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Influenza Vaccination Can Broadly Activate the HIV Reservoir During Antiretroviral Therapy

To the Editors:

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] On 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 nonselectively, that is, from a broad pool of bystander-activated HIV-infected cells.

Here, we used deep sequencing to characterize HIV reactivation after a standard influenza vaccination in a group of 7 people living with HIV who were virally suppressed with ART.

METHODS

Methods available as supplemental Digital Content, http://links.lww.com/QAI/B202.

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 per milliliter for at least the past 6 months, and were virally suppressed at the time of vaccine administration. Their median $CD4^+$ T-cell count was 613 cells/mm^3 (IQR = 327–721). Population characteristics are summarized in Table S2, Supplemental Digital Content, http://links.lww.com/QAI/B202. On enrollment, participants provided a baseline blood sample and then received a 0.5-mL intramuscular injection of a standard influenza vaccine (Fluarix; GSK, La Jolla, CA). Blood samples were collected at baseline and days 2, 4, 7, 14, and 28 after vaccination.

HIV RNA Transcription and Immune Activation After Vaccination

We first measured cell-associated HIV RNA encoding for *gag* at each sampled time point, in duplicate by digital droplet polymerase chain reaction, to determine which showed increased HIV transcription after 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 after 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 (Figures S1a, b, Supplemental Digital Content, http://links.lww.com/QAI/B202).

We next analyzed HLA-DR and CD38 expression (markers of cellular activation) by flow cytometry and found that $CD4^+$ T cells from 3 of the 7 participants (K3, K4, and K5) showed increasing percentages of HLA-DR and CD38 expression during the 4 weeks after vaccination compared with baseline (Figure S1c, Supplemental Digital Content, http://links.lww.com/QAI/B202). 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 (Figure S1d, Supplemental Digital Content, http://links.lww.com/QAI/B202).

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:

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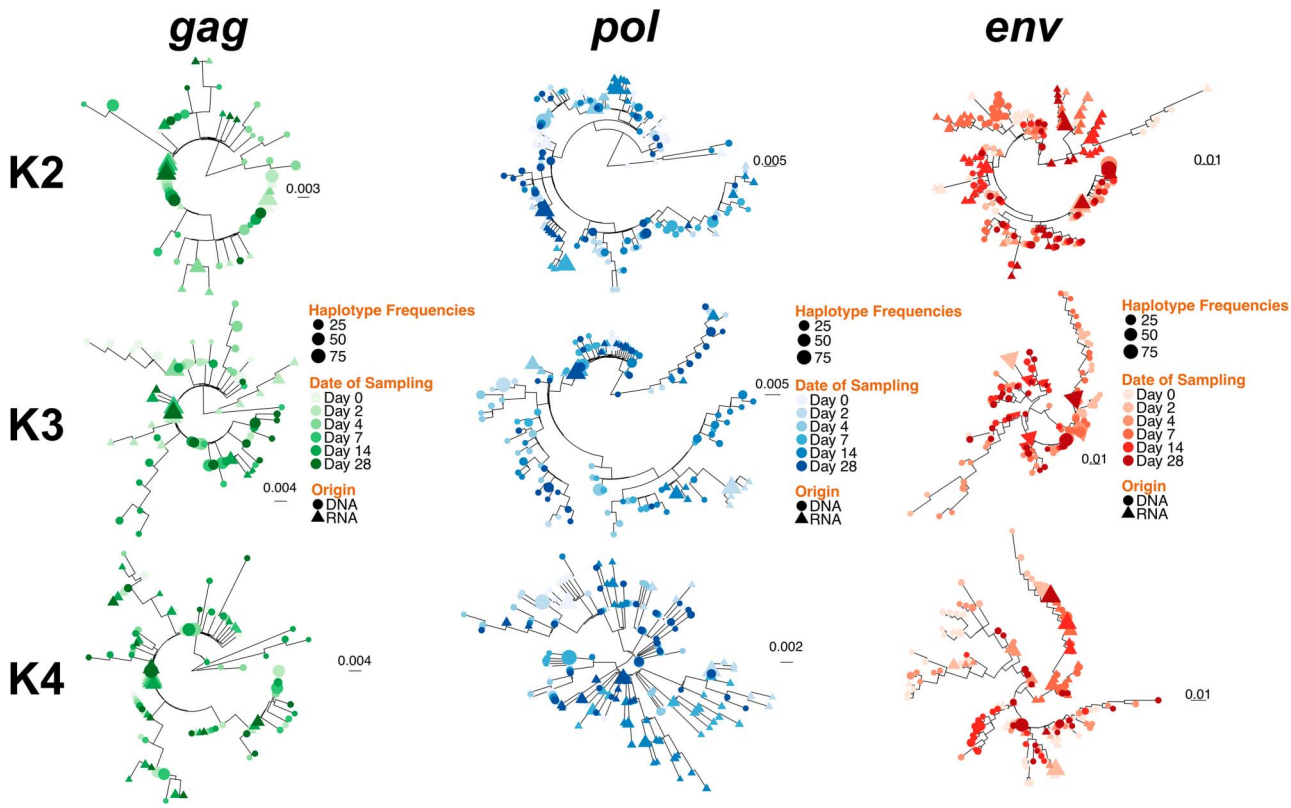

FIGURE 1. Maximum-likelihood phylogenetic reconstruction of sequences generated from longitudinally collected HIV-1 RNA and DNA populations after influenza vaccination. HIV DNA and RNA haplotypes above a minimal frequency threshold of 0.01 were extracted from reads covering the *gag* (left, green), *pol* (middle, blue), and *env* (right, red) regions for individuals 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 (0–28 days from vaccination) is indicated for each variant. Scale bars are in substitutions/site.

198,053–1,494,661). A median of 6 (IQR: 3–11) and 14 (IQR: 8–23) haplotypes per HIV RNA and DNA sample was generated and analyzed. Comparing sequence diversity within the 3 coding regions through 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 prevaccination samples and samples collected 1 month after vaccination (Figure S2, Supplemental Digital Content, http://links.lww.com/QAI/B202). 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 reexpanded haplotypes and a tree-based Slatkin–Maddison test[17] (Table S3, Supplemental Digital Content, http://links.lww.com/QAI/B202). We found no evidence of viral compartmentalization between paired HIV DNA and RNA samples using 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 (Fig. 1), especially compared with those from D0 and D28 in the other 4 participants (Figure S3, Supplemental Digital Content, http://links.lww.com/QAI/B202). Together, these results suggested that the vaccine activated HIV DNA populations for transcription nonselectively.

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 with what has been observed with the HDACi, Vorinostat.[15]

Out of our 7 study participants, K2, K3, and K4 most clearly spiked in their HIV *gag* RNA expression after vaccination (Figures S1a, b, Supplemental Digital Content, http://links.lww.com/QAI/B202). Such variable responsiveness to standard influenza vaccination is common in people living with HIV.[36–39] In these 3 participants, HIV DNA population

diversity did not change during the 4 weeks after vaccination, suggesting that sampling of infected circulating cells did not change appreciably after vaccination (Figure S3, Supplemental Digital Content, http://links.lww.com/QAI/B202). Maximum-likelihood phylogenetic reconstructions of HIV *gag, pol,* and *env* sequences showed extensive overlap between HIV DNA and RNA sequences at all time points (Fig. 1, Figure S4, Supplemental Digital Content, http://links.lww.com/QAI/B202), suggesting that cell-associated HIV RNA sequences were likely derived from a broad, nonselective 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 peripheral blood mononuclear cell.[40]

Our study has several limitations. First, our sample size cannot allow for broad generalization to others who have HIV reactivation after influenza vaccination. Because this was a pilot study and not a clinical trial, some people may have selective activation of HIV reservoirs after 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 3 participants with the best evidence for increased HIV expression after vaccination (Figures S1a, b, Supplemental Digital Content, http://links.lww.com/QAI/B202). 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* after vaccination.[15] Next, our number of sequence reads suggests high levels of polymerase chain reaction amplification, which can introduce primer biases. However, if template selection based on nonideal annealing temperatures occurred, we would expect our data to underestimate the true number of haplotypes and bias in amplification. An underestimation 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. Although 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. Finally, 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. Here, we demonstrated that HIV RNA was broadly expressed from a phylogenetically representative pool of circulating cellular reservoirs after 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 reactivate 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.

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ERRATUM

Multimonth Prescription of Antiretroviral Therapy Among Children and Adolescents: Experiences From the Baylor International Pediatric AIDS Initiative in 6 African Countries: Erratum.

In the August 15, 2018 Supplement 2 issue of Journal of Acquired Immune Deficiency Syndromes in the article by Kim et al, "Multimonth Prescription of Antiretroviral Therapy Among Children and Adolescents: Experiences From the Baylor International Pediatric AIDS Initiative in 6 African Countries", the author Richard S. Wanless should be listed as Richard S. Wanless, MBChB, PhD.

REFERENCE

Caviness AC, Wanless RS, Kim MH, et al. Multimonth Prescription of Antiretroviral Therapy Among Children and Adolescents: Experiences From the Baylor International Pediatric AIDS Initiative in 6 African Countries. *Journal of Acquired Immune Deficiency Syndromes*. 2018; 78: S71-S78.