Host Gene Expression Changes Correlating With Anti–HIV-1 Effects in Human Subjects After Treatment With Peginterferon Alfa-2a

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We investigated whether interferon-inducible genes (IFIGs) with known anti–human immunodeficiency virus (HIV) activity in vitro were associated with in vivo virological response in HIV infection. Nine untreated HIV-1–infected volunteers were treated for 12 weeks with peginterferon alfa-2a. A subset of IFIGs (23 of 47) increased compared with baseline through 6 weeks beyond therapy, and 10 of the 23 IFIGs significantly inversely correlated ($r = -0.7$; $P < .05$) with virological response. The strength of peginterferon alfa-2a–induced IFIG response significantly correlated with declines in HIV load during treatment ($r^2 = 0.87$, $p = .003$). This study links HIV virological response to a specific IFIG subset, a potential prognostic indicator in peginterferon alfa-2a–treated patients with HIV infection.

Interferon (IFN) α plays a major role in innate antiviral immunity against RNA viruses [1]. Previous human trials of treatment for human immunodeficiency virus (HIV) infection with interferon alfa demonstrated antiviral effect but failed to show sustained efficacy (reviewed in [2]). However, none of the standard HIV therapies eradicate HIV infection; instead, they chronically suppress HIV replication in vivo, markedly reducing morbidity and mortality associated with AIDS [3]. Interferon alfa remains the only currently available therapy shown to cure chronic viral disease, as with infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) [4]. Interferon alfa is known to exert its antiviral effect through a myriad of pathways, including augmentation of both innate and adaptive immune system mechanisms [5]. In particular, several IFN-α–induced antiviral molecules of the innate immune system have been shown to mediate antiviral efficacy against HCV (reviewed in [6]), and findings of in vitro studies have suggested that these molecules also affect HIV replication [7]. Recent findings have suggested that anti-HIV activity correlates with IFN-α–induced up-regulation of several genes, including a group of IFN-inducible genes (IFIGs), such as 2′,5′-oligoadenylate synthetase (OAS) and the apolipoprotein B messenger RNA–editing enzyme-catalytic polypeptide–like 3 (APOBEC3) family [8, 9]. These proteins inhibit HIV replication by stimulating endoribonuclease production and hypermutating retroviral RNA [8, 10]. However, evidence supporting the role of these and other IFIGs in anti-HIV activity in vivo is lacking.

Peginterferon alfa-2a is approved for treatment of HCV and HBV infection [10]. Mathematical modeling of HCV viral kinetics during interferon alfa therapy and in vitro HCV replicon assays have suggested that IFN-α primarily combats HCV by blocking production or release of new virions [11]. However, considerable controversy remains regarding the most influential innate antiviral pathways operative against HIV replication or infection [8, 12].

The parent study (AIDS Clinical Trials Group [ACTG] protocol 5192) of this report examined the pharmacokinetic/dynamic profiles, safety, and antiviral activity of 12 weeks of peginterferon alfa-2a administration in HIV-positive, HBV-negative, HCV-negative subjects not receiving antiretroviral therapy (ART) [10]. Viral load (VL) decreased significantly in these patients without apparent correlation with concurrent peginterferon alfa-2a plasma concentrations [10]. The objectives of this study were to validate the relationship between IFIG induction by peginterferon alfa and antiviral effects of peginterferon alfa-2a in HIV-infected individuals, and to explore the role of specific IFIGs in mediating antiviral effects of IFN-α against HIV. Therefore, using peripheral blood mononuclear cells (PBMCs) from patients in the aforementioned peginterferon alfa-2a phase 2 trial [10], we examined the expression profiles for IFIGs and other genes suggested elsewhere to mediate anti-HIV activity in vitro [13, 14].

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Table 1. Factors and Genes Significantly Affected ($P < .05$) by Peginterferon Alfa-2a Treatment in HIV-1–Monoinfected Patients

Factor/Gene (Amplification Method)	P	Log Change in Indicated Factor by Treatment Week, Mean ± SEM				
		0	3	6	12	18
Log_{10} IFN-α (Blood)	3.79×10^{-14}	0.00 ± 0.27	**2.02 ± 0.11**	**2.20 ± 0.05**	**2.22 ± 0.05**	0.16 ± 0.12
IFIG median (bDNA)	8.26×10^{-08}	0.00 ± 0.23	**1.03 ± 0.13**	**1.30 ± 0.12**	**1.35 ± 0.10**	0.14 ± 0.28
IFI27[a] (bDNA)	9.80×10^{-08}	0.00 ± 0.39	**1.92 ± 0.24**	**2.30 ± 0.12**	**2.34 ± 0.20**	0.04 ± 0.37
ISG15[a] (bDNA)	2.48×10^{-07}	0.00 ± 0.22	**0.86 ± 0.12**	**1.20 ± 0.12**	**1.20 ± 0.14**	0.14 ± 0.23
IFI44[a] (bDNA)	4.02×10^{-07}	0.00 ± 0.28	**1.00 ± 0.18**	**1.34 ± 0.16**	**1.39 ± 0.11**	0.06 ± 0.28
MX1[a] (bDNA)	7.19×10^{-07}	0.00 ± 0.31	**1.07 ± 0.19**	**1.49 ± 0.19**	**1.38 ± 0.09**	−0.30 ± 0.4
G1P3[a] (bDNA)	2.22×10^{-06}	0.00 ± 0.25	**0.91 ± 0.13**	**1.17 ± 0.12**	**1.17 ± 0.11**	0.07 ± 0.31
MX2[a] (bDNA)	2.36×10^{-06}	0.00 ± 0.21	0.46 ± 0.13	**0.82 ± 0.14**	**0.70 ± 0.14**	***−0.51 ± 0.21***
Log_{10} VL	3.21×10^{-06}	0.00 ± 0.14	***−0.89 ± 0.32***	***−0.64 ± 0.32***	***−0.72 ± 0.27***	0.26 ± 0.2
IFIT3[a] (bDNA)	5.85×10^{-06}	0.00 ± 0.29	**0.95 ± 0.24**	**1.42 ± 0.18**	**1.30 ± 0.13**	0.38 ± 0.27
EIF2AK2 (bDNA)	7.04×10^{-06}	0.00 ± 0.19	**0.69 ± 0.15**	**0.83 ± 0.15**	**0.97 ± 0.11**	0.11 ± 0.17
ISG20 (bDNA)	1.86×10^{-05}	0.00 ± 0.17	**0.54 ± 0.11**	**0.67 ± 0.1**	**0.81 ± 0.14**	0.01 ± 0.19
SP110[a] (bDNA)	2.12×10^{-05}	0.00 ± 0.09	0.27 ± 0.07	0.34 ± 0.07	0.32 ± 0.06	0.29 ± 0.13
OAS2[a] (bDNA)	2.25×10^{-05}	0.00 ± 0.21	**0.67 ± 0.13**	**1.10 ± 0.2**	**1.03 ± 0.13**	0.43 ± 0.36
Log_2 OAS (Blood)	2.31×10^{-05}	0.00 ± 0.26	**1.55 ± 0.25**	**1.66 ± 0.18**	**1.44 ± 0.24**	**0.86 ± 0.25**
IFIT1[a] (bDNA)	3.38×10^{-05}	0.00 ± 0.25	**0.82 ± 0.22**	**1.32 ± 0.25**	**1.30 ± 0.18**	0.34 ± 0.29
LY6E (bDNA)	4.63×10^{-05}	0.00 ± 0.14	**0.55 ± 0.14**	**0.61 ± 0.07**	**0.72 ± 0.11**	0.04 ± 0.15
MX1 (PCR)	1.51×10^{-04}	0.00 ± 0.87	**1.45 ± 0.71**	**2.01 ± 0.4**	**1.76 ± 0.38**	***−0.75 ± 0.64***
OAS1 (bDNA)	1.64×10^{-04}	0.00 ± 0.25	0.49 ± 0.23	**0.80 ± 0.24**	**0.75 ± 0.19**	0.48 ± 0.32
OAS (PCR)	1.69×10^{-04}	0.00 ± 0.79	**0.69 ± 0.47**	**1.57 ± 0.51**	**1.35 ± 0.63**	***−1.75 ± 0.6***
PLSCR1 (bDNA)	6.17×10^{-04}	0.00 ± 0.14	0.32 ± 0.07	0.39 ± 0.14	**0.61 ± 0.16**	0.28 ± 0.19
APOBEC3A (bDNA)	9.86×10^{-04}	0.00 ± 0.16	**0.71 ± 0.12**	**0.56 ± 0.11**	**0.73 ± 0.09**	0.16 ± 0.23
STAT1 (bDNA)	1.23×10^{-03}	0.00 ± 0.16	0.37 ± 0.13	0.49 ± 0.13	**0.59 ± 0.1**	0.02 ± 0.22
IFITM1 (bDNA)	1.49×10^{-03}	0.00 ± 0.30	0.34 ± 0.21	**0.55 ± 0.19**	0.42 ± 0.19	0.35 ± 0.19
BST2 (PCR)	1.11×10^{-02}	0.00 ± 0.66	**0.65 ± 0.43**	**0.64 ± 0.48**	**0.89 ± 0.27**	***−0.69 ± 0.41***
TNFSF13B (PCR)	1.63×10^{-02}	0.00 ± 0.61	0.04 ± 0.35	***−0.67 ± 0.49***	***−0.56 ± 0.26***	***−2.13 ± 0.52***
SOCS3 (PCR)	2.46×10^{-02}	0.00 ± 0.49	0.39 ± 0.54	***−0.9 ± 0.53***	**0.59 ± 0.41**	**1.22 ± 0.64**
APO3A (PCR)	2.68×10^{-02}	0.00 ± 0.7	**0.79 ± 0.43**	**0.55 ± 0.34**	**0.89 ± 0.24**	***−0.77 ± 0.4***
PKR (PCR)	3.00×10^{-02}	0.00 ± 0.7	**1.14 ± 0.7**	**1.09 ± 0.57**	**1.06 ± 0.62**	0.21 ± 0.71
APO3F (PCR)	4.33×10^{-02}	0.00 ± 0.39	0.03 ± 0.44	0.11 ± 0.44	0.12 ± 0.33	***−1.19 ± 0.4***

Positive increases in gene expression are represented as bold values, decreases in gene expression are represented as bold italicized values.

Abbreviations: APOBEC3, apolipoprotein B messenger RNA–editing enzyme-catalytic polypeptide–like 3; bDNA, branched DNA; BST2, bone marrow stromal cell antigen 2; EIF2AK2, eukaryotic translation initiation factor 2 alpha kinase 2; HIV-1, human immunodeficiency virus type 1; IFI27, interferon alfa inducible protein 27; IFI44, Interferon induced protein 44; IFIG, IFN-inducible gene; IFIT3, interferon induced protein with tetratricopeptide repeats 3; IFITM1, interferon inducible transmembrane protein 1; IFN, interferon; ISG15, Interferon stimulated protein, 15 kDa; ISG20, interferon sensitive gene 20; S LY6E, lymphocyte antigen 6 complex; MX1, Interferon induced GTP binding protein 1; MX2, interferon induced GTP binding protein 2; OAS, 2#,5#-oligoadenylate synthetase; P110, interferon induced protein 40; PCR, polymerase chain reaction; PKR, protein kinase R; PLSCR1, phospholipid scramblase 1; SEM, standard error of the mean; SOCS3, suppressor of cytokine signalling 3; STAT1, signal transducer and activator of transcription 1; TNFSF13B, tumor necrosis superfamily protein 13B; VL, viral load.

[a] Significantly correlated ($r \geq -0.07$; $P < .05$) with VL decline.

MATERIALS AND METHODS

Patients

Thirteen HIV-positive individuals not receiving ART (naive or ≤12 weeks after receiving ART) and without concurrent HBV or HCV infection were enrolled in the study under ACTG Protocol 5192, as described elsewhere [10]. Peripheral blood samples were available for 9 of 13 enrolled subjects at the enrollment and at the 3, 6, 12, and 18 weeks. Subjects received 180 μg of subcutaneous peginterferon alfa-2a weekly (provided by Roche Pharmaceuticals and Amgen) for 12 weeks with 2 scheduled follow-up visits at 1 and 6 weeks after the treatment period (weeks 12 and 18). All subjects signed an institutional review board–approved informed consent, and the study received prior approval as an ACTG New Works Concept Sheet (NWCS 283).

Blood, Plasma, and Serum Samples

Blood samples were collected at baseline and weekly during treatment, with PBMC isolation and HIV-1 RNA quantification before entry and weekly during treatment, as described elsewhere [9, 10].

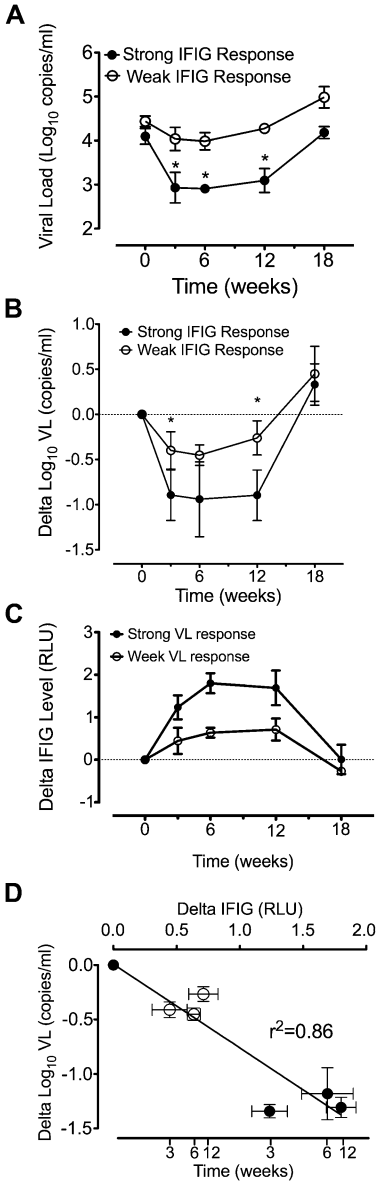

Figure 1. Associations between clinical parameters of interest in subjects grouped by baseline status for each parameter of interest. All data are means ± standard errors of the mean [SEM]; * $P < .05$. *A,* Absolute human immunodeficiency virus (HIV) load over time in subjects grouped according to strength of interferon-inducible gene (IFIG) response to peginterferon alfa-2a (peginterferon alfa-α-2a). *B,* Change in $\log_{10}$ viral load (VL) versus time in the same subjects grouped as in *A. C,* Change in IFIG expression as a measure of strength of IFIG response to peginterferon alfa-2a vs time in subjects grouped by HIV response. One of 5 subjects in the strong IFIG group was in the weak VL group, and vice versa. *D,* Change in $\log_{10}$ VL (ordinate) plotted against the change in IFIG expression (upper abscissa). The IFIG and HIV values shown are means ± SEM for delta VL (vertical error bars) and delta IFIG (horizontal error bars). Subject groupings were by IFIG response as in *A* and by time (indicated by the lower abscissa). Correlation coefficient is shown; the slope of the best-fit line was significantly nonzero ($P = .003$).

IFIG Expression in PBMCs by bDNA Assay

The IFIGs in the PBMCs were quantified by branched DNA (bDNA) techniques with equipment and supplies as described elsewhere [9]. Genes were chosen based on relevance to IFIG expression (reviewed in [9, 14, 15]).

Real-Time Polymerase Chain Reaction

Peripheral blood mononuclear cells were washed and centrifuged, and 30 μL of RNA were extracted using RNeasy Mini Kit (QIAgen) and quantified using spectrophotometry (ThermoScientific). Preparation of complementary DNA and real-time polymerase chain reaction (RT-PCR) were described elsewhere [8]. Data were normalized to baseline within each patient, and the data were summarized by median for each time point.

Statistical Analysis

Host genetic and clinical responses were compared between subjects whose initial parameters were at or above the median and those whose initial parameters were below the median for the given factor of interest. These groups were designated "high/strong" or "low/weak" for each respective parameter. Clinical data were analyzed by analysis of variance with a Bonferroni post hoc test. To analyze gene expression data, pairwise Spearman rank correlation and mixed-model, repeated-measure analyses of variance were performed. The correlation between VL and IFIGs was analyzed by linear regression.

RESULTS

Differential Regulation of Host Genes Involved in Suppression of HIV Infection In Vivo

We used RT-PCR and bDNA methods to amplify 47 genes previously correlated with in vitro antiviral activity and shown to be induced by IFN-α or HIV infection. As shown in Table 1, 23 of these genes demonstrated significant ($P < .05$) changes in expression level with interferon alfa treatment, and increased expression of 10 genes was inversely correlated (Spearman rank, $P < .00001$) with HIV load decline (Table 1). The IFIGs most correlated with HIV virological response were IFI44, IFI27, SP110, MX1, MX2, ISG15, G1P3, OAS2, IFIT3, and IFIT1.

Virological Response to Treatment

Host genetic and virological responses in our subjects were significantly affected by peginterferon alfa-2a treatment (Figure 1). Absolute and relative changes in HIV load were significantly different in subjects grouped by strength of IFIG response (Figure 1*A* and 1*B*). The decline in HIV load was greatest in the subjects with the largest increase in mean IFIG expression levels, whereas those with modest or low IFIG induction levels had smaller declines in HIV load with peginterferon alfa-2a treatment (Figure 1*B* and 1*C*). Most of the subjects in the "strong" and "weak" IFIG response groups were also in the corresponding "strong" and "weak" VL groups. The mean IFIG response

strongly correlated with HIV load response ($r^2 = 0.86$) when the data were analyzed grouped by time and strength of IFIG response (Figure 1*D*).

DISCUSSION

Our data correlate changes in expression of IFN-α–induced genes with antiviral effects in human patients with HIV-1 infection. Of 47 known IFN-α–inducible genes that have demonstrated antiviral or HIV activity in vitro, 23 had significantly altered expression (mostly up-regulated) by therapeutic doses of peginterferon alfa-2a, and 10 of these strongly correlated with declines in HIV load. The lack of genomic response of the remaining 24 genes suggests that these genes are less likely to directly mediate in vivo anti-HIV effects of clinically relevant doses of peginterferon alfa-2a within the time frame investigated. These findings are important because, although the antiviral activity of IFN-α has long been recognized [1], in vivo correlates of interferon alfa treatment and genetic and virological responses are lacking. Understanding the mechanisms of the anti-HIV effects mediated by this treatment could prove an important step forward in the development of alternate treatment strategies for HIV.

Genes with altered expression on interferon alfa treatment in this study included many genes previously shown to be IFN-α inducible and to alter the course of viral infection for non-HIV infections through a variety of mechanisms; some genes also have anti-HIV activity in vitro [9, 14, 15]. In the current study, however, peginterferon alfa-2a treatment failed to significantly affect expression levels for nearly half of the IFN-α–inducible genes studied. Conclusions from this observation must be tempered, however, given the relatively small sample size in our study ($n = 9$) and the finding that some genes demonstrated changes in expression that approached but did not obtain statistical significance owing to high levels of intersubject variability. In addition, the limited blood sampling protocol in this study could miss some physiologically relevant, though transient, changes in expression of IFIGs. This study also did not examine the levels or function of these gene products, which could also change during HIV infection or peginterferon alfa-2a treatment. Despite these limitations, our results clearly support the importance of some genes, while also suggesting that a number of genes previously thought to be important may have little influence on the outcome of interferon alfa treatment of HIV infection in vivo.

The finding that these changes in gene expression correlate with clinically significant effects on VL demonstrates the relevance of our gene expression data. Most of the 10 IFIG genes correlated with HIV load decline are associated with antiviral activity at the transcriptional level or apoptosis, keeping with the observed clinical data. Although the absolute changes in VL were modest, the significant VL differences between groups with weak and strong IFIG responses help substantiate the role of IFIGs in innate anti-HIV immunity. The finding that IFIG response correlated with virological (large versus small viral load decrease) further supports the relevance of IFIGs in anti-HIV virological response. In addition, the strong correlation between mean change in IFIG expression and change in VL on each study day during peginterferon alfa-2a treatment provides significant evidence that the IFIG response to peginterferon alfa-2a in HIV-infected patients directly and negatively affects HIV replication.

In conclusion, there are at least 10 IFIGs that significantly correlate with virological response to peginterferon alfa-2a in HIV-1–infected patients. The effects of peginterferon alfa-2a treatment on the clinical VL parameter suggest that interferon-α may have a role to play in the search for a curative treatment for HIV infection. Our gene expression data complement the clinical data and could serve as a priority list for which genes are likely to be most important. Future work should focus on understanding IFIGs and their individual and collective roles in viral eradication. Such an understanding could provide important insights into immune mechanisms crucial to clearing HIV, an important goal in the global fight against the continuing HIV pandemic.

Notes

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

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