG expression in PBMCs (P value < 0.0001), which was abrogated by the addition of neutralizing anti-IFN α R2a antibodies (P value < 0.0001).

FIG. 3. Inhibition of IFN α signaling in peripheral mononuclear cells by serum from null responders is mediated by soluble IFN α R2. Mean IFN-stimulated gene (ISG) expression ($n=30$) in PBMCs after treatment with 20 IU/mL of IFN α *in vitro* for 60 min with or without serum from NR and SVR in the presence or absence of anti-IFN α R2 antibody were measured by real-time PCR as described in the Materials and Methods section. Expression of each ISG was normalized with that of *GAPDH* and averaged for each experimental condition. As shown in Fig. 4, incubation of serum from NR, but not from SVR resulted in the suppression of IFN α mediated induction of ISG (P value < 0.0001 and > 0.05, respectively). The inhibition of IFN signaling by serum from NR was entirely reversed by addition of antibodies to IFN α R2 (P value < 0.0001), which suggest the mechanism of suppression is mostly, if not entirely, mediated by interactions between IFN α and soluble IFN α R2 present at high levels in the serum of NRs. However, no such effect was observed with SVR, as the levels of sIFN α R2 in the serum of SVR are considerably lower (P value > 0.05).

FIG. 4. (a) Transgene expression in first generation IFN α R2a mice. The single bands in lanes 1–12 in the gel electrophoresis indicate the presence of the IFN α R2a transgene in the CAG-IFN α R2a mice. The double bands in lanes 1–4 of Alb-IFN α R2a mice and lanes 1–7 of Pol2-IFN α R2a mice indicate presence of the IFN α R2a transgene and the control gene. (b) Transgene protein expression in backcrossed IFN α R2a mice. Western blot analysis of serum from transgenic mice was performed as described in the Materials and Methods section. The arrow indicates the presence of IFN α R2a. 1844–1848 are the CAG-IFN α R2a transgenic mice after back cross from the first generation mice. 5716 and 7871 are positive controls. 7242 is the Pol2-IFN α R2a first generation mouse. MW indicates molecular weight ladder and 7232 is the C57BL/6 control without the transgene.

Sera from SVR had minimal effect on the expression of ISG since they had lower levels of sIFN α R2a.

IFN α R2a expression in transgenic mice

Three lines of mice expressing IFN α R2a transgene were generated as described in the methods. Two of them used

TABLE 1. INCREASED INTERFERON STIMULATED GENE EXPRESSION IN sIFN α 2 α TRANSGENIC MICE

	Liver			Lymph node			Spleen		
	Transgenic (avg. Ct $\pm$ SD)	Wild type (avg. Ct $\pm$ SD)	P value	Transgenic (avg. Ct $\pm$ SD)	Wild type (avg. Ct $\pm$ SD)	P value	Transgenic (avg. Ct $\pm$ SD)	Wild type (avg. Ct $\pm$ SD)	P value
IFI27L1	4.04 $\pm$ 0.26	4.36 $\pm$ 0.30	0.1119	9.66 $\pm$ 2.32	6.32 $\pm$ 4.42	0.1939	9.87 $\pm$ 2.04	10.78 $\pm$ 4.71	0.7273
IFITM1	8.82 $\pm$ 0.71	9.69 $\pm$ 0.60	0.0420 ^a	5.20 $\pm$ 0.16	5.67 $\pm$ 0.14	0.0121 ^a	5.32 $\pm$ 0.19	5.12 $\pm$ 0.21	0.1455
IRF3	3.33 $\pm$ 0.45	3.62 $\pm$ 0.43	0.2977	7.79 $\pm$ 1.88	3.52 $\pm$ 3.84	0.1333	6.32 $\pm$ 1.82	6.97 $\pm$ 3.12	0.7273
ISG15	6.80 $\pm$ 0.46	6.80 $\pm$ 0.84	0.8981	7.50 $\pm$ 0.26	6.30 $\pm$ 0.72	0.0121 ^a	7.27 $\pm$ 0.28	6.29 $\pm$ 0.36	0.0091 ^a
ISG20	8.14 $\pm$ 0.51	8.07 $\pm$ 0.30	0.5185	11.26 $\pm$ 2.18	7.24 $\pm$ 3.15	0.0848	7.55 $\pm$ 1.46	7.70 $\pm$ 1.90	0.8636
OAS1b	10.31 $\pm$ 0.88	9.94 $\pm$ 0.36	0.3636	14.35 $\pm$ 2.10	10.44 $\pm$ 3.78	0.1333	12.92 $\pm$ 2.18	11.84 $\pm$ 4.34	0.8889
PLSCR1	9.18 $\pm$ 0.62	8.60 $\pm$ 0.88	0.1469	8.21 $\pm$ 0.13	8.11 $\pm$ 1.37	0.4970	8.19 $\pm$ 0.17	7.43 $\pm$ 0.51	0.0182 ^a
SP110	11.79 $\pm$ 0.56	10.86 $\pm$ 0.58	0.0190 ^a	12.84 $\pm$ 2.97	8.57 $\pm$ 3.53	0.1939	12.73 $\pm$ 2.09	12.39 $\pm$ 4.75	0.8636
STAT1	7.91 $\pm$ 0.78	8.32 $\pm$ 0.60	0.1469	11.77 $\pm$ 2.82	8.94 $\pm$ 3.95	0.3758	11.89 $\pm$ 3.10	11.17 $\pm$ 6.51	0.7273
Total	7.81 $\pm$ 2.67	7.81 $\pm$ 2.41	0.9270	9.84 $\pm$ 3.35	7.24 $\pm$ 3.27	0.0016 ^a	9.07 $\pm$ 3.17	8.64 $\pm$ 3.74	0.2770

^aSignificantly different ($P < 0.05$) between the transgenic and wild-type samples.

ISG, interferon-stimulated gene; avg. Ct $\pm$ SD, average cycle threshold numbers $\pm$ standard deviation.

ubiquitous promoters, while the third used an albumin promoter, restricting the expression of the transgene to the liver. All mice were tested for presence of transgene as shown in Fig. 4a and expression of transgene via western blot in Fig. 4b.

Increased ISG expression in IFN α R2a transgenic mice

To determine the effect of high expression of IFN α R2a on IFN signaling, we performed multiplex PCR for ISG expression using various tissues from CAG-IFN α R2a transgenic mice as shown in Table 1. Total ISG expression in the lymph node was significantly higher (P value=0.0016) compared to wild-type mice. In addition, *IFITM1* (P value=0.0420) and *SP110* (P value=0.0190) were identified to have significantly increased expression in the liver, *IFITM1* (P value=0.0121) and *ISG15* (P value=0.0121) in the lymph node, *ISG15* (P value=0.0091) and *PLSCR1* (P value=0.0182) in the spleen.

Discussion

We describe a novel mechanism mediated by excess levels of soluble IFN α R2 in the serum of patients who subsequently failed to respond to IFN α treatment for hepatitis C. Furthermore, using an *in vitro* system, we demonstrate that excess levels of IFN α R2a can inhibit IFN-mediated antiviral activity against HCV. In this regard, serum from null responders inhibited IFN α signaling in an IFN α R2a specific manner. Moreover, we developed an *in vivo* model of transgenic IFN α R2a mice, which demonstrated enhanced endogenous IFN signaling, suggesting transsignaling as a mechanism of interference of soluble IFN α R2a with JAK/STAT signaling.

In hepatitis C treatment with exogenous IFN α , several theories exist to explain the different host responses (NR versus SVR) to IFN treatment. Although PBMCs from NR have increased baseline ISG expression *in vivo* as demonstrated by DNA microarray and real-time PCR data (Lempicki and others 2006), PBMCs from NR and SVR respond to IFN α similarly *in vitro* (data not shown). This suggests a role for inhibitory mechanisms *in vivo* that determines such a

response. The higher basal levels of soluble IFN α R2a in NR compared to SVR have been observed to possibly affect this IFN-signaling pathway.

The mechanism by which sIFNAR2a would enhance the endogenous ISG expression is not clearly understood. Recent studies have demonstrated that soluble IFN α R2a is one among 3 isoforms of the IFN α R2, which are generated by alternative splicing in both human and mouse and produces the transmembrane IFN α R2b, as well as the complete receptor IFN α R2c (Owczarek and others 1997; Hardy and others 2001; de Weerd and others 2007). In the murine model, IFN α R2a exhibited antagonistic and agonistic properties (Hardy and others 2001). Recombinant IFN α R2a acted as a competitive inhibitor of type I IFN antiproliferative activity in an *in vitro* assay. However, in the murine IFN α R2 knockout thymocytes, recombinant IFN α R2a produced an antiproliferative response, suggesting transsignaling capability of the soluble receptor (Hardy and others 2001). The specific mechanism of soluble IFN α R2a is not clear in its interaction *in vivo* with endogenous and exogenous IFN, especially when administered as therapy. After IFN α treatment in patients with hepatitis C, expression of the IFN α / β receptor in the liver was much stronger in SVR than NR, suggesting host differences in response to IFN (Meng and others 2005). Hence, the soluble form of IFN α R2 receptor soluble may act as important regulators of immune response as in the case on many other such cytokine receptors, including IL-6 and IL-15 (Fernandez-Botran 1991). For example, soluble receptors, IL-6R and IL-15R, have been shown as agonists in enhancing stimulation of target immune cells. Transsignaling is a process by which the soluble component of a heterodimer can interact with the ligand and the membrane bound counterpart resulting in partial activation of the signaling pathway. In this regard, our data demonstrates that sIFN α R2a can also act in such a manner and result in activation of JAK/STAT pathway to enhance endogenous IFN gene signature observed in HIV/HCV coinfecting subjects. Transsignaling has been previously described with both IL-6 and IL-15 receptors. It has been shown that IL-6/sIL-6R complex induces the dimerization of gp130 in cells to regulate intracellular signal transduction (Jones and others 2005). Similarly, the IL-15/IL-15R α complex has been demonstrated to have superior stabilization and increased bioactivity

manifesting in selective induction of natural killer and CD8+ T cells (Rubinstein and others 2006; Bergamaschi and others 2008, 2009). Our data demonstrating that IFNAR2a transgenic mice have enhanced endogenous expression of ISG, is consistent with this hypothesis and could contribute to the IFN signature observed in NRs *in vivo*, most likely by JAK/STAT phosphorylation, resulting in ISGF3-ISRE interactions.

Elevated levels of soluble IFN α R2a (sIFN α R2a) in serum and urine have been associated with hairy cell leukemia and adenocarcinoma, resulting in high resistance to IFN therapy (Novick and others 1992; Ambrus and others 2003). In hepatitis C, where treatment is mainly dependent on IFN therapy, high levels of sIFN α R2a have been associated with poor response rates to pegIFN α and RBV (Mizukoshi and others 1999). In this regard, soluble IFN α R2a may potentially act as a blocking agent in the IFN-signaling pathway. As shown in our experiments, we can block the pathway and inhibit the antiviral activity of IFN. However, it was unclear how the blocking of the pathway led to increased ISG expression. Clinical data suggests that high baseline ISG and *ISG15* are negative predictors for treatment response in hepatitis C to IFN treatment (Chen and others 2005; Sarasin-Filipowicz and others 2008). Therefore, we generated the transgenic mice with overexpression of IFN α R2a, which suggests that IFN α R2a leads to high endogenous IFN signaling.

In our proposed model, high levels of soluble IFN α R2 is associated with a lack of response to exogenous IFN. We propose that the excess amount of IFN α R2a is generated by alternative splicing, probably as a result of a yet unidentified SNP in some but not all HCV-infected individuals. An excess of IFN α R2a then combines with membrane bound IFN α R1 and IFN and leads to phosphorylation of the JAK/STAT pathway, resulting in an enhanced ISG expression (transsignaling) in tissues, such as the liver. Finally, this persistent activation most likely leads to further blunting of JAK/STAT signaling, resulting in a lack of response to exogenous IFN.

Overall, these results suggest that high ISG expression may explain most of the IFN signaling abnormalities described in the hepatic and peripheral lymphatics in patients with hepatitis C. Finally, further studies will extend these observations in an IFN-free treatment paradigm to clarify the role of high ISG expression in predicting treatment response.

Acknowledgments

This research was supported in whole by the Intramural Research Program of the NIH (National Institute of Allergy and Infectious Diseases, National Cancer Institute and the Clinical Research Center). We acknowledge Apath LLC for providing us with J6/JFH-1 HCV clone and Huh7.5 cells.

Disclaimer

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Author Disclosure Statement

None of the authors have any conflicts of interest to report.

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Received 11 March 2013/Accepted 2 June 2013