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Influenza Vaccination Can Broadly Activate the HIV Reservoir During Antiretroviral Therapy

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INTRODUCTION

A major obstacle to an HIV cure is a persistent subset of quiescent, infected cells, known as the latent reservoir [1–3]. Proviruses in their quiescent state remain undetected by the immune system and impervious to antiretroviral therapy (ART) [4, 5]. Upon ART interruption, these proviruses can quickly resume viral replication [6–9]. One unmet need is a means to safely and effectively unmask these infected cells in the setting of ART. Multiple latency reversing agents have been investigated for this purpose, but none has yet demonstrated the ability to significantly reduce the size of the latent reservoir, and most have significant safety concerns [10–14].

We recently reported that clinical vaccines administered to people living with HIV can induce cellular HIV RNA expression during virally suppressive ART [15]. In that study, vaccination was associated with increased immune activation and enhanced HIV-specific responses. However, it remains unknown how vaccine-specific immune responses correlate with HIV activation and whether standard vaccines induce HIV expression selectively from a small pool of antigen-specific, activated HIV-infected cells, or non-selectively, i.e. from a broad pool of bystander activated HIV infected cells.

Here, we used deep sequencing to characterize HIV reactivation following a standard influenza vaccination in a group of seven people living with HIV who were virally suppressed with ART.

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RESULTS

Study Population

Seven participants who had a median duration of HIV infection of 21 years [interquartile range (IQR): 17–31] were included. Their median age was 59 years (IQR: 55–64). All started ART during chronic infection and achieved sustained viral suppression below 20 copies/mL for at least the last 6 months, and were virally suppressed at time of vaccine administration. Their median $CD4^+$ T cell count was 613 cells/mm^3 (IQR= 327–721). Population characteristics are summarized in **SDC Table S2**. Upon enrollment, participants provided a baseline blood sample and then received a 0.5mL intramuscular injection of a standard influenza vaccine (Fluarix®, GSK). Blood samples were collected at baseline and days 2, 4, 7, 14 and 28 following vaccination.

HIV RNA Transcription and Immune Activation Following Vaccination

We first measured cell-associated HIV RNA encoding for *gag* at each sampled time point, in duplicate by digital droplet polymerase chain reaction (ddPCR), to determine which showed increased HIV transcription following vaccination. Participants K2, K3 and K4 had increased copy numbers of *gag* RNA (median increase 256 copies/10^6 cells, Range: 177–924) in the week following vaccination, relative to their baseline measures; there was a median of 852 copies of HIV *gag* DNA (Range: 143–5769) and 590 copies of HIV *gag* RNA (Range: 79–3481) per 10^6 cells in these participants (**SDC Figures S1a and S1b**).

We next analyzed HLA-DR and CD38 expression (markers of cellular activation) by flow cytometry and found that CD4+ T cells from three of the seven participants (K3, K4 and K5) showed increasing percentages of HLA-DR and CD38 expression during the four weeks after vaccination compared with baseline (**SDC Figure S1c**). We also compared the specificity of immune responsiveness of the participants by measuring influenza-specific Immunoglobulin G (IgG) in the serum at baseline and at days 7, 14 and 28. Participants K3, K4 and K7 exhibited the greatest increases in influenza-specific IgG (**SDC Figure S1d**).

Source of HIV Transcription

To better understand the source of detectable cell-associated HIV RNA, we deeply sequenced HIV DNA and RNA populations in blood from participants K2, K3 and K4 at all collected time points. Deep sequencing of the HIV *gag* p24, *pol* reverse transcriptase, and *env* C2-V3 coding regions was performed. After quality filtering, over 50 million reads were analyzed with a median of 320,452 reads/region/sample (IQR: 198,053–1,494,661). A median of 6 (IQR: 3–11) and 14 (IQR: 8–23) haplotypes per HIV RNA and DNA sample were generated and analyzed. Comparing sequence diversity within the three coding regions via pairwise Tamura-Nei 93 distances between reads with at least 100 overlapping base pairs [16] showed no significant changes in sequence diversity in any sequenced HIV coding region between pre-vaccination samples and samples collected one month after vaccination (**SDC Figure S2**). We next assessed viral compartmentalization between HIV DNA and RNA sequences for participants K2, K3 and K4 using a distance-based F_{ST} test on collapsed and re-expanded haplotypes and a tree-based Slatkin-Maddison test [17] (**SDC Table S3**). We found no evidence of viral compartmentalization between paired HIV DNA and RNA

samples via either method. Overall, maximum likelihood phylogenetic trees of the HIV DNA and RNA sequences, and tree topologies exhibited extensive intermingling of sequences between HIV RNA and DNA populations at each time point (**Figure 1**), especially compared with those from D0 and D28 in the other 4 participants (**SDC Figure S3**). Together, these results suggested that the vaccine activated HIV DNA populations for transcription non-selectively.

DISCUSSION

Many current HIV curative strategies have focused on developing methods that induce expression of the virus from infected cells during ART, so that viral proteins are revealed, and cellular reservoirs can be cleared by the host immune response, while ART prevents new cells from being infected [18, 19]. Prophylactic vaccination represents a potential means of transiently activating the immune system, and has been associated with increased levels of cell-free HIV RNA after vaccinations for influenza [20–29], pneumococcus [30–32], tetanus [28, 33], hepatitis B [34] and cholera [35]. Our group recently published data generated from a randomized clinical trial which showed that standard vaccination can increase HIV transcription comparably to what has been observed with the HDACi, Vorinostat [15].

Out of our seven study participants, K2, K3 and K4 most clearly spiked in their HIV *gag* RNA expression following vaccination (**SDC Figure S1a,b**). Such variable responsiveness to standard influenza [36–39]. In these three participants, HIV DNA population diversity did not change during the four weeks following vaccination, suggesting that sampling of infected circulating cells did not change appreciably after vaccination (**SDC Figure S3**). Maximum likelihood phylogenetic reconstructions of HIV *gag, pol* and *env* sequences showed extensive overlap between HIV DNA and RNA sequences at all time points (**Figures 1, S4**), suggesting that cell-associated HIV RNA sequences were likely derived from a broad, non-selective pool of cellular reservoirs of HIV DNA. This is consistent with a study of the HDACi Panobinostat, which showed panmixis of sequences generated from HIV DNA and RNA populations in circulating PBMC [40].

Our study has several limitations. First, our sample size cannot allow broad generalization to others who have HIV reactivation after influenza vaccination. Since this was a pilot study and not a clinical trial, some people may have selective activation of HIV reservoirs following vaccination. Along these lines, increased cell-associated HIV RNA after vaccination may be due to factors other than vaccination. To explore this further, we chose the three participants with the best evidence for increased HIV expression following vaccination (**SDC Figure S1a,b**). In our previous randomized clinical trial of a larger cohort of people receiving a schedule of vaccines, we were able to detect significant increases in copy numbers of cell-associated, unspliced *gag* following vaccination [15]. Next, our number of sequence reads suggest high levels of PCR amplification, which can introduce primer biases. However, if template selection based on non-ideal annealing temperatures occurred, we would expect our data to underestimate the true number of haplotypes and bias in amplification. An under-estimation or biased amplification of templates between HIV RNA and DNA populations would likely increase the observation of compartmentalization and thus selective activation, which we did not detect. Along these lines, we did not barcode

HIV templates before amplification. While this decreased amplification bias, it precluded us from measuring clonal expansion of HIV DNA or large bursts of HIV RNA from a single provirus in our samples; however, such conditions would be expected to also increase the potential for observation of compartmentalization, which we did not detect. Lastly, our compartmentalization analyses may have been confounded by the number of HIV DNA and RNA haplotypes analyzed. To reduce the likelihood of sampling bias, we grouped all time points for each participant. Compartmentalization was detected in some samples when we analyzed each time point individually (data not shown), but this was likely due to limited HIV RNA haplotypes available for analysis at each time point.

The hunt is on for strategies to provoke HIV expression from quiescent cellular reservoirs and then eradicate the exposed, infected cells. Earlier studies examining the effects of clinical vaccinations on people living with HIV suggested that vaccines can potently activate cellular reservoirs of HIV. We demonstrated here HIV RNA was broadly expressed from a phylogenetically representative pool of circulating cellular reservoirs following vaccination. The ability to reactivate a diverse pool of cellular HIV reservoirs will be critical to HIV eradication approaches. Our findings are a proof-of-concept that standard clinical vaccines can broadly re-activate latent HIV from reservoirs suppressed with ART. Standard influenza vaccination will not cure anyone of HIV. However - after further investigation to determine the mechanisms of HIV activation and improve their potency - vaccinations could become a relatively safe addition to various cure efforts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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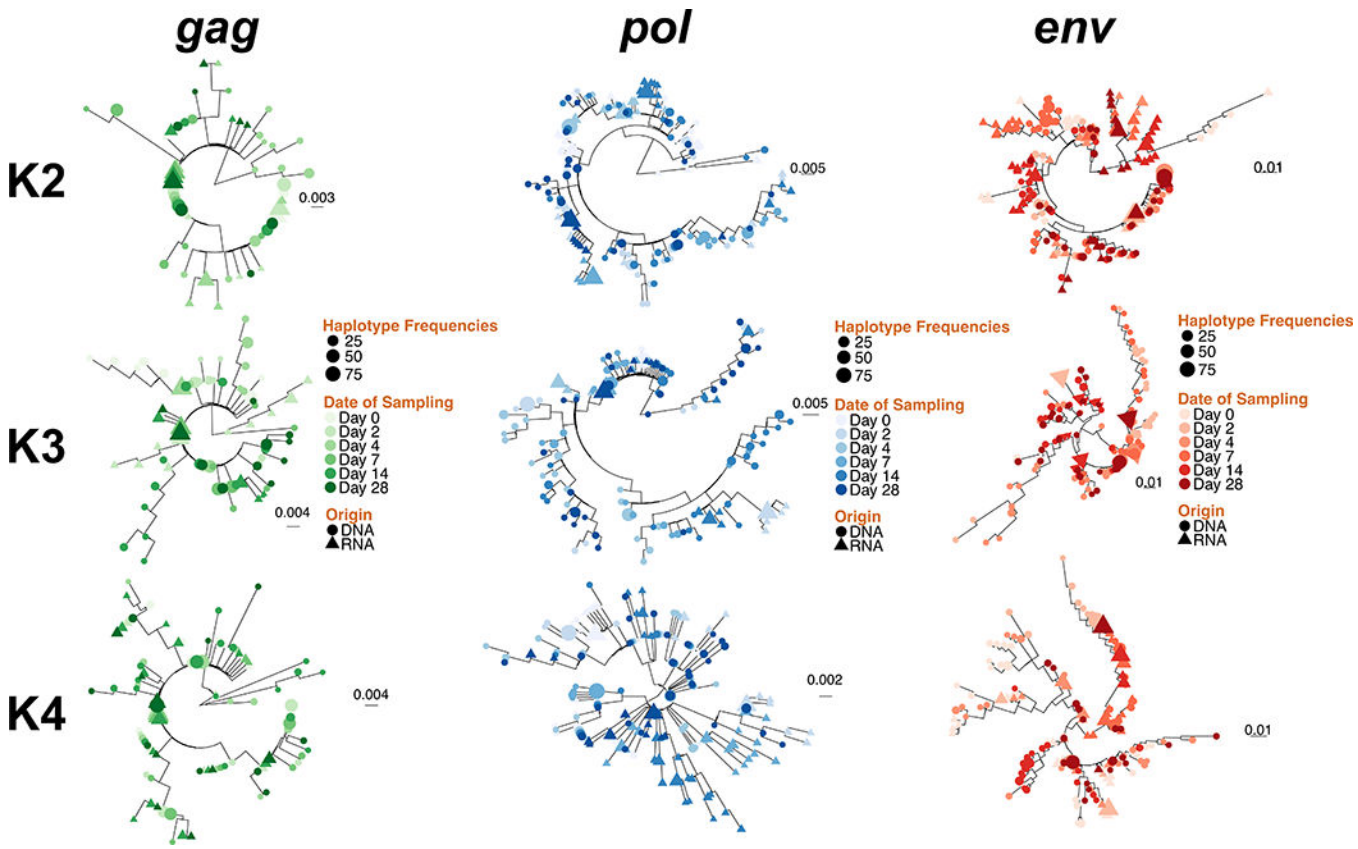

Figure 1. Maximum likelihood phylogenetic reconstruction of sequences generated from longitudinally collected HIV-1 RNA and DNA Populations Following Influenza Vaccination

HIV DNA and RNA haplotypes above a minimal frequency threshold of 0.01 were extracted from reads covering the *gag* (panel A), *pol* (panel B) and *env* (panel C) regions for individual K2, K3 and K4 and were used to construct maximum likelihood phylogenies using FastTree (Price et al., 2009). HIV DNA and RNA haplotypes are depicted in circles and triangles respectively. Time point in days (0 to 28 days from vaccination) is indicated for each variant. Scale bars are in substitutions/site.