Effects of interferon-α treatment on anti-HIV-1 intrinsic immunity in vivo

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ABSTRACT

Interferon-α suppresses HIV-1 replication \textit{in vitro} by inducing cell-intrinsic retroviral restriction mechanisms. We investigated the effects of interferon-α/ribavirin treatment (IFN-α/riba) on 34 anti-HIV-1 restriction factors \textit{in vivo}. Several anti-HIV-1 restriction factors were significantly induced by IFN-α/riba in HIV/HCV-coinfected individuals. Fold-induction of cumulative restriction factor expression in CD4+ T cells was significantly correlated with viral load reduction during IFN-α/riba ($r^2=0.649; \ p<0.016$).

Exogenous interferon-α induces supraphysiologic restriction factor expression, associated with a pronounced decrease in HIV-1 viremia.

Keywords: Interferon-α; intrinsic immunity; retroviral restriction factors; APOBEC3; BST-2; tetherin; TRIM family proteins.
FINDINGS

Detailed analyses of the molecular and immunologic effects of the cytokine interferon-α (IFN-α) may provide insights that contribute to the development of novel prophylactic, therapeutic and curative strategies for HIV-1 infection. Induction of IFN-α expression is a critical first step in the defense against a range of viral infections (1, 2). The antiretroviral activity of IFN-α was demonstrated in vitro soon after the discovery of HIV-1 (3), and several studies have reported that exogenous IFN-α treatment potently suppresses HIV-1 in vivo (4-8). Moreover, IFN-α therapy was recently associated with significant reduction in the size of the HIV-1 latent reservoir (9), suggesting that interferon-associated pathways may be exploited to achieve HIV-1 eradication. The mechanisms underlying the in vivo anti-HIV-1 capacity of IFN-α remain to be thoroughly elucidated.

Cell-intrinsic immune mechanisms likely contribute to the beneficial effects of type I interferon (10). On this front, our laboratory recently reported that the induction of the BST-2/tetherin, APOBEC3G and APOBEC3F cell-intrinsic immune defenses contributes to the IFN-α-mediated suppression of HIV-1 in vivo (6). A large number of additional host restriction factors with anti-HIV-1 activity in vitro have now been identified and characterized. In this study, we perform a comprehensive analysis of the effects of exogenous IFN-α treatment on all as yet established anti-HIV-1 host restriction factors and HIV-1 viremia in vivo.
We examined restriction factor gene expression patterns in longitudinal samples from 89 HIV/HCV-coinfected individuals undergoing pegylated interferon-α/ribavirin combination therapy (IFN-α/riba), and additionally characterized a separate population of interferon-untreated control individuals. We designed and implemented a custom TaqMan Low Density Array (TLDA) to measure the expression of 34 anti-HIV-1 restriction genes (11). We relied on the following two minimal criteria for inclusion in our “Cumulative Restriction” or “CuRe” TLDA: 1) Peer-reviewed, published evidence of direct inhibition of HIV-1 replication in vitro, and 2) Detectable expression in human peripheral blood mononuclear cells. All factors in the CuRe array meet the essential, minimal definition of a host restriction factor, and function in a cell-autonomous manner to suppress HIV-1 replication.

Subjects and specimen processing: Longitudinal samples were collected from 15 HIV/HCV-coinfected individuals enrolled in the Swiss HIV Cohort Study ([SHCS], www.shcs.ch) (12) who underwent IFN-α/riba treatment (Supplementary Table 1). All subjects had PBMC available before, during and after IFN-α/riba treatment, were ART-naïve, and had detectable HIV-1 RNA at baseline. Blood was collected prospectively from 12 HIV-1-infected, (ART-untreated) viremic individuals and 12 HIV-1-uninfected individuals enrolled in the UCSF SCOPE cohort (Supplementary Table 2). PBMC were isolated with Ficoll-Paque PLUS. CD4+ T cells were enriched from fresh PBMCs using the EasySep Human CD4+ T Cell enrichment kit (StemCell Technologies), according to the manufacturer’s instructions. The research was approved by the relevant institutional review boards, and all human participants gave written informed consent.
Gene expression profiling: Total RNA was extracted from PBMC and CD4+ T cells directly after enrichment using Trizol reagent (Invitrogen). RNA was transcribed into cDNA using the SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen). Quantitative real-time PCR utilized custom-made TaqMan® Low Density Arrays (TLDA) from Life Technologies following the manufacturer’s instructions. All assays and their respective target genes are listed in Supplementary Table 3. Thermal cycling was performed using an ABI ViiA™ 7 Real-Time PCR System. Data was analyzed using ABI ViiA™ 7 software. A panel of six housekeeping genes was included in the TLDA plates (GAPDH, 18S, ACTB, PPIA, RPLP0, and UBC). RPLP0 (ribosomal protein, large, P0) was identified as the most stably expressed housekeeping gene using the GeNorm algorithm (13). Therefore, raw cycle threshold (Ct) numbers of amplified gene products were normalized to the housekeeping gene, RPLP0, to control for cDNA input amounts (Supplementary Figure 1). Fold induction was determined using the comparative Ct method (13). APOBEC3G, APOBEC3F, BST2/tetherin and ISG15 relative copy numbers were re-calculated from our previous work (6).

CuRe score calculation: Missing (undetectable) values were imputed using the minimum expression value across samples for each gene. The expression value for the i\textsuperscript{th} gene is notated as e\textsubscript{i}. A reference sample was selected based on having the maximum number of genes that were closest to the median gene expression profile. The reference expression value for the i\textsuperscript{th} gene is notated as r\textsubscript{i}. The CuRe score for a sample is the cumulative fold-
difference in antiviral gene expression with respect to the reference individual, expressed by the following formula:

$$CuRe = \sum_{i=1}^{n} \frac{e_i}{r_i}$$

Statistical Analysis: The paired Wilcoxon test, Mann–Whitney U test and Pearson’s r correlation coefficient were applied to data using GraphPad Prism v5.0c.

We initially examined the effects of IFN-α/riba treatment on HIV-1 plasma viral load. The 15 IFN-α/riba-treated individuals included in this study represent a subset of individuals studied in our previous work on IFN-α effects, chosen based on sample availability (6). Subject characteristics and IFN-α/riba treatment regimens are described in Supplementary Table 1. IFN-α/riba treatment reduced plasma viral load by 0.91 (±0.70) log_{10} copies/mL during treatment, and viremia typically returned to approximate pre-treatment levels following therapy cessation (Supplementary Figure 2). This effect is consistent with previous IFN-α/riba and IFN-α monotherapy studies (4, 5, 7, 8). Seven of 15 individuals were included in analyses of PBMC gene expression, and the remaining eight individuals were included in analyses of CD4+ T cell gene expression. A separate population of 24 IFN-α-untreated individuals (12 HIV-1 uninfected and 12 HIV-1 infected, ART-untreated) enrolled in the SCOPE cohort was additionally characterized as a control group (Supplementary Table 2).

We next implemented the CuRe array to examine the effects of IFN-α/riba treatment on the expression of 34 anti-HIV-1 restriction factors (described in Supplementary Table 3).
in PBMCs and CD4+ T cells. Fourteen out of 34 restriction genes were significantly elevated in unfraccionated PBMCs during the IFN-α/riba treatment period with respect to pre-treatment levels: APOBEC3A, APOBEC3H, IFITM1, IFITM2, IFITM3, ISG15, PKR, HERC5, MOV10, RSAD2 (viperin), TRIM11, TRIM14, TRIM19, and TRIM22 (Figure 1A). An overlapping but distinct set of 15 restriction factors was significantly induced by IFN-α/riba treatment in isolated CD4+ T cells: APOBEC3F, APOBEC3G, BST-2/tetherin, IFITM1, ISG15, PKR, HERC5, MOV10, RSAD2 (viperin), TRIM11, TRIM14, TRIM19, TRIM22, TRIM28, and TRIM32 (Figure 1B). In both unfraccionated PBMCs and CD4+ T cells, induced genes returned to approximate baseline levels post-cessation of IFN-α/riba treatment. Gene-by-gene fold-induction levels (and statistics) observed in PBMCs and CD4+ T cells are presented in Supplementary Table 4.

To infer the contribution of anti-HIV-1 restriction factors to the observed IFN-α/riba-mediated suppression of HIV-1, we examined correlations between the induction of restriction genes and reduction in HIV-1 viremia during the treatment period. We defined the CuRe score (explained above in more details) to represent overall, cumulative restriction factor gene expression across our 34 measured targets. IFN-α/riba-mediated viral load reduction did not exhibit any correlation with induction of the CuRe score in unfractionated PBMCs ($r^2=0.064$, $p=0.584$) (Figure 2A), or induction of any of the 34 restriction factors in PBMCs considered on an individual gene-by-gene basis. IFN-α/riba-mediated viral load reduction exhibited a significant, pronounced correlation with induction of the CuRe score in CD4+ T cells ($r^2=0.649$, $p=0.016$) (Figure 2B). Viral load reduction was significantly correlated with the induction of nine individual anti-HIV-1 restriction genes in CD4+ T cells (Supplementary Figure 3), listed here hierarchically
To investigate the hypothesis that exogenous IFN-α drives supraphysiologic expression of restriction factors, we compared CD4+ T cell anti-HIV-1 restriction factor expression in our SHCS interferon-treated population to CD4+ T cell restriction factor expression in a separate population of 12 HIV-1-infected, (ART-untreated) viremic individuals and 12 HIV-1-uninfected individuals enrolled in the UCSF SCOPE cohort. There were no significant differences between SHCS subjects pre-IFN-α/riba treatment and SCOPE viremic subjects in terms of CuRe score (p=0.464) or ISG-15 expression (p=0.203). However, there was a significant elevation in CuRe score in SHCS subjects during IFN-α/riba treatment with respect to SCOPE viremic (p=0.002) and uninfected subjects (p=0.0003), respectively (Figure 2C). Similarly, ISG-15 expression was significantly higher in SHCS subjects during IFN-α/riba treatment than in SCOPE viremic (p=0.0002) and uninfected subjects (p=0.0002) (Figure 2D), supporting the hypothesis that exogenous IFN-α drives antiviral gene expression to supraphysiologic levels. Gene-by-gene analyses for each of the nine genes associated with IFN-α/riba-mediated suppression of HIV-1 viremia in our study are presented Supplementary Figure 4.
reports on LCMV infection suggest that IFN-α is associated with both beneficial and detrimental disease outcomes, and the overall balance between the various, diverse effects of type I interferon ultimately determines the course of disease (14, 15). This is mirrored in studies of interferon within the context of HIV-1 infection, whereby IFN-α is known to induce several antiretroviral mechanisms that suppress viral replication, but may result in poor disease outcomes due to association with T cell activation and inflammation (17-19). Taken together, these observations suggest that additional work needs to be performed to dissect interferon-associated molecular pathways to identify critical antiretroviral mechanisms and to avoid possible pro-inflammatory consequences.

Endogenous IFN-α is often associated with rapid HIV-1 disease progression and high viral load (17, 18). We hypothesized that the inverse relationship between IFN-α and viral load observed within the context of exogenous IFN-α administration may result from the induction of antiviral genes to supraphysiologic levels not typically encountered in the absence of pharmacological manipulation. Our data strongly support this hypothesis, and suggest that the induction of several restriction factors contributes to IFN-α suppression of HIV-1 \textit{in vivo}. It is provocative that Individual N in our study exhibited the greatest IFN-α/riba induction of the CuRe score, and was the only individual to suppress viral load to undetectable levels for the entire duration of our study (Supplementary Figure 2). Moreover, exogenous IFN-α induces these antiviral mechanisms without appreciably increasing CD4+ T cell activation, which promotes viral transactivation and replication (20). The lack of a relationship between restriction factor induction in PBMCs and viral load reduction suggests that specific consideration of HIV-
1 target cells may be important when evaluating cell-intrinsic immune effects. In addition, a number of innate and adaptive immune mechanisms are likely triggered by IFN-\(\alpha\) which may influence disease outcomes as well. The contribution of non-cell-intrinsic mechanisms to IFN-\(\alpha\) anti-HIV-1 effects should be explored in greater detail.

These data support the concept that the induction of particular intrinsic immune mechanisms may constitute a promising antiretroviral strategy, complementing our previous \textit{in vitro} work (21) and our translational studies of restriction mechanisms in HLA-B*57-positive individuals (22) and HIV-1 elite controllers (11).

\section*{List of abbreviations}

- APOBEC: apolipoprotein B mRNA editing enzyme; BST-2: bone marrow stromal cell antigen 2; SAMHD1: SAM domain and HD domain-containing protein 1; TRIM: tripartite motif; ISG: Interferon-stimulated gene; CDKN1A: cyclin-dependent kinase inhibitor 1A; PAF1: RNA polymerase II associated factor; EIF2AK2: Eukaryotic translation initiation factor 2-alpha kinase 2; HERC5: HECT domain and RLD 5; IFITM: Interferon induced transmembrane; MOV10: Moloney leukemia virus 10, homolog; SLFN11: Schlafen family member 11. TLDA: TaqMan® Low Density Array; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; 18S: 18S ribosomal RNA; ACTB: Beta-actin; PPIA: Peptidylprolyl isomerase A; RPLP0: 60S acidic ribosomal protein P0; UBC: Ubiquitin C.
Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MAM, TL, JG, and SKP performed gene expression experiments and XD performed statistical analyses. AR, BL, SGD and HG coordinated patient recruitment. MAM, SKP, JKW, SGD, JG, MSS, HEAG, and HG designed the studies. MAM and SKP wrote the paper. All authors read and approved the final manuscript.

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The members of the Swiss HIV Cohort Study are:


FIGURE LEGENDS

Figure 1. IFN-α/ribo induction of anti-HIV-1 restriction factors. A) Fold-induction in unfractionated PBMCs. B) Fold-induction in negatively-selected CD4+ T cells. Fold-induction during IFN-α/ribo (green bars) and post-IFN-α/ribo (red bars) were normalized to pre-treatment expression level, as indicated by the dashed line. Mean and standard error are represented in each bar. Asterisks indicate statistically significant differences between during- and pre-therapy expression level based on a paired Wilcoxon test (* = p < 0.05; ** = p < 0.01).

Figure 2. IFN-α/ribo induction of Cumulative Restriction (CuRe) score to supraphysiologic levels correlates with viral load reduction. A) Relationship between HIV-1 viral load reduction during IFN-α/ribo therapy and CuRe score fold-induction in PBMCs; no significant correlation was observed. B) Relationship between HIV-1 viral load reduction during IFN-α/ribo therapy and CuRe score fold-induction in CD4+ T cells. Significance was assessed using Pearson’s r correlation coefficient. C) Comparison of CuRe score between SHCS subjects undergoing IFN-α/ribo therapy and HIV-1-negative and HIV-1-infected (ART-untreated) viremic individuals enrolled in the SCOPE cohort. D) Comparison of ISG-15 expression (marker of interferon exposure) between SHCS subjects undergoing IFN-α/ribo therapy and HIV-1-negative and HIV-1-infected (ART-untreated) viremic individuals enrolled in the SCOPE cohort. Mann-Whitney tests were employed to determine significance.