People with HIV-1 Demonstrate Type 1 Interferon Refractoriness Associated with Upregulated USP18

Sho Sugawara,^{a,b} Ramy El-Diwany,^a Laura K. Cohen,^a Kimberly E. Rousseau,^a Christopher Y. K. Williams,^c Rebecca T. Veenhuis,^a Shruti H. Mehta,^d Joel N. Blankson,^a David L. Thomas,^a Andrea L. Cox,^a Ashwin Balagopal^a

^aDepartment of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

^bCenter for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA

^cUniversity of Cambridge School of Clinical Medicine, Cambridge, United Kingdom

^dDepartment of Epidemiology, Johns Hopkins University School of Public Health, Baltimore, Maryland, USA

ABSTRACT HIV-1 infection persists in humans despite expression of antiviral type 1 interferons (IFN). Even exogenous administration of IFN α only marginally reduces HIV-1 abundance, raising the hypothesis that people living with HIV-1 (PLWH) are refractory to type 1 IFN. We demonstrated type 1 IFN refractoriness in CD4⁺ and CD8⁺ T cells isolated from HIV-1-infected persons by detecting diminished STAT1 phosphorylation (pSTAT1) and interferon-stimulated gene (ISG) induction upon type 1 IFN stimulation compared to those in cells from healthy controls. Importantly, HIV-1-infected people who were virologically suppressed with antiretrovirals also showed type 1 IFN refractoriness. We found that USP18 levels were elevated in people with refractory pSTAT1 and ISG induction and confirmed this finding *ex vivo* in CD4⁺ T cells from another cohort of HIV-hepatitis C virus (HCV) coinfecting persons who received exogenous pegylated interferon- α 2b in a clinical trial. We used a cell culture model to recapitulate type 1 IFN refractoriness in uninfected CD4⁺ T cells that were conditioned with media from HIV-1 inoculated peripheral blood mononuclear cells (PBMCs), inhibiting *de novo* infection with antiretroviral agents. In this model, RNA interference against USP18 partly restored type 1 IFN responses in CD4⁺ T cells. We found evidence of type 1 IFN refractoriness in PLWH irrespective of virologic suppression that was associated with upregulated USP18, a process that might be therapeutically targeted to improve endogenous control of infection.

IMPORTANCE People living with HIV-1 (PLWH) have elevated constitutive expression of type 1 interferons (IFN). However, it is unclear whether this affects downstream innate immune responses. We identified refractory responses to type 1 IFN stimulation in T cells from PLWH, independent of antiretroviral treatment. Type 1 IFN refractoriness was linked to elevated USP18 levels in the same cells. Moreover, we found that USP18 levels predicted the anti-HIV-1 effect of type 1 IFN-based therapy on PLWH. *In vitro*, we demonstrated that refractory type 1 IFN responses were transferable to HIV-1-uninfected target CD4⁺ T cells, and this phenomenon was mediated by type 1 IFN from HIV-1-infected cells. Type 1 IFN responses were partially restored by USP18 knockdown. Our findings illuminate a new mechanism by which HIV-1 contributes to innate immune dysfunction in PLWH through the continuous production of type 1 IFN that induces a refractory state of responsiveness.

KEYWORDS HIV-1, innate immunity, type 1 IFN

Antiretroviral therapy (ART) has enabled people living with HIV-1 (PLWH) to live longer with a better quality of life (1). However, PLWH still have worse outcomes than people without HIV-1 (2), and the reasons underlying these worse outcomes are not fully understood. The major challenge to curing HIV-1 is the persistence of the long-

Citation Sugawara S, El-Diwany R, Cohen LK, Rousseau KE, Williams CYK, Veenhuis RT, Mehta SH, Blankson JN, Thomas DL, Cox AL, Balagopal A. 2021. People with HIV-1 demonstrate type 1 interferon refractoriness associated with upregulated USP18. *J Virol* 95:e01777-20. <https://doi.org/10.1128/JVI.01777-20>.

Editor Viviana Simon, Icahn School of Medicine at Mount Sinai

Copyright © 2021 American Society for Microbiology. All Rights Reserved.

Address correspondence to Ashwin Balagopal, abalago1@jhmi.edu.

Received 18 September 2020

Accepted 19 February 2021

Accepted manuscript posted online 3 March 2021

Published 26 April 2021

lived provirus reservoir that resides in resting CD4⁺ T cells (3). Among experimental therapies to treat PLWH, type 1 interferon (IFN) treatment has been evaluated in various trials and combinations for several decades. Unfortunately, PLWH who were treated with exogenous type 1 IFN have only achieved modest decreases in plasma HIV-1 RNA levels and in the size of the latent reservoir (5, 6). Moreover, PLWH who are coinfecting with hepatitis C virus (HCV) have lower HCV response rates to exogenous pegylated type 1 interferon (PEG-IFN) (7–12). Taken together, these findings suggest that type 1 IFN responses may be systemically modulated by HIV-1 infection, resulting in worsened antiviral control.

Type 1 IFN signaling can be conceptually divided into a sensing arm and an effector arm. In the sensing arm, viral nucleic acids trigger activation of several pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), RIG-I, and cGAS. PRRs then transduce signaling cascades that activate transcription factors such as NF- κ B and IRF3. These transcription factors translocate into the nucleus and upregulate expression of type 1 IFN, which is then released to act in an autocrine or paracrine fashion. The effector arm of signaling is initiated by binding of type 1 IFN to the IFN alpha receptor (IFNAR) heterodimer on the surface of target cells, prompting JAK-STAT signaling. STAT1 and STAT2 are phosphorylated upon type 1 IFN binding with IFNAR, and these phospho-STAT proteins dimerize with each other. The activated phospho-STAT1/2 heterodimer translocates to the nucleus, binds to the transcription factor IRF9, and in complex upregulates expression of hundreds of interferon-stimulated genes (ISGs). Many ISGs are known to have antiviral functions (13–15), such as MX2, which inhibits HIV-1 replication by interfering with nuclear translocation of HIV-1 cDNA (16). Suppressor of cytokine signaling (SOCS) proteins and USP18 mediate a negative feedback loop of type 1 IFN signaling (17). SOCS proteins inhibit type 1 IFN signaling by competing with STAT1, preventing STAT1 phosphorylation (18), while USP18 displaces JAK1 from IFNAR (19). These counter-regulatory molecules can dampen innate immune responses, inducing a refractory state to further type 1 IFN stimulation.

It has previously been demonstrated *in vitro* that HIV-1 regulates the sensing arm of type 1 IFN signaling in several cell types (20–24). However, PLWH show evidence of systemic type 1 IFN signaling, implying that the sensing arm of type 1 IFN signaling is intact in some cell types in PLWH (25–29). We hypothesized that HIV-1 infection may also modulate the effector arm of type 1 IFN signaling, resulting in a type 1 IFN refractory state that is permissive of viral replication.

(This work was presented at the 2019 Conference on Retroviruses and Opportunistic Infection [CROI], Seattle, WA [30], and the 2020 The Japanese Society for AIDS Research Conference [virtual] [31].)

RESULTS

Type 1 IFN responses are refractory in CD4⁺ and CD8⁺ T cells from viremic PLWH. We tested the hypothesis that HIV-1 infection is associated with refractory type 1 IFN responses by directly measuring activation of JAK/STAT signaling, since this is the most upstream event in the effector limb of type 1 IFN signaling after innate immune sensing occurs. We quantified phosphorylation of STAT1 using flow cytometry before and after stimulation with type 1 IFN in CD4⁺ T cells (CD3⁺/CD8[−]) isolated from PLWH (32) (Fig. 1A and B). We then measured the change in STAT1 phosphorylation in peripheral blood mononuclear cells (PBMCs) from people with uncontrolled HIV-1 (HIV-UC), people who were virologically suppressed with ART for ≥ 1 year (HIV-ART), and people without HIV-1 (healthy controls [HC]). We confirmed that there was a significant difference in baseline pSTAT1 level between HIV-UC and HC ($P < 0.05$; Fig. 1C). Upon stimulation of PBMCs with exogenous type 1 IFN, we found that the change in pSTAT1 levels in total CD4⁺ T cells from HIV-UC was significantly less than that in HC ($P < 0.005$; Fig. 1C and D). Interestingly, we also observed that total CD4⁺ T cells from HIV-ART exhibited intermediate changes in pSTAT1 levels with type 1 IFN stimulation, suggesting that virologic suppression does not fully reverse type 1 IFN refractoriness.

We also examined the change in pSTAT1 levels in subsets of T cells, including

FIG 1 CD4⁺ and CD8⁺ T cell subsets from PLWH have reduced pSTAT1 activation with *ex vivo* type 1 IFN stimulation. PBMCs from PLWH with uncontrolled viremia (HIV-UC; *n*=8), ART-controlled viremia (HIV-ART; *n*=7), or healthy controls (HC; *n*=12) were stimulated with type 1 IFN 300 U/ml for 5 min; levels of pSTAT1 in total CD4⁺ T cells were quantified by flow cytometry after type 1 IFN stimulation and compared to levels on cells that were untreated. (A) The gating strategy and (B) representative pSTAT1 histograms are shown. (C) Levels of pSTAT1 with and without type 1 IFN in PLWH. Baseline (circles) and stimulated (squares) pSTAT1 levels from each individual from HIV-UC, HIV-ART, and HC are shown. Mann-Whitney tests were performed to evaluate statistically significant differences (*, *P*<0.05). (D, E, F, G) The changes in pSTAT1 levels (pSTAT1 activation) are shown for (D) total CD4⁺ T cells, (E) activated (CD38⁺/HLA-DR⁺) CD4⁺ T cells, (F) resting (CD38⁻/HLA-DR⁻) CD4⁺ T cells, and (G) total CD8⁺ T cells. Statistical significance between groups was determined by pairwise Wilcoxon tests. (*, *P*<0.05; **, *P*<0.01; ***, *P*<0.005).

activated (HLA-DR⁺ CD38⁺ CD3⁺ CD8⁻ cells) and resting (HLA-DR⁻ CD38⁻ CD3⁺ CD8⁻ cells) CD4⁺ T cells and total CD8⁺ T cells, testing whether type 1 IFN refractoriness was specific to cells that are susceptible to HIV-1 infection or could be found in other cell types more generally, even in cells that are not permissive to HIV-1. Similar to the

results in total CD4⁺ T cells, we found that pSTAT1 levels were reduced in HIV-UC and HIV-ART compared to those in HC in activated CD4⁺ T cells, resting CD4⁺ T cells, and CD8⁺ T cells ($P < 0.05$ for all; Fig. 1E to G). Collectively, these results suggest global type 1 IFN refractoriness in T cells from PLWH, irrespective of virologic suppression.

We confirmed that downstream antiviral responses were also refractory in PLWH by measuring ISG induction using quantitative PCR (qPCR), targeting several ISGs with well-described antiviral functions: *MX2* (16, 33), *ISG15* (33, 34), *PKR* (35), and *BST2* (also known as tetherin) (33, 36). ISG induction was measured in total CD4⁺ T cells that were stimulated with type 1 IFN, using cells from HIV-UC, HIV-ART, and HC. ISG induction was significantly diminished in cells from HIV-UC compared to that in cells from HC (Fig. 2). This phenomenon appeared to result from increased baseline ISG expression in HIV-UC compared to that in HC for *ISG15*, *PKR*, and *BST2*, similar to what has been reported previously (25). Conversely, we observed dampened post-IFN ISG expression in HIV-UC compared to that in HC for *MX2*, *ISG15*, and *PKR* (Table 1). We observed an intermediate amount of ISG expression and induction in CD4⁺ T cells from HIV-ART, consistent with the pSTAT1 results (Fig. 1). Intriguingly, baseline ISG expression (i.e., in the absence of type 1 IFN stimulation) was enhanced in CD4⁺ T cells from HIV-UC, while post-IFN ISG expression was dampened.

Type 1 IFN refractoriness in CD4⁺ T cells is associated with USP18 upregulation.

Next, we explored possible mechanisms that would explain type 1 IFN refractoriness in PLWH. The effector arm of type 1 IFN signaling is counter-regulated by several type 1 IFN regulatory factors, including SOCS proteins and USP18 (17). We speculated that type 1 IFN refractoriness in PLWH may be due to increased intracellular counter-regulatory molecular signaling. We measured baseline mRNA levels of three type 1 IFN counter-regulatory genes, *SOCS1*, *SOCS3*, and *USP18*, in total CD4⁺ T cells. Baseline expression of *SOCS1* and *USP18* was upregulated in CD4⁺ T cells from HIV-UC compared to that in CD4⁺ T cells from HC, while CD4⁺ T cells from HIV-ART exhibited intermediate baseline upregulation of *SOCS1* (Fig. 3A). We correlated baseline expression levels of *SOCS1* and *USP18* with the subsequent induction of pSTAT1 and four ISGs (*MX2*, *ISG15*, *PKR*, and *BST2*) after type 1 IFN stimulation in the same cells. The magnitude of baseline *USP18* expression was inversely correlated with subsequent pSTAT1 activation ($r = -0.47$, $P < 0.05$ for *USP18*; Fig. 3B) and the induction of four ISGs ($r = -0.67$, $P = 3 \times 10^{-4}$ for *MX2*, $r = -0.59$, $P < 0.05$ for *ISG15*, $r = -0.77$, $P < 0.05$ for *PKR*, $r = -0.79$, $P < 0.05$ for *BST2*; Table 2). Notably, the relationship between baseline *USP18* levels and *MX2* induction appeared to be conserved across HIV-UC, HIV-ART, and HC. Baseline *SOCS1* expression was also inversely correlated with pSTAT1 activation and ISG induction.

We confirmed the association between *USP18* and type 1 IFN refractoriness *in vivo* in CD4⁺ T cells from PLWH who received PEG-IFN. We previously demonstrated heterogeneous induction of ISGs in activated CD4⁺ T cells taken from 19 HIV-1/HCV coinfecting people who were treated with PEG-IFN and who had viral kinetic measurements of plasma HIV-1 RNA levels (33). In that study, we performed RNA sequencing (RNA-seq) on activated CD4⁺ T cells taken before and 24 h after a single administration of PEG-IFN. We identified 99 genes that were robustly upregulated in activated CD4⁺ T cells that met the criteria of ISGs, including *MX2*. We interrogated the RNA-seq data set from cells prior to PEG-IFN treatment for reads that mapped to *USP18* or *SOCS1*. We found a strong correlation between the baseline read frequency of *USP18* and the subsequent induction of *MX2* after PEG-IFN ($r = -0.775$, $P = 2 \times 10^{-7}$) but no correlation for *SOCS1* ($r = 0.065$, $P = 0.787$) (Fig. 3C). Next, we performed an unbiased analysis of all gene expression at baseline that correlated inversely with ISG induction (for *MX2*, *PKR* [protein kinase R], and *IFIT3* [IFN-induced protein with tetratricopeptide repeats]). Without prespecifying, *USP18* arose independently within the top 50 genes that exhibited the strongest negative correlation with the subsequent *in vivo* induction of these ISGs with PEG-IFN. In contrast, *SOCS1* was not among the top 50 genes (Table 3).

We reasoned that for refractory type 1 IFN responses to be clinically significant, it

FIG 2 CD4⁺ T cells from PLWH show diminished induction of interferon-stimulated genes (ISG) upon type 1 IFN stimulation. Total CD4⁺ T cells were isolated from PBMCs from HIV-UC (*n*=9), HIV-ART (Continued on next page)

would require an impact on virologic control, for instance by resulting in worse control of HIV-1 by PEG-IFN. Accordingly, we correlated the baseline read frequency of USP18 with the subsequent plasma HIV-1 RNA decline after PEG-IFN treatment. As hypothesized, we found that baseline USP18 expression in activated CD4⁺ T cells was tightly and inversely associated with subsequent decline in plasma HIV-1 RNA levels that appeared to involve MX2 induction: persons with large amounts of baseline USP18 expression in activated CD4⁺ T cells had less MX2 induction and smaller subsequent PEG-IFN-induced decreases in HIV-1 viremia than persons with small amounts of baseline USP18 expression ($r = -0.73$, $P = 0.0018$), whereas baseline SOCS1 expression was poorly correlated with PEG-IFN-induced decreases in HIV-1 viremia ($r = -0.17$, $P = 0.51$) (Fig. 3D).

We performed a similar unbiased analysis of expression of all genes in the activated CD4⁺ T cell transcriptome to identify those that appeared to be negatively correlated with the decline in plasma HIV-1 RNA due to PEG-IFN; USP18 was again found in the top 50 genes inversely correlated with PEG-IFN-induced HIV-1 RNA decline (Table 3). Finally, the Venn diagram analysis of these top 50 genes revealed that USP18 was the only one of four genes (*FAM110A*, *SPATS2L*, *EPSTI1*, and *USP18*) that were among the top 50 genes exhibiting negative correlations with induction of MX2, PKR, and IFIT3 and with a decline in plasma HIV-1 viremia (Fig. 3E). Taken together, these results support our prior findings indicating that baseline USP18 expression in CD4⁺ T cells from PLWH may be a negative regulator of type 1 IFN *in vivo* that both predicts type 1 IFN refractoriness and may have a direct impact on the control of HIV-1 plasma viremia by exogenous PEG-IFN.

Type 1 IFN refractoriness in HIV-1 infection depends on soluble factors. We found supportive evidence that type 1 IFN refractoriness did not require direct infection by HIV-1 of the same cells, since CD8⁺ T cells also demonstrated the refractory phenotype (Fig. 1G). We confirmed this finding in a cell culture model: we used a well-characterized green fluorescent protein-tagged (GFP-tagged) HIV-1 virus (37) to infect primary CD4⁺ T cells from healthy donors. After infection, we sorted GFP⁺ and GFP[−] cells separately and measured baseline ISG levels, a surrogate for pSTAT1 activation that had a larger dynamic range (Fig. 4A). GFP⁺ CD4⁺ T cells showed modestly higher or no changes in ISG expression compared to that in GFP[−] cells (Fig. 4B). Thus, direct infection of CD4⁺ T cells with HIV-1 was neither necessary nor sufficient for changes in ISG expression.

We developed a model of type 1 IFN refractoriness *in vitro* to uncover its underlying mechanisms in PLWH. Since we showed that type 1 IFN refractoriness was independent of direct HIV-1 infection, we hypothesized that soluble mediators might transfer type 1 IFN refractoriness to other cell types. We tested whether conditioned media from *in vitro* HIV-1-infected PBMCs would be sufficient to recapitulate type 1 IFN refractoriness in uninfected target cells. We infected healthy leukopaks with HIV-1 BaL for 3 days and then collected and filtered supernatant through a 0.2 μ m filter to avoid transferring HIV-1-infected cells. Twenty-four hours after incubation, we stimulated target cells with type 1 IFN and quantified the induction of three ISGs (*MX2*, *ISG15*, and *IFIT3*) to test the hypothesis that conditioned supernatant from HIV-1-infected cells is sufficient to dampen type 1 IFN responses in target cells (Fig. 4C). We found refractory ISG induction in target cells incubated with conditioned supernatants from HIV-1-

FIG 2 Legend (Continued)

($n = 7$), and HC ($n = 12$) and were stimulated with exogenous type 1 IFN 10 U/ml for 6 h. Total RNA was extracted after stimulation and the expression levels of four ISGs (*MX2*, *ISG15*, *PKR*, and *BST2*) were measured and compared to RNA from unstimulated cells. Shown are (A) relative expression amounts of each ISG, normalized to *YWHAZ*, *SDHA*, and *RPLP0* housekeeping genes. Each individual is depicted as a separate bar plot without and with type 1 IFN stimulation, arranged identically for both conditions. (B) The induction/fold change of each ISG (calculated by adjusting relative expression without type 1 IFN to relative expression with type 1 IFN) is also shown. Statistically significant differences between the groups were determined by pairwise Wilcoxon tests. (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$).

TABLE 1 ISG expression in CD4⁺ T cells from PLWH. PBMCs from PLWH were thawed and rested overnight. Total CD4⁺ T cells were then isolated and stimulated with 10 U/ml of type 1 IFN. Six hours after stimulation, total RNA was extracted and the levels of four ISGs (*MX2*, *ISG15*, *PKR*, and *BST2*) from cells with and without type 1 IFN were quantified by qPCR. The expression levels were normalized to three housekeeping genes (*YWHAZ*, *SDHA*, and *RPLP0*). Summary table shows relative expression amounts of each ISG in the absence or presence of type 1 IFN stimulation. Mann-Whitney tests were used to evaluate statistically significant differences between groups (n.a., not applicable; n.d., not detectable).

-IFN	Gene	MX2			ISG15			PKR			BST2		
	Group	HIV-UC	HIV-ART	HC	HIV-UC	HIV-ART	HC	HIV-UC	HIV-ART	HC	HIV-UC	HIV-ART	HC
Participant ID	1	0.0633	0.1333	0.0766	0.0317	0.1154	0.0274	0.4029	0.2935	0.1547	0.1324	0.2192	0.135
	2	0.3509	0.4741	0.0633	0.045	0.1104	0.024	0.2707	0.1533	0.1246	0.2669	0.2776	0.1892
	3	0.057	0.0867	0.3645	0.1015	0.0884	0.0327	0.2477	0.418	0.3827	0.204	0.2615	0.0999
	4	0.1832	0.189	0.075	0.3251	0.3595	0.0517	0.6329	0.2484	0.1139	0.3794	0.3291	0.1114
	5	0.106	0.0869	0.0771	0.1426	0.00978	0.0301	0.3763	0.1117	0.1276	0.2629	0.11	0.1127
	6	0.1638	0.1529	0.0636	0.223	0.00564	0.0176	0.3581	0.0694	0.1294	0.167	0.1023	0.1083
	7	0.1942	0.1334	0.0311	0.1586	0.0473	0.0151	0.6046	n.a.	0.3589	0.2785	0.1433	0.1016
	8	0.2614	n.a.	0.0994	0.3761	n.a.	0.0365	0.3268	n.a.	0.1394	0.3008	n.a.	0.0893
	9	0.209	n.a.	0.0678	0.1842	n.a.	0.0348	n.d.	n.a.	0.0946	0.2486	n.a.	0.0664
	10	n.a.	n.a.	0.1468	n.a.	n.a.	0.00859	n.a.	n.a.	0.3124	n.a.	n.a.	0.2368
	11	n.a.	n.a.	0.0952	n.a.	n.a.	0.0135	n.a.	n.a.	0.2394	n.a.	n.a.	0.1057
	12	n.a.	n.a.	0.8151	n.a.	n.a.	n.d.	n.a.	n.a.	0.5704	n.a.	n.a.	0.0715
	median	0.1832	0.1334	0.07685	0.1586	0.0884	0.0274	0.3672	0.20085	0.14705	0.2629	0.2192	0.107
	p value (compared to HC)	0.2255	0.0831	n.a.	0.0002	0.1509	n.a.	0.0159	0.8916	n.a.	0.0002	0.0221	n.a.
+IFN	Gene	MX2			ISG15			PKR			BST2		
	Group	HIV-UC	HIV-ART	HC	HIV-UC	HIV-ART	HC	HIV-UC	HIV-ART	HC	HIV-UC	HIV-ART	HC
Participant ID	1	0.5529	0.6406	0.7855	0.79	0.9075	0.8521	1.0329	1.2592	0.9537	0.6008	0.606	0.612
	2	5.6732	1.6453	0.4498	1.1051	0.527	0.8888	1.4837	1.318	1.459	1.0956	0.4684	0.6177
	3	0.1229	0.4224	2.9046	0.3105	0.9165	0.5279	0.534	0.9488	2.2987	0.3943	0.5922	0.3624
	4	0.6503	0.2813	0.9138	1.0638	0.5158	1.2614	0.5516	0.5854	1.1892	0.6336	0.4012	0.5105
	5	0.362	0.8873	0.838	0.7896	1.0425	1.1668	1.2672	1.8245	1.5026	0.5487	0.7346	0.6406
	6	0.2875	1.494	0.5303	0.3677	1.152	0.7873	0.5384	1.107	1.0905	0.3497	0.5084	0.4561
	7	0.3251	1.8736	0.5874	0.2507	1.3692	1.4389	0.5264	1.1534	2.1361	0.3308	0.6053	0.6962
	8	0.2638	n.a.	1.0395	0.4553	n.a.	1.3931	0.9897	n.a.	1.0668	0.5276	n.a.	0.5038
	9	0.4246	n.a.	0.9648	0.7627	n.a.	1.6857	0.7978	n.a.	0.9433	0.5282	n.a.	0.4149
	10	n.a.	n.a.	0.5987	n.a.	n.a.	1.279	n.a.	n.a.	1.2834	n.a.	n.a.	0.4421
	11	n.a.	n.a.	0.7796	n.a.	n.a.	1.7695	n.a.	n.a.	1.7664	n.a.	n.a.	0.7038
	12	n.a.	n.a.	3.5105	n.a.	n.a.	1.4709	n.a.	n.a.	1.7311	n.a.	n.a.	0.8274
	median	0.362	0.8873	0.81175	0.7627	0.9165	1.2702	0.7978	1.1534	1.3712	0.5282	0.5922	0.56125
	p value (compared to HC)	0.0093	0.9671	n.a.	0.0024	0.1198	n.a.	0.0056	0.3402	n.a.	0.5538	0.8369	n.a.

FIG 3 Expression of counter-regulatory genes, type 1 IFN responses, and HIV-1. (A, B) *Ex vivo* expression of counter-regulatory genes and type 1 IFN responses. (A) Total CD4⁺ T cells were isolated from PBMCs from HIV-UC ($n = 9$), HIV-ART ($n = 7$), and HC ($n = 12$), and

(Continued on next page)

infected cells (sup-LP plus BaL) compared to target cells conditioned with supernatants from HIV-1-uninfected leukopaks (sup-LP without BaL) ($P < 0.05$ for all ISGs; Fig. 4D). As we saw in CD4⁺ T cells from HIV-UC, baseline ISG expression was also increased in target cells conditioned with sup-LP plus BaL compared to that in target cells conditioned with sup-LP without BaL. In addition, total ISG expression upon type 1 IFN stimulation was diminished in cells conditioned with sup-LP plus BaL (Table 4). Next, we investigated whether reduced ISG induction is linked to increased baseline USP18 expression in the same cells. We confirmed that baseline USP18 mRNA levels in target cells prior to type 1 IFN treatment were higher in those conditioned with sup-LP plus BaL than in those conditioned with sup-LP without BaL (Fig. 4E). Taken together, these experiments indicate that type 1 IFN refractoriness results from soluble mediators transferred from HIV-1-infected PBMCs to target cells and is associated with elevated USP18 expression in target cells. Treatment of target cells with antiretrovirals (ARVs) excluded the possibility of direct infection contributing to type 1 IFN refractoriness.

Type 1 IFN production by HIV-1-infected PBMCs perpetuates type 1 IFN refractoriness. We investigated which soluble mediators might potentially upregulate USP18 and transfer type 1 IFN refractoriness to uninfected target cells. Veenhuis et al. previously measured plasma levels of a panel of 17 cytokines in a cohort of people who inject drugs, and upon reanalysis of their data we found increased levels of interleukin 12/23p40 (IL-12/23p40), IL-18, IFN γ , IL-15, eotaxin, MCP-1 (monocyte chemoattractant protein 1), and IP-10 (induced protein 10) in PLWH with uncontrolled HIV-1 compared to those in HIV-1-uninfected participants ($P < 0.005$ for all) (38). In addition to these, we examined type 1 IFN itself since it is known to induce USP18 expression (39, 40) and is not well detected in plasma. We stimulated primary CD4⁺ T cells with each of these cytokines *in vitro* to test which would induce USP18 expression. We found that type 1 IFN and IL-15 induced >2-fold upregulation of USP18 but not IL-12/23p40, IL-18, IFN γ , eotaxin, MCP-1, or IP-10 (Fig. 5A). We then measured the amounts of type 1 IFN, focusing on subtype IFN α 2a, which is known to be elevated during chronic HIV-1 infection (41, 42), and IL-15 in the conditioned supernatants from sup-LP plus BaL and sup-LP without BaL. We found no correlation between IL-15 levels in conditioned supernatants with USP18 expression ($r = 0.49$, $P = 0.18$) nor with the dampening of ISG induction ($r = 0.15$, $P = 0.66$), consistent with a limited role of IL-15 in promoting type 1 IFN refractoriness. In contrast, levels of IFN α 2a in conditioned supernatants exhibited strong positive correlation with baseline USP18 levels ($r = 0.80$, $P = 2 \times 10^{-7}$) and negatively correlated with subsequent MX2 induction ($r = -0.98$, $P = 2 \times 10^{-7}$) in target CD4⁺ T cells (Fig. 5B).

Whereas supernatants from HIV-1-infected PBMCs were sufficient to cause type 1 IFN refractoriness, productive HIV-1 infection in CD4⁺ T cells was not (Fig. 4). Therefore, we reasoned that non-CD4⁺ T cells within populations of PBMCs must be responsible for type 1 IFN production. We hypothesized that PBMCs produce type 1 IFN in

FIG 3 Legend (Continued)

baseline levels of *SOCS1*, *SOCS3*, and *USP18* were quantified by qPCR. Mann-Whitney tests were used to determine statistically significant differences between the groups (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$). (B) pSTAT1 activation (fold change) with type 1 IFN stimulation is shown correlated with baseline *SOCS1* (top) and *USP18* (bottom) in the same cells. Spearman correlation coefficients and P values are shown for each figure. (C, D) *In vivo* expression of counter-regulatory genes, type 1 IFN responses, and plasma HIV-1 RNA decline. (C) Using an RNA-seq data set of CD4⁺ T cells (HLA-DR⁺/CD38⁺) from 19 HIV/HCV coinfecting persons who were given a single dose of pegylated-IFN (PEG-IFN), basal mRNA levels of *USP18* (blue circles) and *SOCS1* (red circles) were quantified and correlated with subsequent MX2 induction in the same cells (calculated by normalizing expression levels 24 h after PEG-IFN to expression levels before PEG-IFN). (D) Plasma HIV-1 RNA declines after PEG-IFN administration are shown in correlation with basal expression of *USP18* (blue circles) and *SOCS1* (red circles) mRNA. The size of each point depicts the relative induction of MX2. Spearman correlation coefficients and corresponding P values are calculated for each. (E) Top 50 genes inversely correlated with MX2, PKR, and IFIT3 induction *in vivo* and PEG-IFN-induced plasma HIV-1 RNA decline. Baseline expression of each gene in RNA-seq data set was correlated with the induction of three ISGs (MX2, PKR, and IFIT3) and plasma HIV-1 RNA decline by PEG-IFN using Spearman correlation. The top 50 genes exhibiting the strongest negative correlations with plasma HIV-1 decline and the induction of three ISGs were selected based on Spearman correlation coefficient in an unbiased analysis. The Venn diagram depicts the list of genes found by this analysis. The numbers in the Venn diagram represent the number of genes that meets each criterion. *USP18* was one of four genes (*FAM110A*, *SPATS2L*, *EPSTI1*, and *USP18*) that arose within the top 50 genes exhibiting negative associations with induction of three ISGs as well as a decline in plasma HIV-1 RNA.

TABLE 2 Spearman correlation coefficients between basal SOCS1 and USP18 expression and ISG induction in PLWH CD4⁺ T cells. Total CD4⁺ T cells are purified from PLWH PBMCs (HIV-UC, *n* = 9; HIV-ART, *n* = 7; and HC, *n* = 12) and stimulated with type 1 IFN. Six hours after stimulation, total RNA was extracted and the levels of *SOCS1*, *USP18*, and four ISGs (*MX2*, *ISG15*, *PKR*, and *BST2*) were measured by qPCR. Relative expression unit of each gene was calculated by normalizing the expression levels by three housekeeping genes (*YWHAZ*, *SDHA*, and *RPLP0*), and baseline *SOCS1* and *USP18* levels were correlated with induction of four ISGs. Spearman correlation was used to calculate correlation coefficients (n.d., not detectable).

Participants		Baseline Expression		ISG induction			
Group	Participant ID	SOCS1	USP18	MX2	ISG15	PKR	BST2
HIV-UC	1	0.091296	0.01225233	5.5021673	24.8901	4.9674313	4.539395
	2	0.143667	0.02652139	16.167223	24.54743	5.0542604	4.105335
	3	0.034237	0.02216151	2.1559644	3.059579	3.4842142	1.932989
	4	0.043234	0.02952949	3.5492151	3.271608	2.2268432	1.670176
	5	0.046391	0.04036364	1.6160816	2.892867	1.7191157	1.337155
	6	0.081475	0.01634508	1.755238	1.649086	1.4306459	2.094588
	7	0.039303	0.03737714	1.6740392	1.580996	1.4700175	1.187834
	8	0.236793	0.01997307	1.0092848	1.210693	1.6367493	1.754224
	9	0.13814	n.d.	2.0314361	4.14106	2.4410469	2.125055
HIV-ART	1	0.065644	0.06114168	4.8065442	7.862565	3.590455	2.765423
	2	0.350503	0.0686308	3.4701548	4.773343	3.2716082	1.687632
	3	0.057016	0.0299251	4.8708267	10.36873	3.5043981	2.264459
	4	0.107075	0.08547818	1.4879575	1.434784	1.4003945	1.219114
	5	0.032035	0.00643787	10.208271	106.6446	7.3445122	6.676846
	6	0.042051	0.00695999	9.7698292	204.1284	9.9062109	4.970301
	7	0.106336	0.01020203	14.041904	28.92343	16.612147	4.225631
HC	1	0.04321	0.00734002	10.255553	35.46523	6.1652811	4.534154
	2	0.045912	0.0061722	7.1066353	32.48421	11.712686	3.264058
	3	0.033843	0.00970201	7.9677184	16.12991	6.0070916	3.62798
	4	0.042787	0.00468836	8.3397261	44.6318	8.4268883	4.213444
	5	0.018001	0.00964034	18.874066	95.39444	5.9518301	6.848715
	6	0.028115	0.00509218	10.452914	38.14265	7.6519812	5.64054
	7	0.020169	0.00267416	14.221483	48.50293	9.9693538	6.244128
	8	0.044348	0.45454657	4.0793332	148.9138	4.1077072	1.867144
	9	0.046151	n.d.	8.1869911	130.6896	7.3785296	6.661437
	10	0.021593	n.d.	12.181895	n.d.	10.440846	4.584189
	11	0.003786	n.d.	4.3069503	24.40605	3.0349372	11.56479
	12	0.034019	n.d.	10.871658	38.71981	11.773734	5.686339
		<i>r</i> with SOCS1	-0.46	-0.48	-0.59	-0.40	
		<i>p</i> value with SOCS1	0.0073	0.0060	0.017	0.00046	
		<i>r</i> with USP18	-0.67	-0.59	-0.79	-0.77	
		<i>p</i> value with USP18	3E-04	0.002	8E-06	3E-06	

TABLE 3 Top 50 genes inversely correlated with ISG induction and IFN-induced plasma HIV-1 viral RNA decline. Activated CD4⁺ T cells (HLA-DR⁺ CD38⁺ cells) were sorted by FACS of PBMCs from 19 HIV/HCV coinfecting individuals who received a single dose of pegylated type 1 IFN (PEG-IFN) treatment, and total RNA was extracted. An RNA-seq analysis was performed on the total RNA from activated CD4⁺ T cells. The induction of three ISGs (*MX2*, *PKR*, and *IFIT3*) was calculated by normalizing the levels of each ISG 24 h after the PEG-IFN treatment to those before the treatment. The baseline level of each gene in individuals prior to PEG-IFN treatment was correlated with the level of ISG induction (posttreatment) and the corresponding magnitude of plasma HIV-1 RNA decline 72 h after the PEG-IFN treatment. Shown are the top 50 genes that exhibited the strongest negative correlation with three ISGs or PEG-IFN-induced plasma HIV-1 RNA declines (National Center for Biotechnology Information Sequence Read Archive accession number [SRP068424](#)).

MX2 induction			PKR induction			IFIT3 induction			HIV-1 RNA decline		
Rank	Gene Name	r	Rank	Gene Name	r	Rank	Gene Name	r	Rank	Gene Name	r
1	IFI44	-0.8385965	1	IFI44	-0.8561404	1	IFIT5	-0.8368421	1	REC8	-0.7843137
2	EPSTI1	-0.8280702	2	PKR	-0.8561404	2	IFIT1	-0.8064942	2	PKFB1	-0.7841583
3	MX1	-0.8263158	3	JAK2	-0.8438596	3	HS2D	-0.8017544	3	SLC29A1	-0.7794118
4	RUFY4	-0.8210526	4	MX1	-0.8333333	4	EPSTI1	-0.7964912	4	SCD	-0.7769608
5	DDX60L	-0.8192982	5	SPATS2L	-0.8315789	5	RUFY4	-0.7894737	5	MOB2	-0.7720588
6	PKR	-0.8192982	6	EPSTI1	-0.8298246	6	DDX60	-0.7877193	6	LRWD1	-0.7720588
7	IFI44L	-0.8175439	7	IFI27	-0.8263158	7	IFI44	-0.7859649	7	MRPS18B	-0.7647059
8	HS2D	-0.8140351	8	ADAR	-0.822807	8	EXOC2	-0.7842105	8	CDC147	-0.7580455
9	IFIT1	-0.8100045	9	OAS3	-0.8210526	9	SP100	-0.7807018	9	MAF1	-0.75
10	ANGPTL2	-0.8038726	10	IFI44L	-0.8105263	10	IFI44L	-0.777193	10	ZNF404	-0.7492337
11	SAMD9	-0.8035088	11	PTGES	-0.8087719	11	PKR	-0.7754386	11	LAP3	-0.7426471
12	EXOC2	-0.7982456	12	IFI6	-0.8087719	12	RSAD2	-0.7754386	12	RUVBL1	-0.7401961
13	RSAD2	-0.7947368	13	USP18	-0.8035088	13	SEMA3G	-0.7736842	13	EPSTI1	-0.7352941
14	CMPK2	-0.7912281	14	HS2D	-0.8	14	RAB23	-0.7731462	14	DCPS	-0.7328431
15	TRIM21	-0.7842105	15	PLSCR1	-0.8	15	GCNT2	-0.7684211	15	MAD2L1	-0.7328431
16	SP100	-0.7842105	16	IFI35	-0.7947368	16	DDX60L	-0.7666667	16	TP53	-0.7230392
17	OAS3	-0.7824561	17	LAP3	-0.7912281	17	EED	-0.7666667	17	MZT2B	-0.7230392
18	DDX60	-0.7824561	18	CMPK2	-0.7877193	18	C4orf27	-0.7666667	18	SQLE	-0.7230392
19	FAM110A	-0.7807018	19	OASL	-0.7877193	19	MX1	-0.7596491	19	HTATIP2	-0.7181373
20	ADAR	-0.7789474	20	ISG15	-0.7824561	20	CMPK2	-0.7596491	20	SPATS2L	-0.7181373
21	OAS1	-0.7789474	21	RPA2	-0.777193	21	HERC6	-0.7578947	21	CHMF5	-0.7132353
22	IFIT5	-0.7789474	22	LY6E	-0.777193	22	FAM110A	-0.7526316	22	SNX17	-0.7083333
23	USP18	-0.7754386	23	SPAG6	-0.7750451	23	IFIT3	-0.745614	23	SMC6	-0.7009804
24	SPATS2L	-0.7701754	24	CHMF5	-0.7684211	24	FANCC	-0.7438596	24	CDC47	-0.7009804
25	IFI27	-0.7684211	25	CPE	-0.7654458	25	RUFY1	-0.7421053	25	STARD3NL	-0.6985294
26	HERC6	-0.7684211	26	L26953	-0.7631579	26	MAP3K1	-0.7421053	26	TRAF3IP1	-0.69816
27	PLSCR1	-0.7649123	27	IFIT1	-0.7582274	27	SAMD9	-0.7385965	27	NCAPG2	-0.6965053
28	EED	-0.7578947	28	FODXL2	-0.7545022	28	TRIM21	-0.7333333	28	IFITM1	-0.6936275
29	IFI6	-0.7561404	29	FAM110A	-0.754386	29	C19orf66	-0.7333333	29	USP18	-0.6936275
30	XAF1	-0.7561404	30	SAMD9	-0.754386	30	AIK30561	-0.7293509	30	IFI6	-0.6911765
31	RAB23	-0.7555946	31	CYC1	-0.754386	31	NSL1	-0.7292673	31	KIF2A	-0.6887255
32	SPATA32	-0.7541706	32	DDX60	-0.7526316	32	USP18	-0.7245614	32	GPANK1	-0.6866954
33	TRIM14	-0.7508772	33	ZSCAN2	-0.7526316	33	XAF1	-0.722807	33	MRPS5	-0.6862745
34	MX2	-0.7473684	34	RUFY4	-0.7491228	34	SAMD9L	-0.7210526	34	NARF	-0.6862745
35	SAMD9L	-0.745614	35	YWHAQ	-0.7491228	35	PARP9	-0.7192982	35	ATP6V0D1	-0.6862745
36	GCNT2	-0.7438596	36	GTF2E1	-0.7491228	36	COQ7	-0.7175439	36	IL12RB2	-0.6813725
37	RUFY1	-0.7421053	37	XAF1	-0.745614	37	GPR156	-0.7163619	37	UNG	-0.6813725
38	IFI16	-0.7403509	38	RSAD2	-0.7421053	38	RAG1	-0.7155656	38	OASL	-0.6789216
39	MAP3K1	-0.7403509	39	TYMP	-0.7421053	39	FGD5-AS1	-0.7125933	39	MRPL28	-0.6789216
40	PARP12	-0.7385965	40	LAMP3	-0.7403509	40	HLF	-0.7122807	40	BST2	-0.6764706
41	PDI6	-0.7368421	41	PRPS2	-0.7403509	41	PLSCR1	-0.7087719	41	BBS7	-0.6764706
42	SERF2-C15orf63	-0.7368421	42	TRIM21	-0.7385965	42	BYSL	-0.7070175	42	MTA2	-0.6764706
43	CHMF5	-0.7333333	43	LRWD1	-0.7368421	43	SPATA32	-0.7067606	43	ANKRD39	-0.6764706
44	FANCC	-0.7333333	44	HERC5	-0.7350877	44	OAS3	-0.7035088	44	HN1	-0.6715686
45	IFIT3	-0.7333333	45	ANGPTL2	-0.7335118	45	DTX3L	-0.7035088	45	FAM110A	-0.6691176
46	HERC5	-0.7315789	46	FAM50B	-0.7304656	46	SERF2-C15orf63	-0.7035088	46	ARPP19	-0.6691176
47	LRWD1	-0.7280702	47	CSAG2	-0.7284378	47	SPATS2L	-0.7017544	47	MRPS9	-0.6683018
48	JAK2	-0.7280702	48	CSAG3	-0.7284378	48	OAS1	-0.7017544	48	HIST2H2AB	-0.6670755
49	SEMA3G	-0.7263158	49	OAS1	-0.7280702	49	MX2	-0.7017544	49	SP100	-0.6666667
50	PARP9	-0.7263158	50	SRGAP2B	-0.7280702	50	IFI35	-0.7	50	OAS2	-0.6642157

FIG 4 HIV-1 associated type 1 IFN refractoriness is not dependent on productive infection. (A, B) HIV-1 infection of CD4⁺ T cells results in limited ISG upregulation. Leukopaks from healthy donors were stimulated with PHA for 3 days; CD4⁺ T cells were then (Continued on next page)

response to HIV-1 infection and release from CD4⁺ T cells and that type 1 IFN is responsible for type 1 IFN refractoriness in target cells (including CD4⁺ T cells) by way of negative feedback. We confirmed that type 1 IFN itself leads to refractory responses by neutralizing type 1 IFN in conditioned supernatants with recombinant B18R, a soluble vaccinia virus type 1 IFN decoy receptor (43), prior to incubating conditioned supernatants with target cells. We showed that preincubation of conditioned supernatants with B18R led to partial recovery of type 1 IFN responses in target cells from some donors (Fig. 5C). Although not statistically significant, type 1 IFN neutralization by B18R modestly diminished the USP18 expression elevated by sup-LP plus BaL (Fig. 5D; $P=0.18$). These results demonstrate that type 1 IFN release during HIV-1 infection contributes to subsequent type 1 IFN refractoriness through negative feedback.

We separately confirmed that PBMCs from PLWH produce type 1 IFN that is associated with type 1 IFN refractoriness. After culturing PBMCs from HIV-UC and HC *ex vivo* and without activation, we collected and filtered their supernatants in 2 aliquots: in the first aliquot we quantified constitutive levels of IFN α 2a production from these cells in the absence of stimulation, while the second was incubated with target cells as we did previously. After incubation, target cells were stimulated with type 1 IFN and ISG induction was quantified. We found that constitutive IFN α 2a levels in PBMC supernatants were negatively correlated with induction of MX2 ($r = -0.64$, $P=0.02$) and ISG15 ($r = -0.70$, $P=0.01$) in target cells (Fig. 5E). Taken together, these results strongly indicate that type 1 IFN refractoriness in PLWH is itself mediated by type 1 IFN. We propose that constitutive type 1 IFN production during HIV-1 infection contributes to a state of type 1 IFN refractoriness.

HIV-1 virions and type 1 IFN refractoriness. We considered other potential soluble mediators during HIV-1 infection that might dampen type 1 IFN responses in uninfected bystander cells (Fig. 6A). Direct infection of target cells was ruled out as a cause of type 1 IFN refractoriness, since target cells were treated with ARVs that inhibit reverse transcriptase. However, inhibition of reverse transcription does not rule out the possibility of virions themselves contributing to type 1 IFN refractoriness in target cells, even in the absence of productive infection. Specifically, because sensing of HIV-1 nucleic acids and capsid proteins has been described (44, 45), we examined whether modifying viral entry or capsid uncoating might affect type 1 IFN refractoriness. We tested whether addition of the HIV-1 fusion inhibitor T20 or a cyclophilin A inhibitor (CypAInh), which partially blocks uncoating of HIV-1 capsid (46, 47), might further modulate type 1 IFN responses. We preincubated uninfected target cells with T20 or CypAInh in addition to the ARVs tenofovir disoproxil fumarate and emtricitabine (TDF plus FTC) and then conditioned cells with sup-LP plus BaL or sup-LP without BaL and treated them with type 1 IFN to measure ISG induction. Compared to ARVs alone, we found that the addition of CypAInh partially restored MX2 induction in target cells that were conditioned with sup-LP plus BaL ($P < 0.05$; Fig. 6B), although we found no effect on ISG15 induction. We found a smaller effect with the addition of T20 plus ART compared to ART alone that was not statistically significant. These results imply that sensing of viral nucleic acids might contribute to type 1 IFN refractoriness in target cells, even in the absence of

FIG 4 Legend (Continued)

isolated and infected with GFP-tagged HIV-1 (HIV-GFP; see Materials and Methods). Three days after infection, GFP⁺ and GFP⁻ cells were sorted by flow cytometry associated cell sorting (FACS). After resting GFP⁺ and GFP⁻ cells for 8 h, cells were lysed and RNA was extracted. ISG expression levels (MX2, ISG15, IP10, and BST2) were quantified by qPCR ($n=4$ donors). (C) Experimental schematic for supernatant experiment. Leukopaks (LP) from uninfected people were infected with HIV-1 BaL for 3 days; uninfected LP were maintained as controls. Supernatants (defined as conditioned supernatants) from HIV-1-uninfected (sup-LP without BaL; $n=10$) or infected LPs (sup-LP plus BaL; $n=10$) were harvested and filtered. Target CD4⁺ T cells from uninfected people were treated with ARVs for 24 h to prevent *de novo* infection and then incubated with sup-LP without BaL or sup-LP plus BaL in the presence of ARVs. After 24 h of conditioning, target cells were stimulated with exogenous type 1 IFN and compared to those that were left unstimulated. (D) ISG induction was calculated by normalizing expression in type 1 IFN stimulated to unstimulated target cells and compared between target cells incubated with sup-LP without BaL or sup-LP plus BaL conditioned supernatants. (E) USP18 expression was measured by qPCR after incubation with sup-LP without BaL or sup-LP plus BaL but before type 1 IFN stimulation to understand the relationship between baseline USP18 levels and subsequent type 1 IFN responses. Pairwise Wilcoxon tests were performed to determine the statistically significant differences between the groups (*, $P < 0.05$).

TABLE 4 Baseline and total ISG expression in target CD4⁺ T cells conditioned with HIV with or without supernatant. Leukopaks (LP) from uninfected people were infected with HIV-1 BaL for 3 days; uninfected LP were cultured as controls. We collected these supernatants (defined as conditioned supernatants) from HIV-1-uninfected (sup-LP without BaL; $n = 10$) or infected LPs (sup-LP plus BaL; $n = 10$) and filtered them through a 0.2 μ m filter. Target CD4⁺ T cells from uninfected people were incubated with ARVs for 24 h so that *de novo* infection is prevented and then conditioned with sup-LP without BaL or sup-LP plus BaL in the presence of ARVs. After 24 h of incubation, we stimulated target cells with exogenous type 1 IFN and compared them to those that were left unstimulated. ISG expression (*MX2*, *ISG15*, and *IFIT3*) was measured by qPCR; ISG expression in type 1 IFN unstimulated and stimulated target cells was tabulated and compared between target cells conditioned with sup-LP minus BaL or sup-LP plus BaL. Pairwise Wilcoxon tests were used to evaluate the statistically significant differences between the groups (n.a., not applicable; n.d., not detectable).

	Gene	MX2		ISG15		IFIT3	
	HIV	-	+	-	+	-	+
Supernatant Donor ID	1	0.0949	0.2623	0.0729	0.486	0.0132	0.2725
	2	0.102	0.1729	0.0736	0.0964	0.0367	0.0578
	3	0.0748	0.1863	0.0323	0.0988	0.0171	0.0448
	4	0.1578	0.478	0.0503	0.4483	0.0267	0.2638
	5	0.0626	0.1021	0.0373	0.0774	0.011	0.0746
	6	0.0629	0.157	0.0279	0.0435	0.0068802	0.0188
	7	0.0658	0.0908	0.0294	0.0394	0.0079033	0.025
	8	0.0537	0.1342	0.0501	0.0464	n.d.	n.d.
	9	0.0806	0.2234	0.0178	0.0701	n.d.	n.d.
	10	0.15	0.1975	0.0933	0.1675	0.2189	0.1768
	median	0.0777	0.1796	0.0437	0.0869	0.01515	0.0662
	p value (compared to HIV-)	n.a.	0.0011	n.a.	0.019	n.a.	0.021
	Gene	MX2		ISG15		IFIT3	
	HIV	-	+	-	+	-	+
Supernatant Donor ID	1	0.8463	0.3356	1.2827	0.4111	0.4535	0.2369
	2	1.2968	0.6894	1.0105	0.4891	0.4788	0.3489
	3	1.3356	0.4345	0.7513	0.317	0.4897	0.2373
	4	1.3249	0.2304	0.8832	0.2675	0.3388	0.1746
	5	1.0317	0.8822	1.3496	0.8274	0.6854	0.4819
	6	1.2694	1.1507	1.2541	1.1975	0.7522	0.6666
	7	0.7859	0.3222	1.3899	0.2919	0.3469	0.1741
	8	0.4165	0.0669	0.5154	0.168	0.2528	0.1186
	9	1.1408	0.3086	0.606	0.1683	0.1593	0.052
	10	0.5468	0.1596	0.4488	0.2117	0.6402	0.2652
	median	1.08625	0.3289	0.94685	0.30445	0.46615	0.2371
	p value (compared to HIV-)	n.a.	0.0039	n.a.	0.0021	n.a.	0.052

bona fide infection. However, there may be differential effects of viral nucleic acid sensing on which ISGs are induced and which remain dampened.

USP18 knockdown and type 1 IFN refractoriness *in vitro*. We developed a model of type 1 IFN refractoriness that commenced with type 1 IFN signaling, involved USP18 upregulation, and terminated in refractory responses to secondary stimulation by type 1 IFN. We examined the role of USP18 in HIV-1-associated type 1 IFN refractoriness,

FIG 5 HIV-1-infected PBMCs produce type 1 IFN that induces USP18 expression and type 1 IFN refractoriness. (A) CD4⁺ T cells from healthy donors were incubated for 6 h with a panel of cytokines, including type 1 IFN, IL-12/23p40, IL-15, IL-18, IFN γ , MCP-1, and IP-10. After incubation, total RNA was extracted and levels of *USP18* were quantified by qPCR. Shown is the fold change in *USP18* expression in comparison to CD4⁺ T cells from the same donors that were incubated with media alone, normalized to a panel of housekeeping genes. Only type 1 IFN and IL-15 incubation resulted in significant induction of *USP18* levels. (B) IFN α 2 α levels were measured by ELISA in sup-LP without BaL ($n=13$; white circles) and sup-LP plus BaL ($n=13$; red circles) and correlated with baseline *USP18* levels (left) and MX2 induction (right) in target CD4⁺ T cells after conditioning. Spearman correlation coefficients were calculated. (C, D) Type 1 IFN neutralization with recombinant B18R, a soluble decoy receptor for type 1 IFN, restored type 1 IFN responses in cells from some individuals. B18R was added to conditioned supernatants from sup-LP without BaL or sup-LP plus BaL for 1 h. Target CD4⁺ T cells were then incubated with these conditioned supernatants for 24 h, following which cells were stimulated with exogenous type 1 IFN. (C) ISG induction (*MX2*, *ISG15*, and *IFIT3*) and (D) baseline *USP18* expression in type 1 IFN unstimulated cells were quantified in comparison to those in target cells that were not stimulated with type 1 IFN. Mann-Whitney tests were used to determine statistical significance between conditions (*, $P<0.05$; $n=11$ for sup-LP without BaL and sup-LP plus BaL, $n=6$ for sup-LP plus BaL plus B18R). (E) IFN α 2 α production and type 1 IFN refractoriness *ex vivo*. Supernatants from cultured PBMCs (rested overnight) that

(Continued on next page)

using RNA interference to blunt the impact of USP18. Western blotting of USP18 protein demonstrated that USP18 protein expression decreased by 70% after knockdown (Fig. 7A). Given the complex feedback between type 1 IFN production and its proposed negative feedback, we addressed the role of USP18 in type 1 IFN refractoriness in three ways. First, we measured the effect of USP18 knockdown on baseline ISG expression by exposing target cells to conditioned supernatants without type 1 IFN stimulation. USP18 knockdown resulted in an increase in baseline ISG expression ($\Delta_{\text{sup-induced}}$) between target cells conditioned with sup-LP without BaL and those conditioned with sup-LP plus BaL ($P < 0.05$ for ISG15 and IFIT3; Fig. 7B and C). Next, we quantified the role of USP18 knockdown in ISG induction upon type 1 IFN administration ($\Delta_{\text{IFN-induced}}$) and found no change with USP18 knockdown (Fig. 7D). Then, we hypothesized that by diminishing constitutive counter-regulation of type 1 IFN responses by USP18, the total type 1 IFN response was restored. We tested this hypothesis by calculating the total amount of ISG mRNA induction with either constitutive or exogenous type 1 IFN ($\Delta_{\text{total-induced}}$). We found that the $\Delta_{\text{total-induced}}$ amount of ISG induction was increased for ISG15 and IFIT3 after USP18 knockdown ($P < 0.05$ for both; Fig. 7E). We reasoned that the $\Delta_{\text{total-induced}}$ calculation of ISG expression modeled how type 1 IFN responses might be enhanced during HIV-1 infection if constitutive USP18 were inhibited *in vivo*. Thus, while we did not find a significant change in $\Delta_{\text{IFN-induced}}$ ISG expression, we did find increases in both $\Delta_{\text{sup-induced}}$ and $\Delta_{\text{total-induced}}$ ISG expression with USP18 inhibition, suggesting that targeting USP18 *in vivo* might result in improved type 1 IFN responses during HIV-1 infection.

DISCUSSION

We show here that primary T cells (CD4^+ and CD8^+) from PLWH demonstrated refractory responses to exogenous type 1 IFN that are strongly associated with upregulation of USP18 in those cells. We found that HIV-1 infection of PBMCs led to release of type 1 IFN and other soluble mediators that increased USP18 levels in uninfected target cells and dampened target cell responses to subsequent type 1 IFN exposure. These mediators include type 1 IFN itself and HIV-1 virions, even in the absence of *de novo* infection of target cells. We confirmed that, *in vivo*, USP18 mRNA levels in CD4^+ T cells are strongly and inversely associated with ISG induction in response to PEG-IFN administered to PLWH. Intriguingly, USP18 levels in CD4^+ T cells were closely linked to the subsequent decline in plasma HIV-1 RNA levels after PEG-IFN administration, suggesting a strong link between USP18 levels and HIV-1 control by type 1 IFN *in vivo*. Surprisingly, we found that a subgroup of PLWH who have been virologically suppressed with ART for >1 year demonstrated continued type 1 IFN refractoriness. Taken together, we describe a persistent defect in innate immune antiviral defenses among PLWH that perpetuates type 1 IFN refractoriness and may promote HIV-1 persistence (5, 46, 48).

Similar to previous findings in PLWH or animal models (25, 50, 65), we found evidence of constitutively upregulated ISG expression in CD4^+ T cells from PLWH. Although these results might have been inferred from previously published results (26–29), to our knowledge ours is the first report to characterize type 1 IFN refractoriness in PLWH. Specifically, we showed that with exogenous type 1 IFN stimulation, both STAT1 phosphorylation and ISG induction were diminished in several cell types taken from PLWH. Several clinical trials have demonstrated that type 1 IFN-based therapy exhibited only modest control of HIV-1 replication and of the size of the latent reservoir (5, 6, 10, 51–54). In PLWH, we demonstrated that the magnitude of USP18

FIG 5 Legend (Continued)

were taken from *ex vivo* HIV-UC ($n=5$) and HC ($n=8$) were collected separately in 2 aliquots. IFN α 2a levels were measured in the first aliquot. The second aliquot was filtered and incubated with target uninfected CD4^+ T cells that were pretreated with ARVs for 24 h. Twenty-four hours after conditioning, uninfected target cells were stimulated with type 1 IFN and ISG induction (MX2 and ISG15) was calculated. Thereafter, supernatant IFN α 2a levels in PBMCs were correlated with ISG induction in uninfected target cells and found to have a significant negative association. Black dots depict induction in cells incubated with conditioned supernatants from HC and blue dots depict induction in cells incubated with conditioned supernatants from HIV-UC.

FIG 6 The effect of HIV-1 virions on type 1 IFN refractoriness. (A) Model diagram. When target cells were incubated with sup-LP plus BaL, soluble mediators, including type 1 IFN and HIV-1 virions, are transferred to the target cells. Both type 1 IFN and HIV-1 virions upregulate USP18 expression, thereby dampening the responses to exogenous type 1 IFN. (B) Target CD4⁺ T cells from HIV-1-uninfected donors were pretreated with the HIV-1 entry inhibitor T20 or with a cyclophilin A inhibitor (CypAInh) in addition to ARVs for 24 h. Target cells were then incubated with sup-LP without BaL (*n* = 5) or sup-LP plus BaL (*n* = 5); T20 (*n* = 4) or CypAInh (*n* = 5) was maintained in the media as well as ARVs. Twenty-four hours after conditioning, target cells were stimulated with exogenous type 1 IFN, and ISG expression was measured and compared to cells that were not stimulated with type 1 IFN. Pairwise Wilcoxon tests were used to determine statistically significant differences between conditions (*, *P* < 0.05).

FIG 7 USP18 contributes to type 1 IFN refractoriness. (A) USP18 knockdown in primary CD4⁺ T cells. Total CD4⁺ T cells were nucleofected with FITC-tagged scramble RNA (si-ctrl) or siUSP18. Twenty-four

(Continued on next page)

expression predicted how effective exogenous type 1 IFN was in suppressing HIV-1 replication. The results point to a role of USP18 in maintaining HIV-1 persistence. Indeed, recent studies demonstrated that USP18 facilitated HIV-1 replication in THP-1 cells and monocyte-derived macrophages (55, 56). Importantly, we observed the same refractory responses in CD8⁺ T cells. A similar trend was also demonstrated by Kwaa et al. where the enhancement of CD8⁺ T cell responses in PLWH by type 1 IFN was limited compared to that of natural killer (NK) cells in PLWH (57). In addition, our findings plausibly explain how HIV-1 infection is associated with type 1 IFN refractoriness. We note, however, that in contrast to our findings, Catalfamo et al. reported that pSTAT1 activation was greater in naive and memory CD4⁺ T cells and CD8⁺ T cells from PLWH with uncontrolled HIV-1 infection than in those from people with controlled infection (58). It is worthwhile noting that their group reported the activated CD4⁺ T cell pSTAT1 mean fluorescence intensity (MFI) after stimulation with IFN α , whereas we also report the change in pSTAT1 activation with IFN α stimulation. Although we did see higher pSTAT1 levels after type 1 IFN stimulation in some HIV-UC compared to those in HIV-ART, the diminished change in STAT1 phosphorylation before and after type 1 IFN stimulation that we observed in HIV-UC and HIV-ART is more consistent with type 1 IFN refractoriness. Indeed, our finding of diminished pSTAT1 activation is corroborated by reduced ISG expression with type 1 IFN stimulation. Furthermore, our findings support those reported by Nguyen et al., who described a statistically insignificant lower proportion of pSTAT1⁺ CD3⁺ CD4⁺ cells in PLWH who had poor immune restoration (i.e., CD4⁺ T cells counts) than in healthy controls (29). Intriguingly, in that study, PLWH who recovered CD4⁺ T cells exhibited an intermediate phenotype, similar to what we demonstrated.

Type 1 IFN from HIV-1-infected PBMCs *in vitro* (Fig. 5C and D) or *ex vivo* (Fig. 5E) was sufficient to increase USP18 levels and to contribute to subsequent type 1 IFN refractoriness. We speculate that plasmacytoid dendritic cells (pDCs), potent type 1 IFN secretors (46, 48), are the main producer of type 1 IFN in HIV-1-infected PBMCs. pDCs have been previously shown to sense HIV-1 nucleic acids through the pattern recognition receptor TLR7, resulting in type 1 IFN production, especially IFN α 2 (46, 59). Notably, type 1 IFN neutralization resulted in partial restoration of ISG induction (Fig. 5C), suggesting that type 1 IFN refractoriness is reversible in PLWH. However, future research is required to demonstrate that pDCs are the source of type 1 IFN in PLWH that contributes to type 1 IFN refractoriness.

Interestingly, T cells from PLWH who were virologically suppressed exhibited an intermediate phenotype between HC and HIV-UC. Because PLWH who are virologically

FIG 7 Legend (Continued)

or 48 hours after nucleofection, cells were then stimulated with type 1 IFN for 24 h and total protein was extracted. Protein levels of USP18 and β -actin were visualized by Western blotting, confirming knockdown of USP18. (B, C, D, E) Target CD4⁺ T cells from healthy donors were nucleofected with scramble RNA (si-ctrl) or siUSP18. After 24 h, target CD4⁺ T cells were incubated with sup-LP without BaL or sup-LP plus BaL for an additional 24 h. Following incubation, target cells were stimulated with exogenous type 1 IFN for 6 h. Levels of ISG expression (*MX2*, *ISG15*, and *IFIT3*) with and without type 1 IFN were quantified by qPCR. The levels of expressions were normalized by three housekeeping genes (*YWHAZ*, *SDHA*, and *RPLP0*). Several comparisons were made to account for the effect of USP18 on conditioning and the effect of USP18 on type 1 IFN stimulation. The first panels in panels C, D, and E show the same raw qPCR data but indicate the different comparisons that are shown in the subsequent panels. (C) The Δ sup-induced expression (blue), defined as the effect of USP18 knockdown on conditioning, was calculated by normalizing ISG expression values in unstimulated target cells that were incubated with sup-LP without BaL from those in target cells incubated with sup-LP plus BaL. (D) The Δ IFN-induced expression (red) is defined as a difference of differences, the change in ISG expression with type 1 IFN stimulation compared to that without type 1 IFN stimulation, normalizing the induction in target cells conditioned with sup-LP without BaL from sup-LP plus BaL. (E) The Δ total expression (purple), defined as the compound change in ISG expression, accounting for the Δ sup-induced expression after conditioning, and the Δ IFN-induced expression with type 1 IFN stimulation. The Δ total expression is calculated as the difference in ISG expression between target cells conditioned with sup-LP without BaL and those conditioned with sup-LP plus BaL, added to the difference in ISG expression between the target cells treated with type 1 IFN and those that were not treated with type 1 IFN. Pairwise Wilcoxon tests were performed to examine statistically significant differences between conditions. ($n = 5$; *, $P < 0.05$).

suppressed still release low levels of HIV-1 virions (60), and because cell-associated HIV-1 DNA and RNA levels decay slowly during ART (61), we speculate that pDC sensing of residual HIV-1 nucleic acids may contribute to constitutive type 1 IFN signaling, either from defective or intact proviruses released from the HIV-1 reservoir (62). We demonstrated that adding fusion or uncoating inhibitors to supernatants partially restored type 1 IFN responses, indicating that HIV-1 virions may also contribute to diminished type 1 IFN responses independent of infection. Therefore, it is plausible that defective viruses can also mediate suppression of type 1 IFN responses. Recently, Bruner et al. demonstrated that defective proviruses can clonally expand in response to CD3/CD28 stimulation, while intact proviruses showed only modest proliferation (63). Therefore, it is possible that low levels of defective virions could be sensed by pDCs, resulting in constitutive type 1 IFN signaling in PLWH who are virologically suppressed with ART (46, 50, 64–69). Further study is required to test whether type 1 IFN refractoriness in virologically suppressed PLWH renders them more susceptible to other viral infections.

Several type 1 IFN regulatory factors have been well-described. Our findings are supportive of the involvement of USP18 in type 1 IFN refractoriness in PLWH. Our observations in PLWH who received PEG-IFN demonstrate that USP18 was strongly and negatively correlated with ISG induction *in vivo*, while SOCS1 levels predicted ISG induction poorly. This is surprising, since HIV-1 infection is associated with increased levels of IFN γ (38), which in turn is known to upregulate *SOCS1* expression (70). Nevertheless, as shown in Table 3, other type 1 IFN regulatory factors may also contribute to type 1 IFN refractoriness and can be explored in different models.

This study has several limitations that are worth noting. We studied a modest number of people intensively, maximizing our understanding of type 1 IFN refractoriness. Our study will therefore need to be repeated in larger numbers of PLWH. In addition, USP18 knockdown increased baseline ISG expression and total ISG induction but did not restore ISG induction to a fixed concentration of type 1 IFN. Because type 1 IFN is both part of the causal pathway of refractoriness and ultimately a measurement of the final outcome, it is challenging to tease out the role of USP18 *in vitro*. Subsequent experiments using a humanized mouse model of HIV-1 and USP18 knockout may resolve the contribution of USP18 to type 1 IFN refractoriness. Furthermore, follow-up studies are needed to demonstrate whether type 1 IFN refractoriness is observed in other immune subsets, including CD14⁺ monocytes. It will also be important to confirm whether pDCs are indeed the cells that produce type 1 IFN that propagates refractoriness.

We report here that PLWH have evidence of constitutive type 1 IFN expression, irrespective of ART with virologic suppression, and that this precipitates a refractory state that is associated with USP18 upregulation and reduces the effect of exogenous type 1 IFN. Type 1 IFN refractoriness appears to be reversible. Thus, we propose that novel therapies which target constitutive type 1 IFN production or USP18 expression may result in improved HIV-1 control. Although type 1 IFN blockade in acute SIV infection worsens disease progression (66), it was recently reported that IFNAR blockade in chronic HIV-1 infection of humanized mice on ART led to decreased plasma HIV-1 RNA levels and the size of the HIV-1 reservoir (50, 68), indicating that manipulation of type 1 IFN signaling could improve HIV-1 control in the setting of chronic infection. Further, IFNAR blockade also restored CD8⁺ T cells from exhaustion in a humanized mouse model of HIV-1 infection (50, 68). This suggests that targeting type 1 IFN production or USP18 expression could also restore the dampened adaptive immune responses in PLWH. To our knowledge, this is the first investigation to demonstrate evidence of type 1 IFN refractoriness in PLWH irrespective of virological suppression.

MATERIALS AND METHODS

Patient samples. Peripheral blood mononuclear cells (PBMCs) from 9 PLWH who were viremic and not treated with antiretrovirals (HIV-UC) and 7 PLWH who were treated with antiretrovirals and virologically suppressed for ≥ 1 year (HIV-ART) were obtained from the Johns Hopkins AIDS Linked IntraVenous Experience Study (ALIVE) cohort and the Johns Hopkins Clinical Cohort. In addition, PBMCs from 9 people who inject drugs (PWIDs) from Johns Hopkins Baltimore Before and After Study of Hepatitis (BBAASH) cohorts, 1 healthy donor, and 2 leukopaks from the Anne Arundel Medical Blood Donor

Center (Anne Arundel, Maryland) were tested as negative controls. PWIDs were included because donors are from the same neighborhoods, race, gender, and ethnic background, and they are exposed from injection drug use.

Quantification of pSTAT1. PBMCs were thawed and rested overnight in RPMI (ThermoFisher) with 10% fetal bovine serum (FBS) (Sigma-Aldrich) (R10). PBMCs from HIV-ART cohorts were rested overnight with 10 μ M tenofovir disoproxil fumarate (TDF) (Selleckchem) and 1 μ M emtricitabine (FTC) (Selleckchem). PBMCs were then stimulated with 300 U/ml of universal type 1 IFN (PBL Assay Science). Five minutes after stimulation, PBMCs were stained with CD3-Alexa Fluor 700 (eBioscience), CD8-APC, HLA-DR-FITC, pSTAT1-BV421 (BD Bioscience), and CD38-BV711 (Biolegend), along with live-dead staining dye eFluor780 (eBioscience). The levels of pSTAT1 in CD3⁺ CD8⁻ cells, CD3⁺ CD8⁻ HLA-DR⁺ CD38⁺ cells, CD3⁺ CD8⁻ HLA-DR⁻ CD38⁻ cells, and CD3⁺ CD8⁺ cells with and without type 1 IFN stimulation were measured by flow cytometry.

qPCR for ISGs and type 1 IFN regulatory factors. PBMCs were thawed and rested overnight. Total CD4⁺ T cells were isolated by using human CD4⁺ T cell isolation kits (Miltenyi Biotec, catalog 130-096-533) and stimulated with 10 U/ml of universal type 1 IFN (PBL Assay Science). Six hours after, cells were collected and total cellular RNA was extracted using Zymo DNA/RNA Duet kits (Zymo Research). Total cellular RNA from CD4⁺ T cells from HC1 and 2, HIV-UC1 and 2, and HIV-ART1 and 2 was extracted using RNeasy plus mini kits Qiagen. Total RNA was then reverse transcribed into cDNA using Multiscribe reverse transcriptase kits (ThermoFisher). The levels of *MX2*, *ISG15*, *PKR*, *BST2*, *SOCS1*, *SOCS3*, and *USP18* cDNA were quantified by quantitative PCR (qPCR). The expression level was normalized to three house-keeping genes (*YWHAZ*, *SDHA*, and *RPLP0*), and induction of ISGs was calculated by using the $\Delta\Delta$ Ct method, normalizing to unstimulated CD4⁺ T cells. In addition, the relative expression unit of each gene was calculated from the Δ Ct. Primers, probes, and qPCR mastermix were obtained from Integrated DNA Technologies.

Collection and expansion of HIV-GFP and HIV-1 BaL. HIV-1 BaL was kindly donated from Joel Blankson's lab and expanded using the protocol modified from what Veenhuis et al. (46) previously described. Briefly, healthy leukopaks were thawed and stimulated with 2.5 μ g/ml of phytohemagglutinin (PHA) (FisherScientific). Three days after activation, CD4⁺ T cells were isolated and 10 to 20 million CD4⁺ T cells were spinoculated with HIV-1 BaL at 1,200 $\times$ g for 2 h. The infected cells were maintained in culture for 10 days, and the supernatant was collected and filtered through a 0.2 μ m filter. The filtered supernatant was then placed on a 20% sucrose solution and ultracentrifuged at 130,000 $\times$ g for 2 h at 4°C. Virus precipitates were then resuspended with RPMI with 1% FBS and rested overnight at 4°C. Alliance HIV-1 p24 ELISA kit (Perkin Elmer) was used to measure the concentration of the harvested HIV-1 BaL.

For collection of GFP-tagged HIV-1 (HIV-GFP), 1 million 293T cells were seeded into 6-well plates and cultured with Dulbecco's modified Eagle medium (Corning) with 10% FBS 1 day prior to transfection. 293T cells were then transfected with 2 μ g of HIV-1 NL4_3 Δ Env enhanced green fluorescent protein (EGFP) reporter vector (NIH AIDS Reagent Program) (37) and 1 μ g of pSVSV-G (kindly donated from Michael Chattergoon's lab) using lipofectamine 2000 (ThermoFisher) following the manufacturer's protocol. Twenty-four hours after transfection the medium was replenished, and after 48 h, the supernatant was harvested and filtered through a 0.2 μ m filter. The concentration of HIV-GFP was determined by Alliance HIV-1 p24 ELISA kit (Perkin Elmer).

ISG induction in HIV-GFP-infected CD4⁺ T cells. Leukopaks from healthy controls were thawed and stimulated with 2.5 μ g/ml of PHA for 3 days. CD4⁺ T cells were then isolated using human CD4⁺ T cell isolation kits (Miltenyi Biotec) and spinoculated with p24 100 ng of HIV-GFP per 1 million cells at 1,200 $\times$ g for 2 h. Three days after infection, cells were stained with CD3-PE (BD-Biosciences), CD8-APC (BD-Biosciences), and 3 μ g/ml propidium iodide (PI) (Nexcelom Biosciences). Live GFP⁺ CD3⁺ CD8⁻ and GFP⁻ CD3⁺ CD8⁻ cells were sorted by fluorescence-activated cell sorting (FACS). Sorted cells were rested for 8 h, and total RNA was extracted. The levels of four ISGs (*MX2*, *ISG15*, *IP-10*, and *BST2*) were measured by qPCR.

Collection of supernatants from *in vitro* HIV-1-infected leukopaks and PLWH PBMCs. Eleven healthy leukopaks were thawed and rested for 5 h and were spinoculated with p24 1 ng of HIV-1 BaL per 1 million cells from a leukopak at 1,200 $\times$ g for 2 h. BaL-infected or -uninfected leukopaks were then cultured in R10. Three days after infection, supernatants from BaL-infected or -uninfected leukopaks were collected. In addition, supernatants from PBMCs from 8 HC and 5 HIV-UC were harvested and filtered through 0.2 μ m filter after the PBMCs were thawed and rested overnight. Supernatant preparations were divided evenly before incubation with target cells, ensuring equivalent exposures to supernatants between different test conditions. The levels of IFN α 2a and IL-15 were quantified by human IFN α tissue culture kit and human IL-15 kit from Meso Scale Discovery (MSD).

Cytokine screening for USP18 upregulation. Total CD4⁺ T cells were isolated from healthy leukopaks and stimulated with 5,000 U/ml universal type 1 IFN, 2.5 μ g/ml recombinant IL-12/23p40, 2.5 μ g/ml IL-15, 2.5 μ g/ml IFN γ , 2.5 μ g/ml MCP-1, 2.5 μ g/ml IP-10 (Peprotech), and 2.5 μ g/ml IL-18 (R & D Systems). Six hours after stimulation, cells were harvested and lysed. cDNA was reverse transcribed and the level of *USP18* was quantified by qPCR.

Supernatant experiment. Uninfected target CD4⁺ T cells were isolated from healthy leukopaks using human CD4⁺ T cell isolation kits (Miltenyi Biotec) and cultured with 10 μ M tenofovir disoproxil fumarate (TDF) and 1 μ M emtricitabine (FTC) (Selleckchem) in addition to 1 μ M T20 (ThermoFisher) or 1.5 nM cyclophilin A inhibitor (CypAinh, Millipore) (46). After 24 h, target CD4⁺ T cells were incubated with supernatants from BaL-uninfected or -infected leukopaks, divided evenly between target wells, or HC or HIV-UC PBMCs with the addition of TDF and FTC, T20, or CypAinh to inhibit various stages of HIV-1 replication. After 24 h of incubation, target CD4⁺ T cells were then stimulated with 10 U/ml of universal type 1 IFN, and 6 h later, cells

were harvested. Total RNA was extracted using Zymo DNA/RNA Duet kits (Zymo Research). Total RNA was reverse transcribed and qPCR was performed for *MX2*, *ISG15*, *IFIT3*, and *USP18*.

For neutralization of cytokines, supernatants from BaL-infected or -uninfected leukopaks were preincubated with recombinant B18R (eBioscience) for 1 h prior to incubation with target cells.

USP18 knockdown. Healthy leukopaks were thawed and rested overnight. After resting, total CD4⁺ T cells were separated by magnetic separation and nucleofected with 5 μ M Accel Green nontargeting control RNA or 5 μ M SMARTpool human USP18 siRNA (Dharmacon) using human T cell nucleofector kit (Lonza) and Amaxa nucleofector (Lonza, Program U-014). Nucleofected cells were transferred to CTS Optmizer T cell expansion SFM (Thermo Fisher) with 10 mM HEPES (Corning) (71). After 24 or 48 h from nucleofection, cells were conditioned with supernatants from HIV-1 BaL-infected or -uninfected leukopaks for 24 h followed by the stimulation with 10 U/ml of universal type 1 IFN for 6 h. The expression levels of three ISGs (*MX2*, *ISG15*, and *IFIT3*) and *USP18* were measured by qPCR.

Correlation among ISG induction, plasma HIV-1 RNA level decline, and baseline USP18 expression. We used archived data from 19 HIV/HCV coinfecting participants who were treated with pegylated-IFN α 2b (PEG-IFN) who also had plasma HIV-1 RNA measurements at baseline and at 12, 24, 72, and 168 h after treatment (National Center for Biotechnology Information Sequence Read Archive accession number [SRP068424](https://www.ncbi.nlm.nih.gov/sra/SRP068424)) (8). In that study, PBMCs were sampled 0 and 24 h after PEG-IFN treatment. CD38⁺ HLA-DR⁺ CD3⁺ CD4⁺ cells were sorted from all 2 aliquots of PBMCs by fluorescence-activated cell sorter (FACS) sorting. Total RNA was extracted and RNA sequencing (RNA-seq) was performed. The level of ISG induction was calculated by dividing fragments per kilobase million (FPKM) value of ISGs after IFN treatment by its baseline FPKM value. ISGs that were induced by PEG-IFN were defined empirically by their change in FPKM values after PEG-IFN. The magnitude of ISG induction was correlated with baseline USP18 expression FPKM values. In addition, baseline USP18 FPKM values were correlated with plasma HIV-1 RNA declines 72 h after PEG-IFN treatment.

Statistics. Pairwise Wilcoxon tests were performed to determine the statistically significant differences before and after treatment with exogenous type 1 IFN. For comparisons between independent groups of HIV-UC, HIV-ART, and healthy controls (HC), Mann-Whitney tests were performed. Spearman's coefficient correlations were performed.

Study approval. The experiments we performed in this project were approved by the institutional review board (IRB) of Johns Hopkins University School of Medicine. Adult participants gave written informed consent for the use of archived PBMCs in this study.

ACKNOWLEDGMENTS

This project was funded by National Institutes of Health grants, including R01 AI116868, R37 DA013806, R01 DA016078, R01 AI108403, R01 AI140789, U19 AI088791, and U01 DA036297, and by the Johns Hopkins University Center for AIDS Research (P30AI094189).

This project was designed by S.S., A.B., and A.L.C., and S.S. performed the majority of the biological experiments. Type 1 IFN ELISA was conducted by L.K.C. and K.R., and S.S. and C.W. developed HIV-GFP infection in primary CD4⁺ T cells and THP-1 cells. R.E. provided the RNA-seq data from type 1 IFN-treated HIV/HCV-coinfecting individuals. Plasma cytokine levels were provided by the work from R.T.V. For PLWH samples, S.H.M. supplied PBMCs from HIV-UC. J.N.B., S.H.M., and A.B. provided blood samples from HIV-ART, and D.L.T., A.L.C., and J.N.B. participated in the planning and interpretation of experiments. S.S. and A.B. drafted the manuscript with input from all coauthors.

The authors declare that no conflict of interest exists.

REFERENCES

- Montaner JS, Lima VD, Harrigan PR, Lourenco L, Yip B, Nosyk B, Wood E, Kerr T, Shannon K, Moore D, Hogg RS, Barrios R, Gilbert M, Krajden M, Gustafson R, Daly P, Kendall P. 2014. Expansion of HAART coverage is associated with sustained decreases in HIV/AIDS morbidity, mortality and HIV transmission: the "HIV Treatment as Prevention" experience in a Canadian setting. *PLoS One* 9:e87872. <https://doi.org/10.1371/journal.pone.0087872>.
- Shiels MS, Cole SR, Kirk GD, Poole C. 2009. A meta-analysis of the incidence of non-AIDS cancers in HIV-infected individuals. *J Acquir Immune Defic Syndr* 52:611–622. <https://doi.org/10.1097/QAI.0b013e3181b327ca>.
- Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, Smith K, Lisiewicz J, Lori F, Flexner C, Quinn TC, Chaisson RE, Rosenberg E, Walker B, Gange S, Gallant J, Siliciano RF. 1999. Latent infection of CD4⁺ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med* 5:512–517. <https://doi.org/10.1038/8394>.
- Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. 2009. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 49:1335–1374. <https://doi.org/10.1002/hep.22759>.
- Pillai SK, Abdel-Mohsen M, Guatelli J, Skasko M, Monto A, Fujimoto K, Yukl S, Greene WC, Kovari H, Rauch A, Fellay J, Battegay M, Hirschel B, Witteck A, Bernasconi E, Ledergerber B, Gunthard HF, Wong JK, Barth J, Battegay M, Bernasconi E, Boni J, Bucher H, Burton-Jeangros C, Calmy A, Cavassini M, Cellerai C, Egger M, Elzi L, Fehr J, Fellay J, Flepp M, Francioli P, Furrer H, Fux C, Gorgievski M, Gunthard H, Haerry D, Hasse B, Hirsch H, Hirschel B, Hosli I, Kahlert C, Kaiser L, Keiser O, Kind C, Klimkait T, Kovari H, Ledergerber B, Martinetti G, Swiss HIVCS, et al. 2012. Role of retroviral restriction factors in the interferon-alpha-mediated suppression of HIV-1 in vivo. *Proc Natl Acad Sci U S A* 109:3035–3040. <https://doi.org/10.1073/pnas.1111573109>.
- Azzoni L, Foulkes AS, Papasavvas E, Mexas AM, Lynn KM, Mounzer K, Tebas P, Jacobson JM, Frank I, Busch MP, Deeks SG, Carrington M, O'Doherty U, Kostman J, Montaner LJ. 2013. Pegylated Interferon alfa-2a monotherapy results in suppression of HIV type 1 replication and

- decreased cell-associated HIV DNA integration. *J Infect Dis* 207:213–222. <https://doi.org/10.1093/infdis/jis663>.
7. Thomas DL. 2008. The challenge of hepatitis C in the HIV-infected person. *Annu Rev Med* 59:473–485. <https://doi.org/10.1146/annurev.med.59.081906.081110>.
 8. Balagopal A, Kandathil AJ, Higgins YH, Wood J, Richer J, Quinn J, Eldred L, Li Z, Ray SC, Sulkowski MS, Thomas DL. 2014. Antiretroviral therapy, interferon sensitivity, and virologic setpoint in human immunodeficiency virus/hepatitis C virus coinfecting patients. *Hepatology* 60:477–486. <https://doi.org/10.1002/hep.27158>.
 9. Carrat F, Bani-Sadr F, Pol S, Rosenthal E, Lunel-Fabiani F, Benzekri A, Morand P, Goujard C, Pialoux G, Piroth L, Salmon-Ceron D, Degott C, Cacoub P, Perronne C, Team AHRIS. 2004. Pegylated interferon alfa-2b vs standard interferon alfa-2b, plus ribavirin, for chronic hepatitis C in HIV-infected patients: a randomized controlled trial. *JAMA* 292:2839–2848. <https://doi.org/10.1001/jama.292.23.2839>.
 10. Torriani FJ, Rodriguez-Torres M, Rockstroh JK, Lissen E, Gonzalez-Garcia J, Lazzarin A, Carosi G, Sasadeusz J, Katlama C, Montaner J, Sette H, Jr, Pisse S, De Pampillis J, Duff F, Schrenk UM, Dieterich DT, Group AS. 2004. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. *N Engl J Med* 351:438–450. <https://doi.org/10.1056/NEJMoa040842>.
 11. Chung RT, Andersen J, Volberding P, Robbins GK, Liu T, Sherman KE, Peters MG, Koziel MJ, Bhan AK, Alston B, Colquhoun D, Nevin T, Harb G, van der Horst C, Team ACTGAS. 2004. Peginterferon alfa-2a plus ribavirin versus interferon alfa-2a plus ribavirin for chronic hepatitis C in HIV-coinfecting persons. *N Engl J Med* 351:451–459. <https://doi.org/10.1056/NEJMoa032653>.
 12. Laguno M, Murillas J, Blanco JL, Martinez E, Miquel R, Sanchez-Tapias JM, Bargallo X, Garcia-Criado A, de Lazzari E, Larrousse M, Leon A, Lonca M, Milinkovic A, Gatell JM, Mallolas J. 2004. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for treatment of HIV/HCV co-infected patients. *AIDS* 18:F27–36.
 13. Doyle T, Goujon C, Malim MH. 2015. HIV-1 and interferons: who's interfering with whom? *Nat Rev Microbiol* 13:403–413. <https://doi.org/10.1038/nrmicro3449>.
 14. Rustagi A, Gale M, Jr. 2014. Innate antiviral immune signaling, viral evasion and modulation by HIV-1. *J Mol Biol* 426:1161–1177. <https://doi.org/10.1016/j.jmb.2013.12.003>.
 15. Sugawara S, Thomas DL, Balagopal A. 2019. HIV-1 infection and type 1 interferon: navigating through uncertain waters. *AIDS Res Hum Retroviruses* 35:25–32. <https://doi.org/10.1089/AID.2018.0161>.
 16. Goujon C, Moncorge O, Bauby H, Doyle T, Ward CC, Schaller T, Hue S, Barclay WS, Schulz R, Malim MH. 2013. Human MX2 is an interferon-induced post-entry inhibitor of HIV-1 infection. *Nature* 502:559–562. <https://doi.org/10.1038/nature12542>.
 17. Ivashkiv LB, Donlin LT. 2014. Regulation of type I interferon responses. *Nat Rev Immunol* 14:36–49. <https://doi.org/10.1038/nri3581>.
 18. Waiboci LW, Ahmed CM, Mujtaba MG, Flowers LO, Martin JP, Haider MI, Johnson HM. 2007. Both the suppressor of cytokine signaling 1 (SOCS-1) kinase inhibitory region and SOCS-1 mimetic bind to JAK2 autophosphorylation site: implications for the development of a SOCS-1 antagonist. *J Immunol* 178:5058–5068. <https://doi.org/10.4049/jimmunol.178.8.5058>.
 19. Malakhova OA, Kim KI, Luo JK, Zou W, Kumar KG, Fuchs SY, Shuai K, Zhang DE. 2006. UBP43 is a novel regulator of interferon signaling independent of its ISG15 isopeptidase activity. *EMBO J* 25:2358–2367. <https://doi.org/10.1038/sj.emboj.7601149>.
 20. Park SY, Waheed AA, Zhang ZR, Freed EO, Bonifacio JS. 2014. HIV-1 Vpu accessory protein induces caspase-mediated cleavage of IRF3 transcription factor. *J Biol Chem* 289:35102–35110. <https://doi.org/10.1074/jbc.M114.597062>.
 21. Doehle BP, Chang K, Fleming L, McNeven J, Hladik F, McElrath MJ, Gale M, Jr. 2012. Vpu-deficient HIV strains stimulate innate immune signaling responses in target cells. *J Virol* 86:8499–8506. <https://doi.org/10.1128/JVI.00424-12>.
 22. Doehle BP, Chang K, Rustagi A, McNeven J, McElrath MJ, Gale M, Jr. 2012. Vpu mediates depletion of interferon regulatory factor 3 during HIV infection by a lysosome-dependent mechanism. *J Virol* 86:8367–8374. <https://doi.org/10.1128/JVI.00423-12>.
 23. Doehle BP, Hladik F, McNeven JP, McElrath MJ, Gale M, Jr. 2009. Human immunodeficiency virus type 1 mediates global disruption of innate antiviral signaling and immune defenses within infected cells. *J Virol* 83:10395–10405. <https://doi.org/10.1128/JVI.00849-09>.
 24. Okumura A, Alce T, Lubyova B, Ezelle H, Strebel K, Pitha PM. 2008. HIV-1 accessory proteins VPR and Vif modulate antiviral response by targeting IRF-3 for degradation. *Virology* 373:85–97. <https://doi.org/10.1016/j.virol.2007.10.042>.
 25. Sedaghat AR, German J, Teslovich TM, Cofrancesco J, Jr, Jie CC, Talbot CC, Jr, Siliciano RF. 2008. Chronic CD4+ T-cell activation and depletion in human immunodeficiency virus type 1 infection: type I interferon-mediated disruption of T-cell dynamics. *J Virol* 82:1870–1883. <https://doi.org/10.1128/JVI.02228-07>.
 26. Aso H, Ito J, Koyanagi Y, Sato K. 2019. Comparative description of the expression profile of interferon-stimulated genes in multiple cell lineages targeted by HIV-1 infection. *Front Microbiol* 10:429. <https://doi.org/10.3389/fmicb.2019.00429>.
 27. Rahmberg AR, Rajakumar PA, Billingsley JM, Johnson RP. 2017. Dynamic modulation of expression of lentiviral restriction factors in primary CD4 (+) T cells following simian immunodeficiency virus infection. *J Virol* 91. <https://doi.org/10.1128/JVI.02189-16>.
 28. Rotger M, Dang KK, Fellay J, Heinzen EL, Feng S, Descombes P, Shianna KV, Ge D, Gunthard HF, Goldstein DB, Telenti A, Swiss HIVCS. Center for HIVAVI. 2010. Genome-wide mRNA expression correlates of viral control in CD4+ T-cells from HIV-1-infected individuals. *PLoS Pathog* 6:e1000781. <https://doi.org/10.1371/journal.ppat.1000781>.
 29. Nguyen TP, Shukla S, Asaad R, Freeman ML, Lederman MM, Harding CV, Sieg SF. 2016. Responsiveness to IL-7 but not to IFN- α is diminished in CD4+ T cells from treated HIV infected patients who experience poor CD4+ T-cell recovery. *AIDS* 30:2033–2042. <https://doi.org/10.1097/QAD.0000000000001161>.
 30. Sugawara S, El-Diwanly R, Blankson JN, Thomas DL, Cox AL, Balagopal A. 2019. HIV-1 infection is associated with increased USP18 and dampened type 1 IFN responses, abstr Conference on Retroviruses and Opportunistic Infection, Seattle, Washington, 4 to 7 March 2019.
 31. Sugawara S, El-Diwanly R, Cohen LK, Rousseau KE, Williams CYK, Veenhuis RT, Mehta SH, Blankson JN, Thomas DL, Cox AL, Balagopal A. 2020. Type 1 interferon refractoriness in people living with HIV-1 is associated with increased USP18, abstr The Japanese Society for AIDS Research Conference, virtual, Japan, 2020.
 32. Lundquist CA, Tobiume M, Zhou J, Unutmaz D, Aiken C. 2002. Nef-mediated downregulation of CD4 enhances human immunodeficiency virus type 1 replication in primary T lymphocytes. *J Virol* 76:4625–4633. <https://doi.org/10.1128/jvi.76.9.4625-4633.2002>.
 33. El-Diwanly R, Soliman M, Sugawara S, Breitwieser F, Skaist A, Coggiano C, Sangal N, Chattergoon M, Bailey JR, Siliciano RF, Blankson JN, Ray SC, Wheelan SJ, Thomas DL, Balagopal A. 2018. CMPK2 and BCL-G are associated with type 1 interferon-induced HIV restriction in humans. *Sci Adv* 4:eaat0843. <https://doi.org/10.1126/sciadv.aat0843>.
 34. Okumura A, Lu G, Pitha-Rowe I, Pitha PM. 2006. Innate antiviral response targets HIV-1 release by the induction of ubiquitin-like protein ISG15. *Proc Natl Acad Sci U S A* 103:1440–1445. <https://doi.org/10.1073/pnas.0510518103>.
 35. Toribio R, Ventoso I. 2010. Inhibition of host translation by virus infection in vivo. *Proc Natl Acad Sci U S A* 107:9837–9842. <https://doi.org/10.1073/pnas.1004110107>.
 36. Neil SJ, Zang T, Bieniasz PD. 2008. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* 451:425–430. <https://doi.org/10.1038/nature06553>.
 37. Zhang H, Zhou Y, Alcock C, Kiefer T, Monie D, Siliciano J, Li Q, Pham P, Cofrancesco J, Persaud D, Siliciano RF. 2004. Novel single-cell-level phenotypic assay for residual drug susceptibility and reduced replication capacity of drug-resistant human immunodeficiency virus type 1. *J Virol* 78:1718–1729. <https://doi.org/10.1128/jvi.78.4.1718-1729.2004>.
 38. Veenhuis RT, Astemborski J, Chattergoon MA, Greenwood P, Jarosinski M, Moore RD, Mehta SH, Cox AL. 2017. Systemic elevation of proinflammatory interleukin 18 in HIV/HCV coinfection versus HIV or HCV mono-infection. *Clin Infect Dis* 64:589–596.
 39. Kang D, Jiang H, Wu Q, Pestka S, Fisher PB. 2001. Cloning and characterization of human ubiquitin-processing protease-43 from terminally differentiated human melanoma cells using a rapid subtraction hybridization protocol. *Gene* 267:233–242. [https://doi.org/10.1016/S0378-1119\(01\)00384-5](https://doi.org/10.1016/S0378-1119(01)00384-5).
 40. Sarasin-Filipowicz M, Wang X, Yan M, Duong FH, Poli V, Hilton DJ, Zhang DE, Heim MH. 2009. Alpha interferon induces long-lasting refractoriness of JAK-STAT signaling in the mouse liver through induction of USP18/UBP43. *Mol Cell Biol* 29:4841–4851. <https://doi.org/10.1128/MCB.00224-09>.

41. Li Y, Sun B, Esser S, Jessen H, Streeck H, Widera M, Yang R, Dittmer U, Sutter K. 2017. Expression pattern of individual IFNA subtypes in chronic HIV infection. *J Interferon Cytokine Res* 37:541–549. <https://doi.org/10.1089/jir.2017.0076>.
42. Lehmann C, Taubert D, Jung N, Fatkenheuer G, van Lunzen J, Hartmann P, Romero F. 2009. Preferential upregulation of interferon-alpha subtype 2 expression in HIV-1 patients. *AIDS Res Hum Retroviruses* 25:577–581. <https://doi.org/10.1089/aid.2008.0238>.
43. Symons JA, Alcamí A, Smith GL. 1995. Vaccinia virus encodes a soluble type I interferon receptor of novel structure and broad species specificity. *Cell* 81:551–560. [https://doi.org/10.1016/0092-8674\(95\)90076-4](https://doi.org/10.1016/0092-8674(95)90076-4).
44. Henrick BM, Yao XD, Rosenthal KL, INFANT Study Team. 2015. HIV-1 structural proteins serve as PAMPs for TLR2 heterodimers significantly increasing infection and innate immune activation. *Front Immunol* 6:426. <https://doi.org/10.3389/fimmu.2015.00426>.
45. Berg RK, Melchjorsen J, Rintahaka J, Diget E, Soby S, Horan KA, Gorelick RJ, Matikainen S, Larsen CS, Ostergaard L, Paludan SR, Mogensen TH. 2012. Genomic HIV RNA induces innate immune responses through RIG-I-dependent sensing of secondary-structured RNA. *PLoS One* 7:e29291. <https://doi.org/10.1371/journal.pone.0029291>.
46. Veenhuis RT, Freeman ZT, Korleski J, Cohen LK, Massaccesi G, Tomasi A, Boesch AW, Ackerman ME, Margolick JB, Blankson JN, Chattergoon MA, Cox AL. 2017. HIV-antibody complexes enhance production of type I interferon by plasmacytoid dendritic cells. *J Clin Invest* 127:4352–4364. <https://doi.org/10.1172/JCI95375>.
47. Campbell EM, Hope TJ. 2015. HIV-1 capsid: the multifaceted key player in HIV-1 infection. *Nat Rev Microbiol* 13:471–483. <https://doi.org/10.1038/nrmicro3503>.
48. Liu YJ. 2005. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu Rev Immunol* 23:275–306. <https://doi.org/10.1146/annurev.immunol.23.021704.115633>.
49. Bosinger SE, Johnson ZP, Folkner KA, Patel N, Hashempour T, Jochems SP, Del Rio Estrada PM, Paiardini M, Lin R, Vanderford TH, Hiscott J, Silvestri G. 2013. Intact type I interferon production and IRF7 function in sooty mangabeys. *PLoS Pathog* 9:e1003597. <https://doi.org/10.1371/journal.ppat.1003597>.
50. Zhen A, Rezek V, Youn C, Lam B, Chang N, Rick J, Carrillo M, Martin H, Kasparian S, Syed P, Rice N, Brooks DG, Kitchen SG. 2017. Targeting type I interferon-mediated activation restores immune function in chronic HIV infection. *J Clin Invest* 127:260–268. <https://doi.org/10.1172/JCI89488>.
51. Asmuth DM, Murphy RL, Rosenkranz SL, Lertora JJ, Kottlil S, Cramer Y, Chan ES, Schooley RT, Rinaldo CR, Thielman N, Li XD, Wahl SM, Shore J, Janik J, Lempicki RA, Simpson Y, Pollard RB, Team ACTG. 2010. Safety, tolerability, and mechanisms of antiretroviral activity of pegylated interferon Alfa-2a in HIV-1-monoinfected participants: a phase II clinical trial. *J Infect Dis* 201:1686–1696. <https://doi.org/10.1086/652420>.
52. Lane HC, Davey V, Kovacs JA, Feinberg J, Metcalf JA, Herpin B, Walker R, Deyton L, Davey RT, Jr, Falloon J. 1990. Interferon-alpha in patients with asymptomatic human immunodeficiency virus (HIV) infection. A randomized, placebo-controlled trial. *Ann Intern Med* 112:805–811. <https://doi.org/10.7326/0003-4819-112-11-805>.
53. Lane HC, Kovacs JA, Feinberg J, Herpin B, Davey V, Walker R, Deyton L, Metcalf JA, Baseler M, Salzman N. 1988. Anti-retroviral effects of interferon-alpha in AIDS-associated Kaposi's sarcoma. *Lancet* 2:1218–1222. [https://doi.org/10.1016/S0140-6736\(88\)90811-2](https://doi.org/10.1016/S0140-6736(88)90811-2).
54. Manion M, Rodriguez B, Medvik K, Hardy G, Harding CV, Schooley RT, Pollard R, Asmuth D, Murphy R, Barker E, Brady KE, Landay A, Funderburg N, Sieg SF, Lederman MM. 2012. Interferon-alpha administration enhances CD8+ T cell activation in HIV infection. *PLoS One* 7:e30306. <https://doi.org/10.1371/journal.pone.0030306>.
55. Taylor JP, Cash MN, Santostefano KE, Nakanishi M, Terada N, Wallet MA. 2018. CRISPR/Cas9 knockout of USP18 enhances type I IFN responsiveness and restricts HIV-1 infection in macrophages. *J Leukoc Biol* 103:1225–1240. <https://doi.org/10.1002/JLB.3MIA0917-352R>.
56. Osei Kuffour E, Schott K, Jaguva Vasudevan AA, Holler J, Schulz WA, Lang PA, Lang KS, Kim B, Haussinger D, Konig R, Munk C. 2018. USP18 (UBP43) abrogates p21-mediated inhibition of HIV-1. *J Virol* 92. <https://doi.org/10.1128/JVI.00592-18>.
57. Kwaa AKR, Talana CAG, Blankson JN. 2018. Interferon alpha enhances NK cell function and the suppressive capacity of HIV-specific CD8(+) T cells. *J Virol* 93. <https://doi.org/10.1128/JVI.01541-18>.
58. Catalfamo M, Wilhelm C, Tcheung L, Proschan M, Friesen T, Park JH, Adelsberger J, Baseler M, Maldarelli F, Davey R, Roby G, Rehm C, Lane C. 2011. CD4 and CD8 T cell immune activation during chronic HIV infection: roles of homeostasis, HIV, type I IFN, and IL-7. *J Immunol* 186:2106–2116. <https://doi.org/10.4049/jimmunol.1002000>.
59. Harper MS, Guo K, Gibbert K, Lee EJ, Dillon SM, Barrett BS, McCarter MD, Hasenkrug KJ, Dittmer U, Wilson CC, Santiago ML. 2015. Interferon-alpha subtypes in an ex vivo model of acute HIV-1 infection: expression, potency and effector mechanisms. *PLoS Pathog* 11:e1005254. <https://doi.org/10.1371/journal.ppat.1005254>.
60. Palmer S, Maldarelli F, Wiegand A, Bernstein B, Hanna GJ, Brun SC, Kempf DJ, Mellors JW, Coffin JM, King MS. 2008. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci U S A* 105:3879–3884. <https://doi.org/10.1073/pnas.0800050105>.
61. Besson GJ, Lalama CM, Bosch RJ, Gandhi RT, Bedison MA, Aga E, Riddler SA, McMahon DK, Hong F, Mellors JW. 2014. HIV-1 DNA decay dynamics in blood during more than a decade of suppressive antiretroviral therapy. *Clin Infect Dis* 59:1312–1321. <https://doi.org/10.1093/cid/ciu585>.
62. Pollack RA, Jones RB, Pertea M, Bruner KM, Martin AR, Thomas AS, Capoferri AA, Beg SA, Huang SH, Karandish S, Hao H, Halper-Stromberg E, Yong PC, Kovacs C, Benko E, Siliciano RF, Ho YC. 2017. Defective HIV-1 proviruses are expressed and can be recognized by cytotoxic T lymphocytes, which shape the proviral landscape. *Cell Host Microbe* 21:494–506. <https://doi.org/10.1016/j.chom.2017.03.008>.
63. Bruner KM, Wang Z, Simonetti FR, Bender AM, Kwon KJ, Sengupta S, Fray EJ, Beg SA, Antar AAR, Jenike KM, Bertagnolli LN, Capoferri AA, Kufera JT, Timmons A, Nobles C, Gregg J, Wada N, Ho YC, Zhang H, Margolick JB, Blankson JN, Deeks SG, Bushman FD, Siliciano JD, Laird GM, Siliciano RF. 2019. A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature* 566:120–125. <https://doi.org/10.1038/s41586-019-0898-8>.
64. Pellerin A, Otero K, Czerkowicz JM, Kerns HM, Shapiro RI, Ranger AM, Otipoby KL, Taylor FR, Cameron TO, Viney JL, Rabah D. 2015. Anti-BDCA2 monoclonal antibody inhibits plasmacytoid dendritic cell activation through Fc-dependent and Fc-independent mechanisms. *EMBO Mol Med* 7:464–476. <https://doi.org/10.15252/emmm.201404719>.
65. Harris LD, Tabb B, Sodora DL, Paiardini M, Klatt NR, Douek DC, Silvestri G, Muller-Trutwin M, Vasile-Pandrea I, Apetrei C, Hirsch V, Lifson J, Brenchley JM, Estes JD. 2010. Downregulation of robust acute type I interferon responses distinguishes nonpathogenic simian immunodeficiency virus (SIV) infection of natural hosts from pathogenic SIV infection of rhesus macaques. *J Virol* 84:7886–7891. <https://doi.org/10.1128/JVI.02612-09>.
66. Sandler NG, Bosinger SE, Estes JD, Zhu RT, Sharp GK, Boritz E, Levin D, Wijeyesinghe S, Makamdop KN, del Prete GQ, Hill BJ, Timmer JK, Reiss E, Yarden G, Darko S, Contijoch E, Todd JP, Silvestri G, Nason M, Norgren RB, Jr, Keele BF, Rao S, Langer JA, Lifson JD, Schreiber G, Douek DC. 2014. Type I interferon responses in rhesus macaques prevent SIV infection and slow disease progression. *Nature* 511:601–605. <https://doi.org/10.1038/nature13554>.
67. Asmuth DM, Abel K, George MD, Dandekar S, Pollard RB, Miller CJ. 2008. Pegylated interferon-alpha 2a treatment of chronic SIV-infected macaques. *J Med Primatol* 37:26–30. <https://doi.org/10.1111/j.1600-0684.2007.00221.x>.
68. Cheng L, Ma J, Li J, Li D, Li G, Li F, Zhang Q, Yu H, Yasui F, Ye C, Tsao LC, Hu Z, Su L, Zhang L. 2017. Blocking type I interferon signaling enhances T cell recovery and reduces HIV-1 reservoirs. *J Clin Invest* 127:269–279. <https://doi.org/10.1172/JCI90745>.
69. Carnathan D, Lawson B, Yu J, Patel K, Billingsley JM, Sharp GK, Delmas OM, Dawoud R, Wilkinson P, Nicolette C, Cameron MJ, Sekaly RP, Bosinger SE, Silvestri G, Vanderford TH. 2018. Reduced chronic lymphocyte activation following interferon alpha blockade during the acute phase of simian immunodeficiency virus infection in rhesus macaques. *J Virol* 92. <https://doi.org/10.1128/JVI.01760-17>.
70. Starr R, Wilson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, Gonda TJ, Alexander WS, Metcalf D, Nicola NA, Hilton DJ. 1997. A family of cytokine-inducible inhibitors of signalling. *Nature* 387:917–921. <https://doi.org/10.1038/43206>.
71. Noel S, Lee SA, Sadasivam M, Hamad ARA, Rabb H. 2018. KEAP1 editing using CRISPR/Cas9 for therapeutic NRF2 activation in primary human T lymphocytes. *J Immunol* 200:1929–1936.