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### Title

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### Permalink

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### Journal

AIDS

### ISSN

0269-9370

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### Publication Date

2026-07-07

### DOI

10.1097/qad.0000000000004580

Peer reviewed

**Impact of Influenza and Pneumococcal Vaccines on Inflammatory Response During  
Suppressive Antiretroviral Therapy**

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Subtitle/Running head: Vaccination and Inflammation During ART

Disclaimer: The contributions of the NIH authors were made as part of their official duties as NIH federal employees, follow agency policy requirements, and are considered Works of the United States Government. However, the findings and conclusions presented in this paper are those of the authors and do not necessarily reflect the views of the NIH or the U.S. Department of Health and Human Services.

Sources of Funding: Department of Veterans Affairs, the James B. Pendleton Charitable Trust, Translational Virology Core at the San Diego Center for AIDS Research (P30 AI036214), HOPE T32 Training (AI007384) and NIH grant AI118422.

Conflicts of interest: Davey Smith consulted for Model Medicines, Gilead Sciences Inc, Pfizer, Hyundai Biosciences, and Palisades. Susan J. Little receives funding paid to her institution by Gilead Sciences Inc. All other authors declare no conflicts of interest.

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## ABSTRACT

**Objective:** We performed a secondary analysis of a randomized clinical trial that evaluated stimulation of HIV reservoir through routine vaccines (NCT02707692) to characterize vaccine-induced inflammatory responses and their association with HIV virologic measures following influenza and pneumococcal vaccination in people with HIV (PWH) on suppressive antiretroviral therapy (ART).

**Design:** Prospective, randomized, double-blinded, placebo-controlled, crossover trial evaluating influenza and pneumococcal vaccines.

**Methods:** 54 PWH on ART were enrolled. Blood was collected at baseline and days 2,4,7,14, and 30 post-vaccinations. Of 41 cytokines quantified by Luminex, 19 with >30% detectable values post-baseline were analyzed. Cellular HIV RNA and DNA were measured by ddPCR. Analyses included Partial Least Squares Discriminant Analysis (PLSDA) and linear mixed-effects models.

**Results:** 53 participants contributed  $\geq 1$  post-vaccination visit. We observed no distinct cytokine profile by vaccine type or placebo. Pneumococcal vaccination induced transient increases in LBP (0.231; 95% CI: [0.126, 0.336];  $p=0.0003$ ) and CRP (0.643; 95% CI: [0.482, 0.804];  $p<0.0001$ ), while influenza vaccination was associated with a modest decrease in RANTES ( $-0.116$ ; 95% CI: [ $-0.188$ ,  $-0.043$ ];  $p=0.0218$ ) compared with placebo. Independent of vaccine type, HIV DNA Gag levels were inversely associated with IL-16 and RANTES.

**Conclusions:** In PWH on suppressive ART, influenza and pneumococcal vaccination elicited mild and transient changes in individual cytokines, though distinct global cytokine signatures were not observed. This finding supports vaccine safety in PWH, showing no broad inflammatory perturbations. Independent of vaccine type, specific cytokines (IL-16 and RANTES) were associated with HIV reservoir measures, suggesting potential roles in reservoir dynamics and immune activation.

**Keywords:** immune response, HIV reservoir, influenza vaccine, pneumococcal vaccine

## INTRODUCTION

In people with HIV (PWH), antiretroviral therapy (ART) reconstitutes immune function by increasing CD4<sup>+</sup> T cell counts, but it does not eliminate latent HIV reservoir.<sup>1,2</sup> One proposed strategy to eliminate the HIV reservoir has been to induce HIV transcription from latently infected cells ("shock and kill").<sup>3</sup> This approach has faced practical limitations in efficacy, safety and implementation.<sup>4</sup>

Vaccine-induced inflammatory response could activate cellular HIV transcription through non-specific bystander stimulation.<sup>5-7</sup> Yet, in our prior randomized trial evaluating influenza and pneumococcal vaccines, we found no meaningful increase in cellular HIV RNA compared to placebo,<sup>8</sup> suggesting minimal reservoir perturbation. Interestingly, we observed an increase in TIGIT<sup>+</sup>PD-1<sup>+</sup> and CD38<sup>+</sup>HLA-DR<sup>+</sup>PD-1<sup>+</sup> T cells following pneumococcal vaccination,<sup>8</sup> consistent with transient T-cell activation.<sup>9</sup> In parallel, we detected a potential transient increase in HIV DNA following influenza

vaccination compared with placebo, despite both vaccines being inactivated formulations.<sup>8</sup> These findings, and prior studies in individuals without HIV, suggest that cytokine responses may differ by vaccine type.<sup>10-16</sup>

Inflammatory cytokines are associated with HIV reservoir size and immune activation in PWH on ART.<sup>17-20</sup> However, less is known of the dynamic changes of these soluble markers during suppressive ART or how they relate to reservoir measurements following immune stimulation.<sup>7,21</sup>

Here, we report a secondary analysis of our randomized, placebo-controlled crossover trial to characterize cytokine patterns following influenza or pneumococcal vaccination and to evaluate associations between cytokine levels and HIV virologic measures in PWH on suppressive ART.

## METHODS

**Study Design and Sampling:** This analysis utilizes data from a prospective, placebo controlled, randomized crossover clinical trial (NCT02707692) evaluating two clinically indicated vaccines (influenza and pneumococcal) as potential immune stimulatory interventions for perturbing the HIV reservoir. A detailed protocol was previously published.<sup>8</sup>

**Cytokine quantification:** A panel of 41 cytokines was quantified in replicate in plasma at baseline and on days 2, 4, 7, 14 and 30 post baseline vaccination using a Luminex assay. Nineteen cytokines with >30% detectability were included in the analyses. See Supplementary Table 1, Supplemental Digital Content, <http://links.lww.com/QAD/D935> for additional information.

**Cellular HIV DNA and HIV RNA measurements:** Nucleic acid was extracted from peripheral blood mononuclear cells, and cellular HIV DNA (total and 2-LTR) and RNA (unspliced and multiply spliced encoding for TatRev) were quantified by ddPCR.<sup>8</sup>

**Statistical approach:** Values below the lower limit of detection (LLOD) were imputed using a left-censored parametric survival model assuming a left-censored log-normal distribution at the replicate level.<sup>22</sup> Ten imputed datasets ( $m = 10$ ) were generated for cytokine analyses.

To evaluate global cytokine patterns, we restricted to participants with  $\geq 1$  post-vaccination visits and used Partial Least Squares Discriminant Analysis (PLS-DA) with change-from-baseline values across influenza, pneumococcal, and placebo conditions. We used a single imputation ( $m = 1$ ) and the replicate mean for PLS-DA.<sup>24</sup>

To examine differences in individual cytokines, we restricted to participants with  $\geq 2$  post-vaccination visits and used linear mixed-effects models including a random intercept for each participant and fixed effects for vaccine type, continuous time since vaccination, and baseline cytokine level. Interaction terms were included when time effects were significant ( $\alpha = 0.10$ ). Multiple imputation was applied ( $m = 10$ ), all replicates were used, and estimates were pooled using Rubin's rules.<sup>25</sup> Models were corrected for multiple comparisons using the False Discovery Rate (FDR).

Associations between cytokines and virologic outcomes were examined using similar mixed-effects models independent of vaccination type. For each cycle, we used baseline HIV reservoir measures and mean baseline cytokine values. Because as many as 31% of HIV RNA and DNA measurements fell below LLOD and were not normally distributed, we log transformed HIV DNA Gag, HIV DNA 2-LTR, HIV RNA Gag, and HIV RNA TatRev. Measurements below the limit of detection were coded as 0. A constant of 1 was subsequently added to all values before log-transformation. Further methodological details are available in Supplementary Table 2, Supplemental Digital Content, <http://links.lww.com/QAD/D935>. Statistical significance for main-effect testing was defined as  $\alpha = 0.05$ .

## RESULTS

**Cohort description:** Of 54 participants, 35 completed all three vaccine/placebo cycles, 47 completed  $\geq 1$  cycle with  $\geq 2$  post-vaccination visits, and 53 had  $\geq 1$  post-vaccination visit. Participants were mostly men (83%), Hispanic (50%), and non-Hispanic White (35%) and ranged in age from 24 to 65 with an average age of 45 years. See Supplementary Table 3, Supplemental Digital Content, <http://links.lww.com/QAD/D935> for additional information.

**Global cytokine patterns following vaccination:** Fifty-three participants contributed at least one post-vaccination visit to the PLS-DA analysis. The model did not reliably classify vaccination type, with balanced error rates  $>60\%$  for the first three components, indicating no global cytokine signature distinguishing influenza, pneumococcal, or placebo.

**Effect of vaccine administration on individual cytokines:** In exploratory analyses including 53 participants and 648 follow-up measurements, pneumococcal vaccination was associated with transient increases in Lipopolysaccharide-Binding Protein (LBP) (0.231, 95% CFI: [0.126, 0.336],  $p = 0.0003$ ) and C-Reactive Protein (CRP) (0.643, 95% CFI: [0.482, 0.804],  $p = <0.0001$ ) compared with placebo. Both markers declined over time (LBP:  $-0.013/\text{day}$ ; CRP:  $-0.035/\text{day}$ ;  $p < 0.001$ ). Additionally, influenza vaccination was associated with a small but statistically significant decrease in RANTES ( $-0.116$ ; 95% CI:  $[-0.188, -0.043]$ ,  $p = 0.0218$ ) relative to placebo. See Table 1 for LBP, CRP and RANTES levels post vaccination. See Supplementary Table 4, Supplemental Digital Content, <http://links.lww.com/QAD/D935> for additional cytokines levels post vaccination.

**Cytokine levels and their impact on HIV virologic outcomes:** As shown in **Table 2**, across 54 participants and 138 time points, HIV DNA Gag levels were inversely associated with IL-16 ( $-0.658$ ; 95% CI:  $[-1.054, -0.261]$ ,  $p = 0.0126$ ) and RANTES ( $-0.506$ ; 95% CI:  $[-0.779, -0.233]$ ,  $p = 0.0067$ ), independent of vaccine type. No consistent associations were observed for HIV RNA measures. See Supplementary Table 5-8, Supplemental Digital Content, <http://links.lww.com/QAD/D935> for additional information.

## DISCUSSION

In this secondary analysis of a randomized crossover trial, influenza and pneumococcal vaccination in PWH on suppressive ART induced mild and transient changes in select inflammatory markers without

generating a distinct global cytokine signature. Pneumococcal vaccination was associated with short-lived increases in LBP and CRP, whereas influenza vaccination was associated with a modest reduction in RANTES. Importantly, these findings reinforce that routinely recommended inactivated vaccines are safe and do not induce broad or sustained inflammatory activation in PWH receiving effective ART—an observation of relevance in an era of increasing vaccine skepticism.

Although prior studies in individuals without HIV have demonstrated vaccine-specific transcriptional and cytokine responses,<sup>12,13,15,16,26,27</sup> we did not observe separable global cytokine profiles distinguishing influenza, pneumococcal, and placebo conditions. This may reflect the fact that chronic immune activation in treated HIV infection can attenuate acute cytokine shifts following vaccination.<sup>28,29</sup> In addition, the relatively constrained inflammatory potential of inactivated vaccine platforms may limit systemic perturbations detectable in plasma.<sup>30,31</sup> Moreover, age-related immune senescence may also influence vaccine responsiveness in PWH,<sup>32</sup> although our sample size did not permit age-stratified analyses.

While our primary analysis showed no meaningful increase in cellular HIV RNA following vaccination, we previously observed transient increases in activated T-cell subsets, expressing TIGIT<sup>+</sup>PD-1<sup>+</sup> and CD38<sup>+</sup>HLA-DR<sup>+</sup>, after pneumococcal vaccination, as well as a possible transient increase in total HIV DNA following influenza vaccination.<sup>8</sup> The present findings demonstrate that systemic cytokine changes were modest and not globally coordinated, suggesting that subtle cellular activation – rather than broad inflammatory cascades – may underlie any transient reservoir fluctuations. Collectively, these data provide mechanistic reassurance that routine inactivated vaccination does not induce sustained immune activation or major reservoir disruption in PWH on suppressive ART.

Independent of vaccine type, we observed inverse associations between total HIV DNA and IL-16 and RANTES. Although correlative, these findings are biologically plausible IL-16 is a natural ligand for CD4 and can induce intracellular signaling in CD4<sup>+</sup> T cells and macrophages.<sup>33-37</sup> Prior studies show that IL-16 engagement of CD4 may reduce CD4 surface expression and limit HIV transcriptional activity.<sup>35,36,38-40</sup> IL-16 signaling has also been associated with transient activation of CD4<sup>+</sup> T cells accompanied by inhibitory functional programs, potentially involving STAT6-dependent pathways.<sup>35,38</sup>

In parallel, RANTES (CCL5) can inhibit viral entry through CCR5 competition.<sup>41,42</sup> Consistent with this mechanism, IL-16 signaling has also been shown to modulate HIV co-receptor expression, including downregulation of CCR5,<sup>36</sup> suggesting a broader role in regulating receptor availability and viral entry.

Together, these observations raise the possibility that discrete immune regulatory pathways – rather than generalized systemic inflammation – may influence HIV reservoir stability during suppressive ART.

This study has several limitations. First, immune cell phenotyping was performed and reported in prior analyses;<sup>8</sup> integration of those datasets with the present cytokine-focused analysis was beyond the

scope of this secondary study. Accordingly, we cannot directly attribute circulating cytokine changes to specific immune cell subsets, particularly given that cytokines often induce highly cell-type-specific transcriptional responses. Second, total HIV DNA represents a broad surrogate of proviral burden and does not distinguish intact from defective proviruses. Third, many cytokine and virologic measurements fell below the lower limit of detection (LLOD). To address this, we used multiple imputation and mixed-effects modeling, which account for left censoring and within-participant correlation and are less biased than simple substitution approaches.<sup>23</sup> Cytokines with statistically significant associations (LBP, CRP, and RANTES) had complete observed values, mitigating concerns that imputation biased the primary findings. Fourth, cytokine variability is influenced by clinical and demographic factors,<sup>21</sup> and the predominantly male composition of the cohort limits generalizability, particularly given known sex-based differences in immune activation and vaccine responsiveness.<sup>43,44</sup> Additionally, both vaccines evaluated were inactivated formulations designed to minimize systemic inflammation.<sup>45,46</sup> The limited inflammatory signal observed may therefore reflect intrinsic platform characteristics rather than absence of immune responsiveness in PWH on suppressive ART. More immunogenic platforms or intensified vaccine regimens could potentially induce more robust perturbations, although safety considerations remain paramount.

Finally, this exploratory secondary analysis was not designed to test a shock-and-kill paradigm or to definitively characterize reservoir modulation. Rather, its primary contribution lies in empirically demonstrating that routine influenza and pneumococcal vaccination does not induce broad inflammatory activation in virologically suppressed PWH. In this context, the findings are confirmatory yet clinically meaningful. Future studies integrating immune phenotyping, transcriptional profiling, and reservoir-specific assays will be required to further delineate the interplay between immune activation and HIV persistence.

## ACKNOWLEDGEMENTS

This work was supported by the Department of Veterans Affairs, the James B. Pendleton Charitable Trust, Translational Virology Core at the San Diego Center for AIDS Research (P30 AI036214), HOPE T32 Training (AI007384) and grant from the National Institutes of Health: AI118422.

Renato Bobadilla performed analysis and wrote the primary version of the manuscript. Tara L. Tenenbaum performed the secondary analysis and wrote the primary version of the manuscript. Sara Gianella and Davey Smith conceptualized the study design, generated data, performed analysis and reviewed the manuscript. Christophe Vanpouille conceptualized the study design and performed analysis. Alan Wells conceptualized the study design, performed the secondary analysis, and reviewed the manuscript. Antoine Chaillon participated in conceptualizing the study design, performed the bioinformatics analysis and reviewed the manuscript. Leticia Muttera participated in conceptualizing the study design, administered study drug and reviewed the manuscript. Liliana Harkness, Joseph Lonergan, Susan J. Little participated in conceptualizing the study design, enrolled study participants and reviewed the manuscript.

## DISCLAIMER

The contributions of the NIH authors were made as part of their official duties as NIH federal employees, follow agency policy requirements, and are considered Works of the United States Government. However, the findings and conclusions presented in this paper are those of the authors and do not necessarily reflect the views of the NIH or the U.S. Department of Health and Human Services.

## CONFLICTS OF INTEREST

Davey Smith consulted for Model Medicines, Gilead Sciences Inc, Pfizer, Hyundai Biosciences, and Palisades. Susan J. Little receives funding paid to her institution by Gilead Sciences Inc. All other authors declare no conflicts of interest.

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**Table 1: Overall difference in mean cytokine levels (RANTES, LBP and CRP) post vaccination (N = 53 participants and 648 timepoints)**

|                                                      | RANTES                                  |        |                               | LBP                                     |         |                               | CRP                                     |         |                               |
|------------------------------------------------------|-----------------------------------------|--------|-------------------------------|-----------------------------------------|---------|-------------------------------|-----------------------------------------|---------|-------------------------------|
| Fixed effects                                        | Model coefficient (95% CI) <sup>a</sup> | P*     | Adjusted P (FDR) <sup>‡</sup> | Model coefficient (95% CI) <sup>a</sup> | P*      | Adjusted P (FDR) <sup>‡</sup> | Model coefficient (95% CI) <sup>a</sup> | P*      | Adjusted P (FDR) <sup>‡</sup> |
| Vaccines                                             |                                         |        |                               |                                         |         |                               |                                         |         |                               |
| Saline                                               | Reference                               |        |                               | Reference                               |         |                               | Reference                               |         |                               |
| Influenza                                            | -0.116 (-0.188 to -0.043)               | 0.0017 | 0.0218                        | 0.093 (-0.010 to 0.195)                 | 0.0768  | 0.2428                        | 0.116 (-0.041 to 0.274)                 | 0.1475  | 0.2803                        |
| Pneumococcal                                         | -0.071 (-0.146 to 0.004)                | 0.0623 | 0.2152                        | 0.231 (0.126 to 0.336)                  | <0.0001 | 0.0003                        | 0.643 (0.482 to 0.804)                  | <0.0001 | <0.0001                       |
| Level of cytokine on day of vaccination <sup>b</sup> | 0.350 (0.286 to 0.413)                  | 0.0000 |                               | 0.336 (0.286 to 0.386)                  | <0.0001 |                               | 0.438 (0.376 to 0.501)                  | <0.0001 |                               |
| Days from vaccine to visit                           | 0.001 (-0.002 to 0.004)                 | 0.5060 |                               | 0.003 (-0.002 to 0.007)                 | 0.2329  |                               | 0.003 (-0.004 to 0.010)                 | 0.4567  |                               |

|                                                                                |                             |   |   |                                   |        |  |                                   |         |  |
|--------------------------------------------------------------------------------|-----------------------------|---|---|-----------------------------------|--------|--|-----------------------------------|---------|--|
| <b>Vaccines *</b><br><b>Days from</b><br><b>vaccine to</b><br><b>visit</b>     |                             |   |   |                                   |        |  |                                   |         |  |
| <b>Saline *</b><br><b>Days from</b><br><b>vaccine to</b><br><b>visit</b>       | <b>Referenc</b><br><b>e</b> |   |   | <b>Referenc</b><br><b>e</b>       |        |  | <b>Referenc</b><br><b>e</b>       |         |  |
| <b>Influenza *</b><br><b>Days from</b><br><b>vaccine to</b><br><b>visit</b>    | -                           | - | - | -0.005 (-<br>0.012 to<br>0.002)   | 0.1443 |  | -0.009 (-<br>0.020 to<br>0.001)   | 0.0771  |  |
| <b>Pneumococcal *</b><br><b>Days from</b><br><b>vaccine to</b><br><b>visit</b> | -                           | - | - | -0.013 (-<br>0.020 to -<br>0.006) | 0.0003 |  | -0.035 (-<br>0.045 to -<br>0.024) | <0.0001 |  |

**Legend:** CI, confidence interval. \* Unadjusted *P* value. ‡ Adjusted *P* value for multiple comparisons across cytokines by False Discovery Rate. <sup>a</sup>Pooled coefficients estimated from multivariable, mixed-effects linear regression. Coefficients with Adjusted *P*<0.05 imply an overall difference in mean cytokine level compared to saline. <sup>b</sup>Corresponds to the day of vaccination for each cycle.

**Table 2: Association between HIV DNA Gag and cytokine level (IL-16 and RANTES) (N = 54 participants and 138 timepoints)**

|                                                        | IL-16                         |            |                                 | RANTES                        |            |                                 |
|--------------------------------------------------------|-------------------------------|------------|---------------------------------|-------------------------------|------------|---------------------------------|
| Fixed effects                                          | Model coefficient<br>(95% CI) | <i>P</i> * | Adjusted<br><i>P</i> (FDR)<br>‡ | Model coefficient<br>(95% CI) | <i>P</i> * | Adjusted<br><i>P</i> (FDR)<br>‡ |
| Mean cytokine level on day of vaccination <sup>b</sup> | -0.658 (-1.054 to -0.261)     | 0.0013     | 0.0126                          | -0.506 (-0.779 to -0.233)     | 0.0004     | 0.0067                          |
| Vaccines                                               |                               |            |                                 |                               |            |                                 |
| Saline                                                 | Reference                     |            |                                 | Reference                     |            |                                 |
| Influenza                                              | -0.211 (-0.520 to 0.098)      | 0.1798     |                                 | -0.214 (-0.520 to 0.093)      | 0.1697     |                                 |
| Pneumococcal                                           | -0.176 (-0.496 to 0.145)      | 0.2797     |                                 | -0.250 (-0.566 to 0.066)      | 0.1197     |                                 |

**Legend:** CI, confidence interval. \*Unadjusted *P* value. ‡Adjusted *P* value for multiple comparisons across cytokines by False Discovery Rate. <sup>a</sup>Pooled coefficients estimated from multivariable, mixed-effects linear regression. Coefficients with Adjusted *P* < 0.05 imply an association in the expected change of HIV DNA Gag for a one-unit change in mean cytokine level. <sup>b</sup>Corresponds to the day of vaccination for each cycle.