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BRIEF REPORT

Short-term Treatment With Interferon Alfa Diminishes Expression of HIV-1 and Reduces CD4$^+$ T-Cell Activation in Patients Coinfected With HIV and Hepatitis C Virus and Receiving Antiretroviral Therapy

Sara Morón-López,[1] Elisabet Gómez-Mora,[1] Maria Salgado,[1] Dan Ouchi,[1] Maria C. Puertas,[1] Víctor Urrea,[1] Jordi Navarro,[3] Antoni Jou,[2] Mercedes Pérez,[3] Cristina Tural,[2] Bonaventura Clotet,[1,2,5] Luis J. Montaner,[6] Julià Blanco,[1,5] Manuel Crespo,[3,a] and Javier Martinez-Picado[1,4,5,a]

[1]AIDS Research Institute IrsiCaixa, Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Universitat Autònoma de Barcelona, and [2]Fundació Lluita Contra la SIDA, Badalona, [3]Infectious Diseases Department, Hospital Universitari Vall d'Hebron, and [4]Institució Catalana de Recerca i Estudis Avançats, Barcelona, and [5]Universitat de Vic–Universitat Central de Catalunya, Vic, Spain; and [6]The Wistar Institute, Philadelphia, Pennsylvania

Long-term treatment with interferon (IFN) alfa plus ribavirin decreases the proviral human immunodeficiency virus type 1 (HIV) DNA level. However, the short-term impact of IFN alfa on persistent HIV and its effects on immune activation after antiretroviral therapy remain unknown. Our study showed that the cell-associated HIV RNA level and CD4$^+$ T-cell activation decreased in the IFN group (n = 10). No changes were detected in levels of residual plasma viremia, replication-competent reservoirs, proviral DNA, or 2–long-terminal repeat circles, although *APOBEC3G*, *TRIM5α*, *BST2*, and *TRIM22* were upregulated in the IFN group. These data suggest that short-term treatment with IFN alfa combined with RBV decreases HIV expression, in part through inhibition of HIV transcription by TRIM22 and decrease in T-cell activation.

Keywords. IFN alfa; HIV/HCV coinfection; HIV persistence; HIV RNA expression; TRIM22; Siglec-1; immune activation; CD4$^+$ T-cell subpopulations.

Combination antiretroviral therapy (cART) quickly and persistently suppresses viral replication but cannot eliminate the latent reservoir. Therefore, complementing cART with new immunotherapeutic and chemotherapeutic interventions has been proposed as a means of reactivating viral latency, reducing the size of the viral reservoir, and, ultimately, achieving functional cure [1].

Interferon (IFN) alfa is a type I interferon with potent antiviral activity. Until recently, IFN alfa combined with ribavirin (RBV) was the standard treatment for infection with hepatitis C virus (HCV) [2]. Furthermore, long-term treatment with IFN alfa might reduce the size of the human immunodeficiency virus type 1 (HIV) reservoir [3–5]. However, the short-term mechanism by which IFN alfa diminishes the viral reservoir, as well as the effects IFN alfa on activation and persistent HIV expression, remain unknown.

In a cohort of patients coinfected with HIV and HCV and receiving suppressive cART, we analyzed variations in the cell-associated HIV RNA level, the replication-competent virus level, expression of specific IFN-α–stimulated genes (ISGs) encoding HIV restriction factors, and expression of the protein Siglec-1, which mediates HIV transinfection [6], in peripheral CD4$^+$ T cells. We also assessed changes in the residual plasma viremia level, the total HIV reservoir level, the 2–long terminal repeat (LTR) circle level, distribution of peripheral T-cell subsets, and expression of activation and exhaustion markers during treatment with IFN alfa–RBV. Our objective was to identify a short-term mechanism by which IFN alfa could impact the HIV reservoir.

METHODS

Study Design

We performed an open, prospective, nonrandomized cohort study at 2 university hospitals in Barcelona, Spain. The study population comprised 21 HIV/HCV-coinfected patients receiving suppressive cART for at least 6 months; HCV treatment in 10 patients involved pegylated IFN alfa-2a (180 μg/week; Pegasys, Roche) and RBV (800–1200 mg twice daily; the IFN group), and HCV treatment in 11 patients was deferred (the control group). The participants gave their written informed consent, and the institutional review boards and the Catalonian Health Department (code: FHU-INT-2013-01) approved the protocol.

We collected whole-blood samples (80 mL) at study days 0 and 28. Peripheral blood mononuclear cells (PBMCs) were analyzed by flow cytometry. For the other analyses, CD4$^+$ T cells were isolated using negative immunomagnetic selection (Miltenyi).

Cell-Associated HIV Messenger RNA (mRNA)

One-step reverse-transcription droplet-digital polymerase chain reaction (ddPCR) analysis (BioRad) was used to quantify HIV RNA, with primers/probe sets located in the viral 5′ long

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[a]M. C. and J. M.-P. contributed equally to this work.

Correspondence: J. Martinez-Picado, AIDS Research Institute IrsiCaixa, Badalona, Spain (jmpicado@irsicaixa.es).

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terminal repeat and Gag and in the housekeeping gene TATA-binding protein (*TBP*) [7].

Ultrasensitive Plasma Viremia

Samples containing 4–5 mL of plasma were ultracentrifuged at 170 000*g* at 4°C for 30 minutes before quantification using the Abbott Real-Time HIV assay (Abbott Molecular). Serial dilutions of a positive control down to 5 HIV RNA copies/mL were used as a standard curve to infer quantitative values from raw quantitative PCR cycle threshold (C_T) data.

Quantitative Viral Outgrowth Assay (qVOA)

The replication-competent reservoir was measured in a limiting dilution cell culture assay [7, 8], with a final $p24^{Gag}$ readout. The frequency of infectious HIV units per million $CD4^+$ T cells was determined using IUPMStats v.0.3 (Rosenbloom's software), based on the maximum likelihood method [9].

Proviral HIV DNA

The proviral reservoir was quantified using cell-lysed extracts to measure the housekeeping gene *RPP30* and total cell-associated HIV DNA (ddPCR with the 5′LTR and Gag primers/probe sets) [7, 10].

HIV 2-LTR Circles

To estimate the level of residual new infections, we quantified the number of 2-LTR circles by ddPCR with 2-LTR [11] and *RPP30* primers/probe sets in lysed extracts of $CD4^+$ T cells.

mRNA Expression of *APOBEC3G, TRIM5α, BST2,* and *TRIM22*

RNA from $CD4^+$ T cells was reverse transcribed and amplified using TaqMan reagents (Applied Biosystems) for the panel of genes and for the housekeeping gene *GUSB*. Fold induction ($\Delta\Delta C_T$) was determined by normalizing expression at day 28 relative to that at day 0.

Distribution and Activation of T-Cell Subsets

Cryopreserved PBMCs were labeled with Viability Dye eFluor 506 (eBioscience) and antibodies against CD3, CD4, CD8, CD45RA, CD27, CCR7, CD38, HLA-DR, PD-1 (Becton Dickinson), and Siglec-1 (bioNova).

T-cell subpopulations were classified by the expression of CD45RA, CCR7, and CD27 [12]. Activation markers (CD38 and HLA-DR) and exhaustion markers (PD-1) were analyzed within each T-cell subset. The percentage of Siglec-1–expressing monocytes ($CD14^+CD3^-$ cells) was also measured.

Statistical Analysis

Differences between groups were analyzed using the Mann–Whitney and Kruskal–Wallis tests. Changes within a group were evaluated using the Wilcoxon signed rank test. For categorical variables, independence between groups was studied using the Fisher exact test. The Wald test was used to compare slopes between groups in a mixed regression model. The Spearman test and Winsor correlation test were used to evaluate the association between variables in independent and paired samples, respectively. Censored matched pairs of ultrasensitive viral load were analyzed using the paired Prentice–Wilcoxon test. Statistical significance was set at 5% for all the tests. The analyses were performed with R (v3.0.2) and GraphPad (v5.01).

RESULTS

Patients

The study population comprised 21 HIV/HCV-coinfected patients recruited at Hospital Vall d'Hebron and Hospital Germans Trias i Pujol. One patient from the control group, with detectable HIV at day 0, was excluded from the final analysis.

Baseline demographic and clinical characteristics of patients included in the IFN group and the control group were comparable (Table 1). Most patients were men, with a median age of

Table 1. Patient Characteristics and Clinical Results

Characteristic	Control Group (n = 10)	IFN Alfa Group (n = 10)	*P* Value[a]
Age, y	47 (45–53)	47 (41–51)	.430
Women	1 (10)	2 (20)	1.000[b]
Regimen			
2-drug based	2 (20)	4 (40)	.629[b]
3-drug based	8 (80)	6 (60)	.629[b]
PI based	8 (80)	7 (70)	1.000[b]
Time since diagnosis, y	23.5 (20.2–24.0)	24.0 (19.8–25.2)	.570
Duration of suppressed HIV load, y	4.5 (2.5–5.8)	4.5 (2.2–7.8)	.940
HCV load, IU/L			
Day 0, $\times 10^6$	4.1 (1.9–4.9)	2.5 (1.3–4.5)	.190
Day 28, $\times 10^4$	NA	3.7 (0.2–77.8)	
P[c]		.049	
$CD4^+$ T cells			
Absolute count, cells/μL			
Day 0	447 (295–670)	437 (318–624)	.820
Day 28	570 (480–650)	310 (203–370)	.010
P[c]	1.000	.009	
Percentage			
Day 0	21.5 (18.3–30.8)	22.5 (21.0–37.5)	.450
Day 28	25.0 (22.0–33.0)	28.1 (22.5–36.7)	.760
P[c]	1.000	.444	
$CD8^+$ T cells			
Absolute count, cells/μL			
Day 0	1050 (1010–1200)	650 (585–1130)	.370
Day 28	1190 (660–1310)	440 (378–523)	.005
P[c]	.813	.031	
Percentage			
Day 0	54.6 (42.2–60.0)	49.0 (38.7–58.9)	.620
Day 28	56.0 (37.0–59.0)	43.5 (39.2–57.9)	.850
P[c]	.892	.652	

Data are no. (%) of subjects or median value (interquartile range). *P* values of ≤.05 were considered statistically significant.

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus type 1; IFN, interferon; IU, infectious units; NA, not applicable; PI, protease inhibitor.

[a] By the Kruskal–Wallis test (using GraphPad Prism v5.01) unless otherwise indicated, for comparison between groups.

[b] By the Fisher exact test (using R v3.02), for comparison between groups.

[c] By the Wilcoxon signed rank test (using GraphPad Prism v5.01), for comparison between day 0 and day 28.

47 years, HIV infection for >20 years, and HIV suppression maintained by cART for a median of 4.5 years (Table 1).

No serious adverse events were reported, and the initial dose of IFN alfa was maintained during the study.

Cell-Associated HIV mRNA and Residual Viremia Levels

The quantification of cell-associated HIV RNA revealed low-level viral transcription in peripheral $CD4^+$ T cells in all patients. The median expression of HIV/*TBP* in $CD4^+$ T cells decreased 9-fold at day 28 in the IFN group ($P = .049$), whereas no significant changes were observed in the control group (Table 2 and Supplementary Figure 1*A*).

We measured the residual plasma viremia level to evaluate whether the decline in cell-associated viral transcription decreased production of virus. We detected plasma viremia in 90% of patients. However, we observed no significant changes in any group during the study (Table 2, Supplementary Figure 1*B*), suggesting that short-term treatment with IFN alfa does not reduce the residual viremia level, despite its effect on viral transcription.

Replication-Competent HIV Reservoir, Total HIV DNA, and 2-LTR DNA Circles

To evaluate the effect of IFN alfa on the size of the replication-competent reservoir, we measured the infectious units per million cells in a qVOA of $CD4^+$ T cells. We detected replication-competent viruses in 90% of patients. However, we did not detect a significant decrease at day 28 in any group (Table 2 and Supplementary Figure 1*C*).

We also detected circulating $CD4^+$ T cells harboring proviral DNA in all patients. The count remained stable in both study groups (Supplementary Figure 1*D*).

We quantified the number of 2-LTR circles to estimate the level of residual replication. We detected HIV episomes in 90% of patients. This parameter remained stable in both study groups (Supplementary Figure 1*E*).

Taken together, the data suggest that short-term treatment with IFN alfa did not affect the replication-competent HIV reservoir, the total proviral HIV reservoir, or the number of residual new infections.

Expression of ISGs

As most of the genes upregulated by IFN-α are host restriction factors, we analyzed the effect of IFN alfa on the restriction factors TRIM22, BST2, TRIM5α, and APOBEC3G, which inhibit HIV replication. The mRNA expression of all these host restriction factors increased at day 28 of treatment with IFN alfa–RBV (Table 2 and Supplementary Figure 2). Conversely, no changes were detected in the control group. Moreover, we observed significant differences between the groups in the expression of TRIM22 and TRIM5α at day 28 ($P < .001$ and $P = .025$, respectively; Table 2 and Supplementary Figure 2).

Given that Siglec-1 is a type I IFN-α–induced protein that plays a key role in HIV spread through mDC-CD4 synapsis [6], we measured changes in expression of Siglec-1 in monocytes. We observed a significant increase in Siglec-1 protein expression at day 28 ($P = .002$), with significant intergroup differences ($P < .001$; Table 2 and Supplementary Figure 2).

Table 2. Effect of Interferon (IFN) Alfa Combined With Ribavirin on Markers of Human Immunodeficiency Virus Type 1 (HIV) and IFN Alfa–Stimulated Gene Expression

Marker	Control Group (n = 10)	IFN Alfa Group (n = 10)	*P* Value[a]
Cell-associated HIV RNA			
Ratio of HIV RNA to housekeeping gene			
Day 0	0.13 (0.07–1.45)	0.92 (0.23–3.29)	.143
Day 28	0.13 (0.05–0.42)	0.25 (0.02–0.96)	.912
P[b]	.432	.049	
Ultrasensitive plasma HIV load, copies/mL			
Day 0	0.90 (0.20–6.33)	2.40 (0.88–4.65)	.463
Day 28	0.20 (0.20–3.53)	0.65 (0.20–1.65)	.967
P[c]	.147	.108	
Infectious units per 10^6 $CD4^+$ T cells			
Day 0	0.42 (0.23–8.29)	1.61 (0.23–4.45)	.676
Day 28	0.42 (0.10–12.59)	1.11 (0.40–2.02)	.870
P[b]	.813	.359	
Total HIV DNA load, copies/10^6 $CD4^+$ T cells			
Day 0	1302 (606–2879)	1425 (561–2567)	.853
Day 28	1274 (594–1860)	1669 (589–2985)	.393
P[b]	.065	.131	
2-LTR HIV DNA level, copies/10^6 $CD4^+$ T cells			
Day 0	10.7 (0–15.7)	4.5 (0–15.7)	.847
Day 28	4.8 (2.3–10.8)	8.5 (2.7–34.7)	.383
P[b]	.426	.138	
Day 28 ISG expression, fold change from day 0			
TRIM22	1.15 (0.81–1.38)	2.02 (1.58–3.07)	<.001[d]
P[b]	.322	.002	
BST2	1.04 (0.81–1.20)	1.96 (1.17–2.43)	.067[d]
P[b]	.922	.037	
TRIM5α	0.96 (0.66–1.19)	1.67 (1.27–1.98)	.025[d]
P[b]	.695	.002	
APOBEC3G	0.89 (0.68–1.17)	1.24 (1.13–1.52)	.091[d]
P[b]	.432	.004	
Cells expressing Siglec-1			
Percentage of cells	1.01 (0.89–1.34)	1.54 (1.13–3.63)	.016[d]
P[b]	.492	.002	
Geometric mean percentage	1.09 (0.86–1.33)	5.85 (3.69–15.02)	<.001[d]
P[b]	.432	.002	

P values of ≤.05 were considered statistically significant.

Abbreviations: ISG, IFN-α–stimulated gene; LTR, long terminal repeat.

[a] By the Kruskal–Wallis test (using GraphPad Prism v5.01) unless otherwise indicated, for comparison between groups.

[b] By the Wilcoxon signed rank test (using GraphPad Prism v5.01), for comparison between day 0 and day 28.

[c] By the paired Prentice–Wilcoxon test (using MiniTab v16.2.3), for comparison between day 0 and day 28.

[d] By the Wald test (using R v3.02), for comparison between groups.

Analysis of $CD4^+$ T-Cell Subsets, Activation, and Exhaustion Markers

Significant changes in absolute $CD4^+$ and $CD8^+$ T-cell counts were seen in the IFN group between day 0 and day 28 ($P = .009$ and $P = .031$, respectively) and between the groups at day 28

($P = .010$ and $P = .005$; Table 1). In $CD4^+$ T cells, significant decreases were observed in all subsets except for T_{EMRA} cells. The analysis of the subset frequencies showed a slight increase in the T_{CM} and T_{EM} subsets ($P = .048$ and $P = .037$, respectively) and a reduction in the T_{TM} subpopulation ($P = .037$) in the IFN group at day 28 of treatment; however, none of the differences were statistically significant between the groups.

A general decrease in the expression of activation markers was observed in $CD4^+$ T cells from the IFN group (Supplementary Figure 3*A*). The frequency of HLA-DR^+CD38^+ was significantly lower in the IFN group than in the control group for the whole $CD4^+$ T-cell population and for all the subsets except T_{EMRA} cells (Supplementary Figure 3*A*). Conversely, the frequency of HLA-DR^+CD38^+ cells remained unchanged in the whole $CD8^+$ T-cell population (data not shown). We did not observe any significant difference in PD-1 percentages in the treated group, either for total $CD4^+$ T cells or for individual subsets (Supplementary Figure 3*B*). The data suggest that short-term IFN alfa treatment reduces peripheral $CD4^+$ T-cell activation.

DISCUSSION

Our study is the first to evaluate the short-term effect of IFN alfa–RBV on multiple virological and immunological parameters generally evaluated during interventions to reduce the HIV reservoir. Quantification of cell-associated HIV RNA at baseline revealed persistent low-level viral transcription in peripheral $CD4^+$ T cells in all patients, even though they had been receiving suppressive cART for a median of 4.5 years. Moreover, treatment with IFN alfa–RBV decreased transcription of HIV RNA in circulating $CD4^+$ T cells while simultaneously reducing T-cell activation and increasing expression of ISGs. It seems that the decrease in T-cell activation could contribute to a decrease in viral transcription and mRNA translation [13]. However, these changes could also be associated with IFN alfa–driven migration of T cells from peripheral blood to lymphoid tissue [14]. Although we observed slight changes in the relative frequency of $CD4^+$ T-cell subsets, it remains to be determined which T-cell subsets mainly contribute to HIV RNA transcription under cART.

Finally, upregulation of host restriction factors induced by IFN is expected to reduce viral replication. In our study, TRIM22, a nuclear restriction factor able to inhibit HIV transcription [15], was markedly upregulated in the IFN group, potentially contributing to the decrease in viral transcription.

Notwithstanding this, the lack of changes in ultrasensitive plasma viremia level suggests that the effect of short-term IFN alfa on peripheral $CD4^+$ T cells with decreased viral transcription may not extend to all tissue cells that might also contribute to residual plasma viremia.

In contrast with the results of previous studies where IFN alfa was administered for longer than 28 days [3, 4], we did not detect any change in the size of the HIV reservoir. Treatment with IFN alfa–RBV was previously shown to lead to a moderate but significant and sustained decline in HIV DNA in $CD4^+$ T cells from HIV/HCV-coinfected patients receiving cART [4]. Moreover, Azzoni et al [3] found a drop in the level of integrated HIV DNA in IFN alfa–treated subjects who controlled viral rebound after interruption of cART. Furthermore, although a significant decrease in the level of 2-LTR circles was reported in patients receiving suppressive cART who were treated with IFN alfa–RBV [5], levels of episomes remained unchanged in the present study. Thus, the short period of treatment in our study (28 days) might explain these differences in total proviral DNA and 2-LTR levels, suggesting that administration of IFN alfa–RBV could have a differential effect over time. Moreover, we observed no correlation between the virological variables analyzed. We cannot exclude, however, that some of the negative findings in this study could also be due to issues regarding sample size and assay precision.

We conclude that 28 days of treatment with IFN alfa can trigger a reduction in HIV transcription in HIV/HCV-coinfected patients receiving suppressive cART, through upregulation of viral restriction genes, mainly *TRIM22*, and a decrease in T-cell activation. Although neither residual viremia nor replication-competent virus levels changed at day 28 of treatment, the effects of longer treatment periods remain to be determined. Therefore, after 28 days of treatment, IFN alfa acts more as a viral suppressor than as a reactivating agent. Studies on long-term therapy with IFN in patients receiving ART are necessary to elucidate the mechanism by which IFN alfa–mediated viral suppression extends from cell-associated RNA to DNA and whether it affects replication-competent reservoir levels.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

Acknowledgments. S. M.-L. and E. G.-M acquired, analyzed, and interpreted the data and wrote the manuscript; M. S. and M. C. P. contributed to the acquisition and analysis of data; D. O. and V. U. performed the statistical analysis; J. N., A. J., M. P., C. T., B. C., and M. C. contributed to clinical study design and performance and reviewed the manuscript; L. J. M. contributed to data interpretation and reviewed the manuscript; J. B. contributed to data analysis and interpretation and reviewed the manuscript; and M. C. and J. M.-P. participated in the study design and interpretation of data and wrote the manuscript.

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