Effect of SARS-CoV-2 vaccine booster on HIV reservoirs and immune markers

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Abstract

We investigated effects of the SARV-CoV-2 booster vaccination on HIV reservoir size, immune markers, and host immune responses in PWH receiving ART. Our data suggest that the SARS-CoV-2 booster vaccine is not likely to replenish the persistent HIV reservoir nor provide an immunologic environment to facilitate active HIV expression/replication.

Keywords: HIV, SARS-CoV-2, booster vaccine, HIV reservoirs, immune markers and responses.

INTRODUCTION

The clinical outcomes of coronavirus diseases 2019 (COVID-19) in people with HIV (PWH) are variable. Early data involving small sample sizes showed no increased risk [1], but a recent meta-analysis demonstrated an increased risk of COVID-19 mortality in PWH [2]. Nonetheless, administration of SARS-CoV-2 vaccines elicits strong immunogenicity in PWH with minimal adverse effects [3]. Despite both HIV and SARS-CoV-2 being positive-sense single stranded RNA viruses, one major difference between these two viruses is that most individuals infected with SARS-CoV-2 are able to clear the virus whereas HIV persists in PWH despite years of antiretroviral therapy (ART) that allows near complete virologic suppression and control. In this regard, the persistent HIV reservoir in the $CD4^+$ T cell compartment of the vast majority of PWH receiving ART) is one of the major impediments to the eradication of the virus [4]. Previous studies have shown that vaccination against common pathogens, such as influenza, may modulate immunologic and virologic parameters in PWH receiving ART [5]. Given that one of the proposed mechanisms of HIV persistence is antigen-mediated clonal expansion and homeostatic proliferation of an existing pool of $CD4^+$ T cells carrying intact HIV provirus, it is of interest to investigate whether the SARV-CoV-2 booster vaccination modulates immunologic and virologic parameters in PWH receiving ART.

In the present study, we set out to investigate the effects of the SARV-CoV-2 booster vaccine on HIV reservoir size, immune markers, and host immune responses to HIV and SARS-CoV-2 in a cohort of HIV positive individuals receiving ART following the SARS-CoV-2 booster vaccination.

METHODS

Study Participants

Our study cohort comprised of nine PWH who had previously received a two-dose series of either the Moderna or Pfizer-BioNTech mRNA-based SARS-CoV-2 vaccines. Subsequently, the study participants received their homologous booster dose between November 2021 to January 2022 and had blood drawn 2-4 weeks prior and 10-14 days post vaccination. Given the booster was administered in the same interval to all 9 study participants, we chose this time frame to study the effect of the SARS-CoV-2 vaccination on HIV reservoirs and immune parameters.

Patient Consent Statement

All participants provided written informed consent. Blood was collected in accordance with protocols approved by the Institutional Review Board of the National Institutes of Health.

Measurements of antibody response

Plasma levels of total immunoglobulin (Ig) against SARS-Co-V2 were determined using the ProcartaPlex Human Coronavirus Ig Total Panel 11-Plex assay (ThermoFisher Scientific) and the xMAP INTELLIFLEX system (Luminex) according to the manufacturers' instructions.

Quantitation of HIV reservoirs

The dynamics of viral reservoirs carrying total HIV DNA, intact HIV proviral DNA, and cell-associated HIV RNA in the $CD4^+$ T cells of study participants was assessed as described in Supplementary Data.

Examination of Immune Parameters

Peripheral blood mononuclear cells (PBMCs) were isolated from blood by Ficoll-Hypaque density gradient centrifugation. Expression of surface markers for immune activation and exhaustion was assessed by flow cytometry (Supplementary Data). Data were acquired on an Aurora cytometer using the SpectroFlo software (Cytek Biosciences) and analyzed using FlowJo version 10.7.1.

Immune responses to HIV and SARS-CoV-2

Frequencies of polyfunctional (IFN-γ^+TNF-α^+MIP-1ß$^+$) HIV Gag-specific CD8$^+$ T cells were determined by the intracellular cytokine staining assay (see Supplementary Data). Levels of SARS-CoV-2-specific CD4$^+$ T cells were determined by the Activation Induced Marker (AIM) assay (see Supplementary Data). Data were acquired on an Aurora cytometer using the SpectroFlo Software (Cytek Biosciences) and analyzed using FlowJo version 10.7.1.

Statistical Analysis

Statistical significance was determined by the two-sided Wilcoxon matched-pairs signed-rank test using Prism 9.3.1 (GraphPad).

Results

We examined the impact of the SARS-CoV-2 booster vaccine on HIV reservoirs and immune parameters. Six participants received Moderna mRNA-1273 and three participants received Pfizer/BioNTech BNT162b2 COVID-19 vaccines for their 1st and 2nd doses and booster

shot (Table 1 and Figure 1A). Of note, one study participant (07) had detectable plasma viremia (108 copies/ml) prior to receiving the booster shot (Table 1) despite sustained virologic suppression (<40 copies/ml) prior to participating in the current study.

We first assessed antibody responses (Ig) to the SARS-CoV-2 booster vaccination in the study participants. As expected, the level of plasma antibodies to the nucleocapsid protein did not change following the booster vaccine ($P = 0.65$). However, the levels of plasma antibodies to the receptor-binding domain (RBD), S1 subunit, and Spike protein increased significantly ($P = 0.003$) following the administration of the booster vaccine (Figure 1B).

Previous studies have demonstrated that routine vaccination against common pathogens can modulate HIV reservoirs and phenotypic immune markers [5]. To this end, we evaluated the effect of the SARS-CoV-2 booster vaccine on the frequency of $CD4^+$ T cells carrying total HIV DNA, intact HIV proviral DNA, and cell-associated HIV RNA. As shown in Fig. 1C, no significant differences were found in the levels of these three viral parameters 10-14 days following the booster vaccination.

To investigate the impact of the booster vaccine on immune parameters, we performed high-dimensional flow cytometric analyses on T cells of the study participants. Intensities and frequencies of TIGIT, PD-1, CD226 and CD38/HLA-DR on $CD8^+$ T cells remained unchanged following the booster vaccination (Figure 1D).

Finally, we evaluated T cell responses to HIV and SARS-CoV-2 in the study participants following the booster vaccination. Frequencies of polyfunctional (IFN-γ^+TNF-α^+MIP-1ß$^+$) HIV Gag-specific $CD8^+$ T cells remained unchanged between pre- and post-boost timepoints (Figure 1E). We performed an activation induced marker (AIM) assay to measure SARS-CoV-2 Spike-specific $CD4^+$ and $CD8^+$ T cells following the booster vaccination (Figure 1F). No significant

differences were found in the frequencies of the total Spike-specific $CD4^+$ ($P = 0.82$) and $CD8^+$ T cells ($P > 0.99$); however, Spike-specific circulating $CD4^+$ T follicular helper (cTfh) cells declined following the booster vaccination ($P = 0.004$).

DISCUSSION

The persistence of HIV in the $CD4^+$ T cells of PWH receiving ART is a formidable obstacle to the eradication of the virus and/or achieving sustained virologic remission in the absence of antiretroviral drugs [4]. Although precise mechanisms of HIV persistence remain to be fully delineated, it has been shown that antigenic stimulation that leads to clonal expansion of a pre-existing pool of $CD4^+$ T cells carrying intact HIV proviral DNA could be responsible for its longevity in PWH despite years of clinically effective ART. In this regard, it has been demonstrated that routine vaccination against common pathogens, such as influenza, could modulate the degree and extent of viral expression/production in HIV-infected $CD4^+$ T cells in vivo [5]. Recent studies on SARS-CoV-2 outcomes in PWH have shown variable findings, possibly due to multiple factors including age, race, $CD4^+$ T cell counts, antiretroviral drug regimens, and vaccination status [1, 6, 7]. Nonetheless, SARS-CoV-2 vaccination in PWH has been shown to be safe and effective [8, 9]. Given that the mRNA-based SARS-CoV-2 vaccines induce robust immune responses [3], we set out to investigate whether these vaccines could alter the dynamics of immunologic and virologic parameters in PWH who are receiving ART. Given that the booster vaccine was administered in the same interval to all nine study participants, we chose this time frame to study the effects of the SARS-CoV-2 vaccination on immune and HIV reservoirs.

The antibody response to the booster vaccine was robust in all but one study participant. Of note, Participant 2, whose antibody responses remained largely unchanged following the booster vaccination, had been previously diagnosed with SARS-CoV-2 and had higher initial antibody levels prior to the vaccination. A more modest antibody response to SARS-CoV-2 booster vaccination in people previously infected with SARS-CoV-2 is also consistent with recent findings [10]. In contrast to the antibody response and reports of HIV-uninfected individuals [11], we did not observe a booster vaccine-induced increase in SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells. Levels of T cell surface markers and HIV-specific $CD8^+$ T cells also remained unchanged following the booster vaccination. However, the frequency of SARS-CoV-2 Spike-specific $CD4^+$ cTfh cells declined post-boost. The explanation for the lack of or reduced T cell responses to the booster vaccine is not clear and needs to be further investigated. Previous reports of two doses of SARS-CoV-2 mRNA vaccines in PWH have been shown to induce Spike-specific T cell responses [12], thus it may be that repeated SARS-CoV-2 vaccination may have contributed to the unresponsiveness of these cells. Considering the relatively mild T cell responses to SARS-CoV-2 following the booster vaccination, it is not surprising that the overall size of persistent HIV reservoir did not change over time in our study participants.

One of the major caveats of this study was that we could not perform HIV reservoir and immunologic analyses following the first and second doses of the SARS-CoV-2 vaccination. We were unable to bring study participants to our clinic due to the pandemic-associated restrictions imposed by the National Institutes of Health. Other caveats include a small sample size, again in part associated with pandemic restrictions, and the lack of female participants in our study. Despite these shortcomings, our data suggest that the SARS-CoV-2 booster vaccine is not likely

to replenish the persistent HIV reservoir nor provide an immunologic environment that may facilitate active HIV expression/replication in PWH receiving ART.

FOOTNOTES

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Potential conflicts of interest. All authors declare no competing financial interests.

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Figure legend

Figure 1. Dynamics of immunologic and virologic parameters in study participants prior to and 10-14 days following SARS-CoV-2 booster vaccination. *A.* Study schema. *B.* Comparison of antibody titers against SARS-CoV-2 nucleocapsid protein, receptor-binding domain (RBD), S1 subunit, and Spike protein. *C.* Levels of total HIV DNA, intact HIV proviral DNA, and cell-associated HIV RNA prior to and following SARS-CoV-2 booster vaccination. *D.* Frequencies of TIGIT, PD-1, CD226, and CD38HLA-DR on $CD8^+$ T cells prior to and following SARS-CoV-2 booster vaccination. *E.* Effect of the SARS-CoV-2 booster vaccination on the frequency of IFN-γ^+TNF-α^+MIP-1ß$^+$ HIV Gag-specific $CD8^+$ T cells in study participants. *F.* Frequencies of spike-specific $CD4^+$ and $CD8^+$ T cells (left panel) and spike-specfic $CD4^+$ cTfh cells (right panel) prior to and following SARS-CoV-2 booster vaccination.

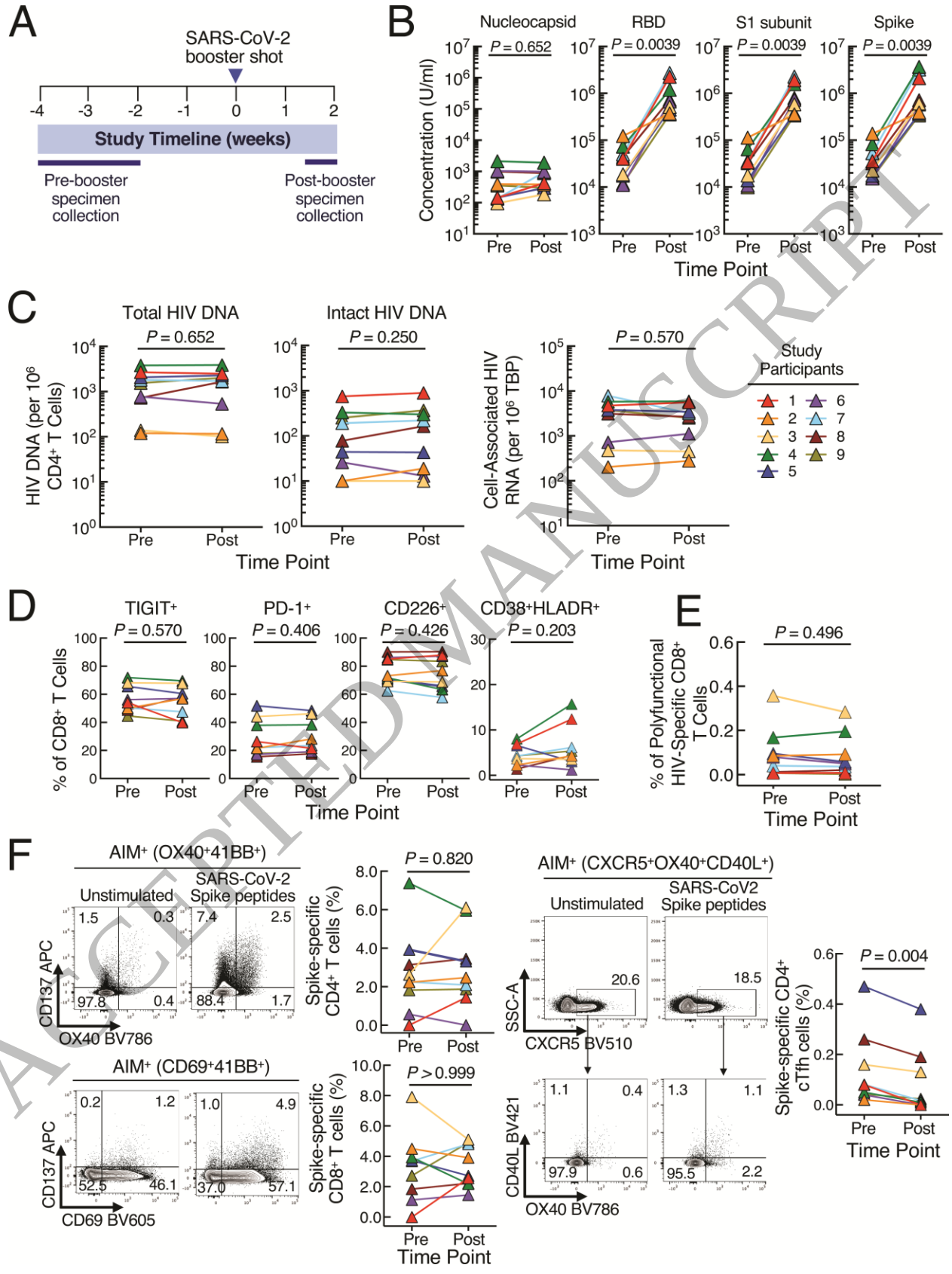

Table 1. Characteristics of study participants

Participant	Age	Gender	Race	SARS-CoV-2 Vaccine	Plasma Viremia (copies/mL)		$CD4^+$ T cell count ($cells/mm^3$)	
					Pre-Boost	Post-boost	Pre-Boost	Post-boost
1	55	Male	White	Moderna	<40	<40	860	800
2	27	Female	Hispanic	Moderna	<40	<40	1,526	1,323
3	43	Male	Hispanic	Moderna	<40	<40	NA	949
4	40	Male	Hispanic	Pfizer-BioNTech	<40	<40	NA	597
5	49	Male	Hispanic	Pfizer-BioNTech	<40	<40	443	655
6	53	Male	Black	Moderna	<40	<40	503	576
7	49	Male	Hispanic	Moderna	108	<40	617	902
8	34	Male	Hispanic	Moderna	<40	<40	852	785
9	31	Male	Mixed	Pfizer-BioNTech	<40	<40	307	383

NA: Not available