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Standard vaccines increase HIV-1 transcription during antiretroviral therapy

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Abstract

Objectives—Curative strategies using agents to perturb the HIV reservoir have demonstrated only modest activity, whereas increases in viremia after standard vaccination have been described. We investigated whether vaccination against non-HIV pathogens can induce HIV transcription and thereby play a role in future eradication strategies.

Design—A randomized controlled trial (NCT00329251) was performed to compare the effects of clinical vaccines with placebo on HIV transcription and immune activation.

Methods—Twenty-six HIV-infected individuals on suppressive antiretroviral therapy were randomized to receive a vaccination schedule ($n = 13$) or placebo ($n = 13$). Cell-associated RNA and DNA were extracted from peripheral blood mononuclear cells, and HIV was quantified by droplet digital PCR using primers for *gag* and 2-LTR (for HIV DNA), unspliced *gag* RNA (*gag* usRNA), multisplliced *tat-rev* RNA (*tat-rev* msRNA) and *polyA* mRNA.

Results—Significant increases in *gag* usRNA after influenza/hepatitis B vaccination ($P = 0.02$) and in *gag* usRNA ($P = 0.04$) and *polyA* mRNA ($P = 0.04$) after pneumococcus/hepatitis B vaccination were seen in vaccinees but not controls. HIV DNA and plasma HIV RNA did not change in either group. Increases in CD4⁺ and CD8⁺ T-cell activation markers ($P = 0.08$ and $P < 0.001$, respectively) and HIV-specific CD8⁺ responses ($P = 0.04$ for p24 *gag*, $P = 0.01$ for p17 *gag* and $P = 0.04$ for total *gag*) were seen in vaccinees but not controls.

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Conflicts of interest

There are no conflicts of interest.

Conclusion—In this study, vaccination was associated with increases in HIV cell-associated RNA and HIV-specific responses during antiretroviral therapy. Using standard vaccines to stimulate HIV transcription may therefore be a useful component of future eradication strategies.

Keywords

cell-associated HIV RNA; HIV eradication; HIV latency; latency reversal; vaccines

Introduction

The latent viral reservoir is widely regarded as the main obstacle to eradication of HIV type 1 (HIV-1) infection [1,2]. Integrated proviral forms in CD4⁺ T cells demonstrate lifelong persistence and the potential to reactivate even in individuals with prolonged complete viral suppression. Proviruses in their quiescent integrated state remain undetected by the immune system and impervious to currently available antiretroviral therapy (ART) [3,4]. Therefore, it appears that complete eradication of HIV infection would require an additional intervention capable of unmasking and clearing these latently infected cells.

One potential strategy, labeled ‘shock and kill’, involves activating latent provirus to transcribe HIV RNA and produce viral proteins, thereby leading to recognition and killing of infected cells [2,5]. Multiple latency reversing agents (LRAs) have been investigated for this purpose. Initial efforts with T-cell activating compounds IL-2 and anti-CD3 succeeded in reactivating latent HIV, but with considerable associated toxicity [6,7]. Subsequent efforts have used compounds that induce HIV gene expression without triggering global T-cell activation; these include histone deacetylase inhibitors [8–11], immune modulators [12,13] and others [14]. Trials of these nonactivating candidate LRAs have shown varied effects in terms of induction of HIV transcription that are modest in comparison with T-cell activators [15,16]. Effects on viral protein translation and virion production are less encouraging, with studies suggesting that nonactivating LRAs may face posttranscriptional blocks in resting CD4⁺ T cells [17]. In addition, even if their induction of HIV transcription were to match that of T-cell activators, it is possible that reversal of viral latency in this context would not be sufficient to bring about cell death [18].

HIV-infected individuals are at increased risk for communicable diseases, and therefore vaccination against common pathogens is an important part of their routine clinical care [19]. Previous studies have found transient increases in levels of plasma HIV-1 RNA after vaccination for influenza [20–25], pneumococcus [23,26], tetanus [27,28], hepatitis B (Hep B) [29,30] and cholera [31], whereas others have shown no effect [32–35]. Literature in the field has been extensively reviewed by our group (Supplemental Fig. 1, <http://links.lww.com/QAD/A952>) and others [36,37] with the common conclusion that most studies demonstrating postvaccination increases in plasma HIV RNA do so within 7–14 days of vaccination, although heterogeneity in study design, study population, inherent vaccine characteristics and assay sensitivities likely account for some variability in observed results. One randomized controlled trial studied whether standard vaccines could induce HIV-specific responses and thereby influence viral rebound dynamics after ART interruption [38]. Although vaccination was not associated with delayed or reduced viral rebound, an

increase in immune activation was described in the vaccine arm. We hypothesized that changes in HIV dynamics at the cellular RNA level may have occurred after vaccination. We therefore measured levels of cell-associated HIV-1 RNA (HIV ca-RNA) in peripheral blood mononuclear cells (PBMCs) of participants receiving standard vaccines or placebo to determine whether vaccination is capable of inducing HIV transcription during suppressive ART.

Materials and methods

Study design

A randomized, double-blinded, placebo-controlled clinical trial was performed at the Hospital Clinic of Barcelona, Spain [38]. The primary end point of the original trial was time to viral rebound after structured treatment interruption. Twenty-six HIV-infected participants on suppressive ART were randomized to receive either a schedule of standard vaccines ($n = 13$) or placebo ($n = 13$) over a period of 12 months. The vaccination schedule included standard doses of seven distinct clinically approved vaccines against a total of 10 different agents (Fig. 1): Hep B, hepatitis A (Hep A), influenza, pneumococcus, varicella zoster virus (VZV), measles-mumps-rubella (MMR), and tetanus-diphtheria (TD). The placebo group received 0.5 ml of intramuscular 0.9% sodium chloride at identical time points. Blood samples were drawn on a monthly basis; in case visits coincided with vaccination time points, blood was first drawn and followed by vaccine delivery.

Ethics statement

Written informed consent was obtained from all participants before study enrollment. Approval was obtained from the local institutional ethics review board (Hospital Clínic de Barcelona, Barcelona, Spain), and the study was registered in the NIH public clinical trials database (NCT00329251).

Measurement of HIV DNA and RNA in peripheral blood mononuclear cell

Whole blood was processed to obtain PBMC by density gradient separation. PBMCs were cryopreserved in aliquots of at least 5×10^6 cells and were later viably thawed for nucleic acid extraction and flow cytometric assessment. Baseline samples (study month 0) were not available due to usage in prior studies [38,39]. For flow cytometry, approximately 0.2×10^6 PBMCs were stained with CD4⁺-FITC, CD8⁺-PE, CD45-PerCP-Cy5.5 and CD3-APC (BD Biosciences, San Diego, California, USA) and acquired in an Accuri flow cytometer (BD Biosciences, San Diego, CA, USA) to assess the percentage of CD4⁺ T cells in each sample. Next, DNA and RNA were dually extracted from the remaining sample using an AllPrep DNA/RNA Mini Kit (Qiagen, Germantown, Maryland, USA). Levels of HIV DNA were measured by ddPCR in triplicate using an extensively validated assay [40] with gag and 2-LTR-specific primers, normalized to a standard host DNA marker (RPP30). HIV ca-RNA was also measured by ddPCR, as previously described [40] using primer/probe sets for unspliced (*gag* usRNA), multisplliced (*tat-rev* msRNA) and all fully elongated and correctly processed HIV mRNA molecules (*polyA* mRNA) [41]. HIV DNA and cell-associated RNA (ca-RNA) values were normalized by percentage of CD4⁺ T cells, as determined by flow cytometry.

Measurement of plasma HIV RNA

Plasma HIV RNA levels were determined at monthly study visits using the Amplicor HIV-1 Monitor Ultra Sensitive Specimen Preparation Protocol Ultra Direct Assay (v1.5, Roche, Somerville, New Jersey, USA) with a limit of quantification of 200 copies/ml. Plasma samples that fell below the limit of quantification were retested with the same kit with a limit of detection of 20 copies/ml.

Immunological tests

Several immunological parameters were measured at 3-month intervals (months 0, 3, 6, 9 and 12) as part of the original study [38]. Briefly, flow cytometric analysis of T-cell subsets [naive ($CD45RA^+CD45RO^-$) and memory ($CD45RA^-CD45RO^+$) $CD4^+$ and $CD8^+$ T cells], cellular activation ($CD25^+$ and $CD38^+HLA-DR^+$), proliferation ($Ki67^+$) and replicative senescence ($CD28^-CD57^+$), and HIV coreceptor expression ($CXCR4^+$ and $CCR5^+$) was performed as previously described [38]. ELISPOT (enzyme-linked immunospot) assays [42] were used to assess IFN- γ release of $CD8^+$ T cells in the presence of different HLA class-I restricted synthetic peptides representing HIV *gag*, *pol*, *env* and *nef* proteins and also vaccine-specific antigens. Spot-forming units were counted using an AID ELISPOT reader (Autoimmun Diagnostika GmbH, Germany).

Statistical analysis

Statistical analysis was performed in Prism (version 6.0; Graphpad Software, La Jolla, California, USA) and statistical software package R. Comparisons between groups were performed using the Wilcoxon and Mann–Whitney tests for paired and unpaired continuous variables, respectively. Correlations were examined using the Spearman test. Average effect (on HIV transcription) per vaccine (or placebo) was calculated by multiple regression of logdomain HIV ca-RNA levels onto predictor variables representing vaccine boost and temporal decay.

Results

Study participants

In this randomized control trial [38], participants receiving vaccines (vaccinees; $n = 13$) and those receiving placebo (controls; $n = 13$) showed similar demographic and clinical characteristics at baseline (Table 1).

Vaccination associated with increases in HIV cell-associated RNA

Overall, measurements of the three transcripts were significantly correlated (*gag* usRNA and *polyA* mRNA $r0.76$, $P < 0.0001$; *gag* usRNA and *tat-rev* msRNA $r0.36$, $P < 0.0001$; *tat-rev* msRNA and *polyA* mRNA $r0.31$, $P < 0.0001$). *Gag* usRNA was the most consistently detectable transcript (detectable in 93% of all samples), significantly more so than *polyA* mRNA (detectable in 77% of samples, $P < 0.001$) and *tat-rev* msRNA (detectable in 35% of samples, $P < 0.0001$).

The effects of individual vaccines are summarized in Table 2. Significant increases in *gag* usRNA from prevaccine levels were seen after administration of influenza/Hep B [median

fold-change 2.4 (1.3–7.4), $P = 0.02$; Fig. 2a] and pneumococcus/Hep B vaccines [median fold-change 7.0 (2.4–22.7), $P = 0.04$; Fig. 2c] and in *polyA* mRNA after administration of pneumococcus/Hep B vaccines [median fold-change 20.9 (3.0–79.7), $P = 0.04$]. In contrast, controls did not show significant increases in *gag* mRNA at corresponding time points (Fig. 2b). *Gag* usRNA increased in 10 of 12 vaccinees after influenza/Hep B vaccination, versus seven of 11 controls, and seven of eight vaccinees after pneumococcus/Hep B vaccination, versus one of one control. In a longitudinal analysis of all study time points, vaccinees experienced overall significant increases in transcript levels with each vaccine administered [average effect per vaccine 1.7 (1.4–2.3), $P = 0.004$], whereas controls did not [average effect per vaccine 0.6 (0.1–1.6), $P = 0.19$; Fig. 2d and e and Supplemental Fig. 2, <http://links.lww.com/QAD/A952>]. The difference between groups was significant ($P = 0.03$).

Plasma HIV RNA and cell-associated HIV DNA do not change with vaccination

At baseline, two of 13 vaccinees had plasma HIV RNA greater than 20 copies/ml as compared with four of 13 controls (Supplemental Fig. 3A and B, <http://links.lww.com/QAD/A952>). Plasma HIV RNA did not increase significantly after any vaccine or placebo administration, and viral load detectability (>20 copies/ml) was not associated with higher *gag* mRNA levels ($P = 0.50$). Detection of 2-LTR circles, which may suggest active rounds of viral infection with abortive integration events [43], did not change significantly after any vaccine or placebo administration (Supplemental Fig. 3C, <http://links.lww.com/QAD/A952>). There were also no significant changes in total HIV DNA in either arm during the study period ($P = 0.89$; Supplemental Fig. 3D, <http://links.lww.com/QAD/A952>).

Vaccination and T-cell responses

The percentage of CD4⁺ T-cell counts did not change significantly in vaccinees ($P = 0.11$) or controls ($P = 0.42$) during follow-up (Supplemental Fig. 4, <http://links.lww.com/QAD/A952>). CD4⁺ cell counts at baseline did not correlate with the magnitude of HIV ca-RNA induction after influenza/Hep B vaccination ($P = 0.90$) or pneumococcus/Hep B vaccination ($P = 1.0$), nor did they correlate with average transcriptional effect per vaccine ($P = 0.17$).

As vaccination effects on the HIV reservoir may be mediated by cellular activation, we measured markers of T-cell activation at months 0, 3, 6, 9 and 12. There was a trend for an increasing proportion of CD4⁺CD38⁺HLA-DR⁺ cells over the course of the study in vaccinees ($P = 0.08$; Fig. 3a) but not controls ($P = 0.47$). However, there was no association between CD4⁺ T-cell activation and HIV ca-RNA levels ($P = 0.64$). The frequency of CD8⁺CD38⁺HLA-DR⁺ cells increased significantly in vaccinees ($P < 0.001$; Fig. 3b) but not controls, with the greatest increase between months 0 and 3 ($P = 0.005$) and months 9 and 12 ($P = 0.02$). There was a trend for increased CD8⁺ T-cell activation correlating with increased levels of HIV ca-RNA ($r 0.50$, $P = 0.09$). Curiously, expression of the T-cell proliferation marker Ki67 significantly increased in both CD4⁺ and CD8⁺ T cells between months 0 and 12 in both vaccinees ($P = 0.008$ and $P = 0.003$) and controls ($P = 0.02$ and $P = 0.001$). As memory T-cell proliferation is a part of the expected vaccine response, we looked for evidence of increased memory CD4⁺ and CD8⁺ T cells circulating in peripheral blood. Proportions of memory CD4⁺ T cells in vaccinees increased significantly between months 0

and 9 ($P < 0.001$) and then decreased between months 9 and 12 ($P = 0.07$). Similarly, memory CD8⁺ T cells in vaccinees increased in the first 9 months ($P = 0.006$) and decreased thereafter ($P = 0.03$).

Finally, we evaluated whether vaccination increased HIV transcription enough to generate an immune response. We found increases in HIV-specific responses in vaccinees after influenza/Hep B and pneumococcus/Hep B vaccinations. Specifically, HIV-specific CD8⁺ T-cell responses to p24 gag ($P = 0.04$), p17 gag ($P = 0.01$) and total gag ($P = 0.04$) significantly increased between months 0 and 3 of the study (Fig. 3c–e). These returned to baseline by month 6. Controls showed no change in HIV-specific CD8⁺ T-cell responses throughout the study.

Discussion

The ‘shock and kill’ method is a possible strategy to unmask and eradicate cells latently infected with HIV [2,5]. This strategy requires a reversal of latency sufficient to produce a measurable decrease in the HIV reservoir. No agent that can be used safely *in vivo* has thus far been able to achieve this benchmark. Standard vaccines may be an attractive option, as vaccines form an important part of routine clinical care for the HIV-infected individuals, are by their nature immune stimulatory and may be associated with transient increases in plasma HIV RNA. Their potential to activate the viral reservoir and their impact on overall viral burden during ART have yet to be fully explored. In this study, we measured HIV ca-RNA as a measure of HIV transcription in participants receiving standard vaccines or placebo injections. We found significant increases in HIV transcription after the administration of influenza/Hep B and pneumococcus/Hep B vaccinations, with no changes in the control group. Furthermore, vaccinees showed increased levels of CD4⁺ and CD8⁺ activation, as well as enhanced HIV-specific responses after vaccination, whereas controls showed no measurable changes.

Our results suggest that certain standard vaccines may be capable of inducing HIV transcription in the setting of ART. We postulate that the most likely mechanism is through a vaccine-elicited generalized inflammatory response with cytokine production [44,45] that in turn leads to activation of bystander cells (including those harboring latent HIV), rather than arising solely from the activation of HIV-infected vaccine-specific T cells. It remains unclear why effects would be seen after some vaccines and not others, but several factors could account for this observation. First, different vaccines vary in their adjuvant components, mechanism of action and overall immunogenicity, which could affect both the magnitude and kinetics of HIV reactivation [23]. Second, previous exposure to antigen may contribute. Although most participants in this study had evidence of previous exposure to varicella, measles, rubella, tetanus and diphtheria, the largest effects were associated with pneumococcus, for which six of eight participants sampled were seronegative at baseline, and seasonal influenza, against which most or all participants would have been immune-naïve. These findings recapitulate previous studies that demonstrated greater increases in plasma HIV RNA among individuals who mounted successful immune responses to vaccines [21,24,26]. Finally, the timing of the vaccines may be important, as significant HIV transcription was seen after the first two vaccines administered but not after subsequent

vaccines. There was also a gradual increase in median HIV ca-RNA levels in vaccinees throughout the study that was not seen in controls, suggesting that each subsequent vaccination may have contributed incrementally, if not significantly, to transcription activation.

Despite previous evidence that vaccines are associated with significant increases in plasma HIV RNA, we found no discernable differences. The main caveat to this observation was that plasma HIV RNA measures were taken 30 days after each injection, and several previous studies demonstrated a return of levels to baseline within 4 weeks of vaccination [21,24,26]. The seemingly transient kinetics of RNA relative to that of ca-RNA could reflect the intrinsic sensitivities of the three measures: transcripts are produced in greater abundance than virions, not all transcripts are translated and packaged into virions and defective proviruses may be capable of transcription but not virion production. It is also possible that ca-RNA increases may reflect replication by cell-to-cell transfer without production of detectable free virus.

The differences in behaviors of the three transcripts measured (*gag* usRNA, *polyA* mRNA and *tat-rev* msRNA) are interesting and reflect an area of uncertainty in the field of HIV latency. In this study, we found that although levels of all three transcripts were significantly correlated, detectability varied greatly amongst the transcripts with *gag* usRNA being the most consistently detectable transcript, followed by *polyA* mRNA and lastly *tat-rev* msRNA. In-vivo studies of different transcripts have shown similar findings [15,46–48]. Multiple mechanisms have been postulated to explain these differences, including differences in relative abundance of transcripts, preferential production of different transcripts depending on stage of the HIV replication cycle [49], presence of read-through transcripts that falsely elevate detection of conventional *gag* usRNA assays (although levels of read-through transcripts have been found to be modest in relation to processive transcripts [48,50]), transcription of defective virions that can produce usRNA but not msRNA or *polyA* mRNA [51] and cross-reactivity of usRNA with packaged virion RNA (although this comprises a negligible proportion total usRNA [46,48]). Newer data suggest that transcriptional interference may also contribute to differential transcript expression, with cellular activation selectively increasing unspliced and spliced transcripts but not read-through or total transcripts, and blocks to elongation, completion and splicing leading to higher levels of early transcripts (such as *gag* usRNA here), as compared with msRNA [50]. Ultimately, there is no consensus over which transcript serves as the most accurate surrogate marker of viral replication and therefore of true latency reversal.

There is also no consensus over the extent of latency reversal that needs to be achieved to produce the desired effects of enhancing HIV-specific immune function and producing a measurable decrease in the size of the HIV reservoir. A critical question raised is whether induction of HIV transcription alone could be sufficient to lead to immune clearance of infected cells. Multiple mechanistically distinct compounds that function at different levels of HIV transcriptional control can achieve a form of ‘upstream’ latency reversal [1], but a growing body of evidence suggests that these compounds face posttranscriptional blocks that could prevent translation of viral proteins in resting CD4⁺ T cells [17]. One study suggested that releasing transcriptional blocks in quiescent cells results in the predominance of read-

through transcripts [15], but this would not account for the significant increase in correctly processed transcripts (*polyA* mRNA) seen here and in other studies after LRA administration [16]. Additional mechanisms that could block virion production include impaired nuclear export of viral mRNAs [52], inefficient RNA splicing [53] and microRNA-mediated translational silencing [54]. Regardless, there is evidence that a large proportion of proviruses, including those in resting cells or with mutations in *tat*, are capable of expressing viral proteins [48,53], and protein expression alone may be sufficient to generate HIV-specific immune responses [55].

Another consideration is the ability to clear infected cells. Shan *et al.* [18] elegantly demonstrated that productive infection of resting CD4⁺ T cells did not cause cell death through either viral cytopathic effects or cytotoxic T lymphocyte (CTL) killing, whereas cells that were in an activated state rapidly died after viral reactivation. Also, certain LRAs have demonstrated functional inhibition of CTLs that could further impair killing of infected cells even if antigen presentation were achieved [56]. These factors could account for the lack of correlation between LRA activity and directed immune responses seen in various clinical trials [8–11]. Encouragingly, our study found significant increases in HIV-specific cellular responses in vaccinees between months 0 and 3 of the study, which was also the period in which the most significant increases in HIV ca-RNA were noted. This could reflect HIV-antigen stimulation, potentiated by the T-cell activating effects of vaccination, driving expansion and/or restoration of function of HIV-specific CTLs. Acting together, these mechanisms could improve elimination of infected cells after HIV reservoir perturbation with vaccines. Therapeutic HIV vaccines have shown similar promise in eliciting HIV-specific immune responses and thereby enhancing clearance of infected cells. One clinical trial combining the HIV vaccine Vacc-4x zpromising results in reducing the size of the latent viral reservoir [57]. We propose that the known safety profile and immune stimulatory effects of standard vaccines could make them ideal candidates for use in combination with ‘shock and kill’ strategies.

Limitations in our study include small sample size, single sampling time points after each vaccine and suboptimal sampling timing based on previous study findings. Multiple vaccines were administered at certain time points, making it difficult to evaluate the effect of single vaccines. Samples at certain time points were unavailable due to the retrospective design of the study. Most notably samples from month 0 (study baseline) were not available for analysis, which prevented comparisons of two prevaccination time points. As a result, the earliest available time point (month 1) was after the first administration of the Hep B vaccine (delivered at month 0). Our analysis of HIV ca-RNA therefore considered every vaccine (or combination of vaccines, if several were administered together) separately, comparing only prevaccination and postvaccination values. The retrospective design of the study further meant that samples were subjected to cryopreservation and thawing prior to analysis, although presumably the effects would be comparable in both placebo and vaccination arms. Finally, HIV DNA was used as a surrogate marker of the viral reservoir. This may not be the most sensitive marker of reservoir size given the large proportion of defective virus that constitutes the total HIV DNA population [51]. It would be worthwhile to determine whether vaccination is associated with changes in the replication-competent viral reservoir as measured by the viral outgrowth assay. Nonetheless, our findings are encouraging in that

the level of induced HIV RNA transcription is similar to that seen with leading candidate LRAs [8–11], with the added benefit of potentiating HIV-specific immune responses. As demonstrated elsewhere, effective HIV eradication strategies will likely require a combination of stimulatory agents to achieve a sufficiently robust latency reversal [15].

In conclusion, we demonstrated that the administration of standard vaccines is associated with measurable increases in HIV ca-RNA in the context of successful ART. Vaccination is also associated with increases in overall T-cell activation and HIV-specific CTL function. Taken together, we conclude that immune stimulatory methods might be a useful adjuvant as part of a combination or tiered approach to HIV-1 eradication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Diagrammatic representation of vaccination schedule

Red bar indicates study duration with study visits labeled (months 0–12). Syringes denote vaccination time points; text below describes vaccines delivered at each time point. Hep B, hepatitis B; MMR, measles–mumps–rubella; TD, tetanus–diphtheria; VZV, Varicella zoster virus.

Fig. 2. Changes in HIV cell-associated RNA with vaccination

(a–c) Changes in *gag usRNA* at time points corresponding to vaccine or placebo administration: vaccinees after influenza/hepatitis B vaccination (a), controls at matched time points to influenza/hepatitis B vaccination (b) and vaccinees after pneumococcus/hepatitis B vaccination (c). Filled circles represent individual participants. Different colors are assigned to each vaccinee, with color-coding preserved throughout. Controls are denoted by blue filled circles. P values by Wilcoxon test. (d) Median *gag usRNA* levels with interquartile ranges of vaccinees (red line and bars) and controls (blue line and bars) throughout study period. Vaccinee and control data for each month coincides chronologically but are layered so as to display medians and ranges without overlap. Black arrows denote vaccination time points. (e) Average effect (on HIV transcription) per vaccine (or placebo). Regression coefficient with interquartile range represented by box plots, P value by Mann–Whitney test.

Fig. 3. T-cell responses with vaccination

(a and b) Percentage of CD4⁺CD38⁺HLA-DR⁺ T cells (a) and CD8⁺CD38⁺HLA-DR⁺ T cells (b) in vaccinees (red filled circles) and controls (blue filled circles). P values by Wilcoxon test for within-group comparisons (vaccinees only and controls only) and Mann-Whitney test for intergroup comparisons (vaccinees versus controls); only P values less than 0.1 are shown. (c–e) Changes in CD8⁺ T-cell IFN- γ production in vaccinees as measured by enzyme-linked immunospot (spot-forming units) in response to stimulation with p24 gag (c), p17 gag (d) and total gag (e) between study months 0 and 3. P values by Wilcoxon test. Color-coding is preserved throughout and with respect to Fig. 2. (f–h) Changes in CD8⁺ T-

cell IFN- γ production in response to p24 gag (f), p17 gag (g) and total gag (h) between study months 0 and 3 in controls.

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Table 1

Baseline characteristics of study participants.

	Vaccinees (<i>n</i> = 13)	Controls (<i>n</i> = 13)	<i>P</i> value ^a
Age [years, median (IQR)]	38 (29–41)	40 (38–52)	0.06
Men, <i>n</i> (%)	11 (85)	10 (77)	1.00
Risk factor, <i>n</i> (%)			0.16
Homosexual	9 (69)	5 (38)	
Heterosexual	4 (31)	5 (38)	
IVDU ^b	0	3 (23)	
Estimated duration of HIV-1 infection [years, median (IQR)]	4.6 (2.1–7.9)	6.6 (3.3–11.0)	0.60
Time on ART [years, median (IQR)]	2.4 (1.2–4.6)	4.5 (1.6–6.3)	0.11
ART			0.11
NNRTI-based regimen, <i>n</i> (%)	3 (31)	7 (54)	
PI-based regimen, <i>n</i> (%)	10 (69)	5 (38)	
Other regimen, <i>n</i> (%)	0	1 (8)	
Nadir CD4 ⁺ T-cell count [cells/μl, median (IQR)]	414 (373–514)	411 (384–530)	0.74
Absolute CD4 ⁺ T-cell count at month 0 [cells/μl, median (IQR)]	987 (767–1072)	898 (712–1073)	0.57
Plasma viral load at month 0 [log ₁₀ copies/ml, median (IQR)]	1.28 (1.28–1.28)	1.28 (1.28–1.4)	0.45

ART, antiretroviral therapy; IQR, interquartile range; NNRTI, non-nucleoside reverse transcriptase inhibitor. PI, protease inhibitor.

^a*P*value of Mann–Whitney test for continuous variables and Fisher's exact test for categorical variables.^bIVDU; intravenous drug use.

Table 2

Fold-change increases in HIV transcripts after different vaccinations.

		Participants (n)	Median fold-change (gag)	P value	Median fold-change (polY)	P value
Vaccinees	Influenza/Hep B	12	2.4	0.02	3.8	0.56
	Pneumococcus/Hep B	8	7.0	0.04	20.9	0.04
	VZV/Hep A	10	1.6	0.06	1.7	0.13
	VZV/Hep B	8	0.6	0.38	1.3	0.74
	MMR	12	1.1	0.97	0.2	0.43
	TD	9	1.3	0.50	2.1	0.05
Controls	Influenza/Hep B	11	1.7	0.12	2	0.09
	Pneumococcus/Hep B	1	8.0	N/A	4.4	N/A
	VZV/Hep A	6	0.6	0.44	0.7	0.56
	VZV/Hep B	4	0.2	0.63	0.5	0.13
	MMR	9	0.8	0.38	1.4	0.08
	TD	11	1	0.70	0.3	0.46

Hep A, hepatitis a; Hep B, hepatitis B; MMR, measles-mumps-rubella; TD, tetanus-diphtheria; VZV, Varicella zoster virus.