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Authors

Lund, Jennifer M
Broliden, Kristina
Pyra, Maria N
et al.

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HIV-1-Neutralizing IgA Detected in Genital Secretions of Highly HIV-1-Exposed Seronegative Women on Oral Preexposure Prophylaxis

Jennifer M. Lund,[a,b] Kristina Broliden,[c] Maria N. Pyra,[b] Katherine K. Thomas,[b] Deborah Donnell,[a,b] Elizabeth Irungu,[d] Timothy R. Muwonge,[e] Nelly Mugo,[d] Madhuri Manohar,[f] Marianne Jansson,[g] Romel Mackelprang,[b] Mark A. Marzinke,[f] Jared M. Baeten,[b,h,i] Jairam R. Lingappa,[b,h,j] for the Partners PrEP Study

Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washingon, USA[a]; Department of Global Health, University of Washington, Seattle, Washington, USA[b]; Department of Medicine, Karolinska Institute, Stockholm, Sweden[c]; Partners in Health Research and Development, Kenya Medical Research Institute, Thika, Kenya[d]; Infectious Disease Institute, Makerere University, Kampala, Uganda[e]; Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA[f]; Department of Laboratory Medicine, Lund University, Lund, Sweden[g]; Department of Medicine, University of Washington, Seattle, USA[h]; Department of Epidemiology, University of Washington, Seattle, Washington, USA[i]; Department of Pediatrics, University of Washington, Seattle, Washington, USA[j]

ABSTRACT

Although nonhuman primate studies have shown that simian immunodeficiency virus/simian-human immunodeficiency virus (SIV/SHIV) exposure during preexposure prophylaxis (PrEP) with oral tenofovir can induce SIV immunity without productive infection, this has not been documented in humans. We evaluated cervicovaginal IgA in Partners PrEP Study participants using a subtype C primary isolate and found that women on PrEP had IgA with higher average human immunodeficiency virus type 1 (HIV-1)-neutralizing magnitude than women on placebo (33% versus 7%; $P = 0.008$). Using a cutoff of ≥90% HIV-1 neutralization, 19% of women on-PrEP had HIV-1-neutralizing IgA compared to 0% of women on placebo ($P = 0.09$). We also estimated HIV-1 exposure and found that the proportion of women with HIV-1-neutralizing IgA was associated with the level of HIV-1 exposure ($P = 0.04$). Taken together, our data suggest that PrEP and high levels of exposure to HIV may each enhance mucosal HIV-1-specific humoral immune responses in sexually exposed but HIV-1-uninfected individuals.

IMPORTANCE

Although there is not yet an effective HIV-1 vaccine, PrEP for at-risk HIV-1-uninfected individuals is a highly efficacious intervention to prevent HIV-1 acquisition and is currently being recommended by the CDC and WHO for all individuals at high risk of HIV-1 acquisition. We previously demonstrated that PrEP use does not enhance peripheral blood HIV-1-specific T-cell responses in HIV-exposed individuals. Here, we evaluate for cervicovaginal HIV-neutralizing IgA responses in genital mucosal secretions of HIV-exposed women, which is likely a more relevant site than peripheral blood for observation of potentially protective immune events occurring in response to sexual HIV-1 exposure for various periods. Furthermore, we assess for host response in the context of longitudinal quantification of HIV-1 exposure. We report that HIV-neutralizing IgA is significantly correlated with higher HIV-1 exposure and, furthermore, that there are more women with HIV-1-neutralizing IgA in the on-PrEP group than in the placebo group.

The development of novel human immunodeficiency virus type 1 (HIV-1) prevention strategies that reduce HIV-1 susceptibility and impart long-term immune protection is a high priority. Four randomized, placebo-controlled clinical trials, conducted among diverse geographic and at-risk populations, have demonstrated that HIV-1-uninfected persons taking a daily oral antiretroviral as preexposure prophylaxis (PrEP)—either tenofovir (TDF) alone or coformulated with emtricitabine (TDF/FTC)—are at substantially reduced risk of HIV-1 acquisition (1–4). In recognition of this, WHO has recently recommended PrEP use for all individuals at substantial risk of HIV-1. Since PrEP will increasingly be used as part of standard care in the context of HIV-1 vaccine studies, it is essential to better understand the potential effects of PrEP on the host immune response to HIV-1.

While the primary mechanism of protection afforded by PrEP is thought to be through direct antiviral activity, it has been hypothesized that by blocking initial viral replication, PrEP might permit enhanced presentation of HIV-1 to the immune system and the subsequent development of HIV-1-specific adaptive immune responses. This hypothesis has been supported by three nonhuman primate studies, which reported the presence of simian immunodeficiency virus (SIV)-specific T-cell responses in a majority of animals that received PrEP prior to SIV exposure (5–7), yet studies in humans did not find that PrEP enhanced systemic HIV-specific T-cell responses (8).

Since the genital mucosa is the portal for HIV-1 entry by sexual transmission, mucosal immune responses directed at HIV-1 in this site could be particularly effective at preventing productive infection of HIV-1. In addition, recent identification of HIV-1-specific antibodies as a potential correlate of protection from

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Address correspondence to Jennifer M. Lund, jlund@fredhutch.org.

J.M.L. and K.B. contributed equally to this article.

HIV-1 infection in the RV144 vaccine trial (9) has led to an intensive focus on anti-HIV-1 humoral immunity, with increasing interest in the mucosal humoral response and its role in protection from HIV-1 infection. Some studies have not found mucosal IgA that either binds to or neutralizes HIV-1 (10–13), yet others have reported that HIV-1-exposed seronegative (HESN) women have measurable levels of anti-HIV-1 IgA responses in cervicovaginal secretions (14–23). Specifically, elevated levels of secretory IgA within the female reproductive tract were found to be associated with host resistance to HIV-1 among HIV-1-exposed uninfected commercial sex workers with a large number of clients per day (19). Furthermore, genital HIV-neutralizing IgA was associated with reduced HIV-1 acquisition (18), and HESN women were five times more likely to have neutralizing IgA in cervicovaginal secretions than low-risk control women (23). Thus, it appears that HIV-neutralizing IgA present in female genital secretions could be a correlate of protection from natural HIV-1 infection. Finally, a recent study found that HIV-1-neutralizing IgA was increased in genital swabs from uncircumcised men who did not acquire HIV-1 compared to samples from HIV-1 seroconverters that were collected prior to infection (24).

Here we report on HIV-1-neutralizing IgA in cervicovaginal samples collected from a subset of women from a placebo-controlled trial of PrEP and the effect of oral PrEP and quantifiable exposure to HIV-1.

MATERIALS AND METHODS

Experimental design and study participants. The Partners PrEP Study (ClinicalTrials.gov no. NCT00557245) was a randomized, placebo-controlled clinical trial of daily oral tenofovir disoproxil fumarate (TDF) and tenofovir-emtricitabine (TDF/FTC) PrEP among 4,747 HIV-1-uninfected members of heterosexual HIV-1 serodiscordant couples (i.e., one partner HIV-1 infected and one HIV-1 uninfected at enrollment) from Kenya and Uganda. The study procedures were described previously (1). The placebo arm of this study was discontinued after an interim analysis demonstrated efficacy of PrEP (1). The trial was then continued to compare the safety and efficacy of the two active drug arms by rerandomizing participants in the placebo arm to either TDF or TDF/FTC. Samples from women randomized to the placebo arm used in this study were collected prior to the rerandomization.

For the present study, we used all available cervical and vaginal swab samples that had been collected from individual HIV-1-uninfected women enrolled in follow-up at two study sites (Thika, Kenya, and Kampala, Uganda) in the Partners PrEP Study, who consented to additional genital mucosal sampling. Seventy-seven women contributed samples at 112 visits. Of these, 43 samples were from visits on PrEP, 51 were from visits 2 months after PrEP use had ceased (with 35 women contributing both PrEP and post-PrEP samples); and 18 were from placebo visits. This allowed us to evaluate whether there are mucosal antibody differences between individuals on PrEP versus placebo and whether responses persist or wane after discontinuation of PrEP.

A validated HIV-1 exposure score was calculated at each study visit as previously reported (25), using gender, age, circumcision status, and unprotected sex frequency, modified to incorporate the longitudinal rather than enrollment plasma HIV-1 RNA level of the HIV-1-infected stable partner since our samples were collected as long as 3 years after enrollment. Visit-level exposure scores ranged from −3 to 4, with a score of 0 representing the average risk of infection among the entire cohort of HIV-1 serodiscordant couples. In a serodiscordant cohort with similar distribution of exposure scores, a 1-unit increase in exposure scores represented a 2.7-fold change in HIV-1 acquisition risk (25). For 3 samples obtained during PrEP use, 6 samples post-PrEP, and 4 samples from placebo, exposure scores were not available; these samples did not contribute to analyses with exposure scores.

The procedures of the Partners PrEP Study, including collection of samples for immunologic assays, were approved by the institutional review boards of the University of Washington and collaborating site institutions; participants provided written informed consent. Analytical work was conducted by staff blind to treatment status, and samples from the PrEP and placebo groups were tested in the same batches.

IgA isolation from cervicovaginal swabs. To limit the risk of protease digestion of antibodies in the genital secretions, the samples were immediately kept cold and subsequently frozen within a few hours. Any digestion that may have occurred despite these precautions could theoretically have resulted in underestimation of the HIV-neutralizing IgA activity; however, all samples were handled blind to study drug randomization.

Two separate Dacron swabs were used to collect cervical and vaginal mucous samples: one swab was placed into the endocervical opening and gently rotated, and a second Dacron swab was placed along the lateral vaginal mucosal wall and gently rotated. Each swab was placed in a cryovial with 0.5 ml phosphate-buffered saline (PBS) and stored at −80°C. Separate cervical and vaginal aliquots from the same individual were combined prior to IgA isolation. IgA1 (IgA) was purified from thawed samples as previously described (15) with minor modifications. A total of 200 μl of supernatant was diluted 1:4 in PBS (pH 7.4) and added to 400 μl jacalin-agarose beads (ImmunKemi, Stockholm, Sweden). The diluted sample and the jacalin-agarose beads were mixed for 2 h at 4°C followed by centrifugation. Beads were then thoroughly washed with PBS (pH 7.4). The bound IgA was eluted overnight at room temperature by adding 1 ml 0.8 M D-galactose (pH 7.5), and supernatants with purified IgA were thereafter collected without further quantification or normalization. All fractions were stored at −80°C.

HIV-1 neutralization assay. HIV-1 neutralization assays were performed as described previously (24), with the laboratory investigators blind to the specimen characteristics. Briefly, the subtype C HIV-1 R5 primary isolate ZA97009 was obtained through the NIH AIDS Reagent Program (Rockville, MD). Due to limitation in sample volume, we could only test one of the NIH AIDS Reagent Program reference isolates and therefore selected one that represents a broadly reactive HIV-1-neutralizing response. Three virus dilutions were used in each assay to allow evaluation at 10 to 50 50% tissue culture infective doses ($TCID_{50}$). Peripheral blood mononuclear cells (PBMCs) from healthy blood donors, which were always pooled from a minimum of two donors to avoid donor variability, were stimulated by phytohemagglutinin (PHA) and cultured in medium with interleukin 2 (IL-2) at a concentration of 10^5 cells/microtiter plate well. The undiluted IgA samples were tested for neutralization capacity in duplicate, with virus production analyzed in a p24 antigen enzyme-linked immunosorbent assay (ELISA) (Murex HIV antigen monoclonal antibody [MAb] kit; Abbott Diagnostics, Abbott Park, IL). Reference serum samples were always included as calibrators to control for interassay variability. The neutralizing activity in the PBMC assay was defined as the percentage of reduction in p24 antigen concentration compared to wells in which the virus isolate was incubated in the presence of control samples representing low-risk HIV-1-seronegative Swedish blood donors (mean optical density [OD] value of the four control samples). A few (<5%) test samples had an OD value exceeding the mean value of the control samples. For these test samples (thus scoring as a negative neutralization value), the percentage of neutralization was set to 0%. Unblind IgA purified cervicovaginal samples from four HIV-seronegative women from the Partners PrEP Study cohort and an international standard MAb mixture with known HIV-neutralizing capacity (Trimab; Centre for AIDS Reagents [CFAR] NIBSC, United Kingdom) were included in each assay.

TFV quantification. Tenofovir (TFV) quantification in serum and cervicovaginal lavage (CVL) fluid was performed via previously described liquid chromatographic-tandem mass spectrometric (LC-MS/MS) analyses. The assays' lower limits of quantification (LLOQs) were 0.31 ng/ml and 5 ng/ml for serum and CVL fluid, respectively, and assays were vali-

TABLE 1 Associations between PrEP status and HIV-1-neutralizing IgA

Sample group (*n*)	IgA-90, % (*n*)[a]	*P* value by Fisher's exact test	% IgA neutralization, mean % (SD)	*P* value from linear mixed model[b]
All available samples				
On PrEP (43)	19 (8)	Reference	33 (38)	Reference
Post-PrEP (51)	8 (4)	0.13	26 (33)	0.29
Placebo (18)	0 (0)	0.09	7 (18)	0.008
Women with PrEP and post-PrEP				
On PrEP (35)	17 (6)	Reference	33 (38)	Reference
Post-PrEP (35)	9 (3)	0.47	22 (31)	0.20

[a] IGA-90 represents ≥90% neutralization in both wells.
[b] The *P* value is from the linear mixed model to adjust for correlation in outcomes from the same participant.

dated in accordance with recommendations of the FDA document *Guidance for Industry: Bioanalytical Method Validation* (26). TFV concentrations in eluates were determined using the CVL calibration curve. To assess the comparability of this method for eluates, TFV was first spiked at 15, 100, and 1,000 ng/ml. Results were compared to those from CVL samples spiked at the same concentrations, and the percentage of difference between the two matrices ranged from −7.4 to −2.8%.

Statistical analysis. Neutralization was analyzed in two ways: (i) by percentage of IgA neutralization, or the mean level of HIV-1 neutralization in duplicate wells, a continuous scale from 0 to 100%, and (ii) by IgA-90, or a binary indicator of samples with duplicate wells showing ≥90% HIV-1-neutralization. Associations between treatment status (PrEP versus placebo and PrEP versus post-PrEP) and IgA-90 positivity were tested using Fisher's exact test. Although the HIV-1 exposure score was analyzed as a continuous covariate, bivariate descriptive statistics between HIV-1 exposure score and IgA-90 positivity are presented in 4 categories (with exposure score cutoffs of −1, 0, and 1) to demonstrate possible trends. The association between exposure score and IgA-90 positivity was tested using a generalized estimating equation (GEE) model with logistic link, where outcome represents IgA-90 and predictor represents exposure score, and including robust standard errors to adjust for correlation arising from those participants contributing specimens on both PrEP and post-PrEP.

Associations with percentage of IgA neutralization were estimated using a linear effects model with outcome representing the percentage of IgA and predictor representing the treatment group or exposure score, incorporating a random intercept for each participant to adjust standard errors for within-person correlation. We examined whether the relationship between exposure score and percentage of IgA neutralization depends on PrEP status by adding interaction terms between exposure score and PrEP use categories. A significant interaction term would mean that the effect of HIV-1 exposure on percentage of IgA depends on whether the participant is on PrEP, and the effect of PrEP on percentage of IgA depends on how much HIV-1 exposure there is.

Comparisons of both IgA-90 and percentage of IgA neutralization by treatment group were also performed, limited to the subset of women on PrEP with measurements both before and after discontinuing the drug. All analyses were done using SAS 9.4 (SAS Institute, Cary, NC).

RESULTS

Women providing samples had been with their partners for an average of 10.7 years at enrollment in the parent study. Among the HIV-infected partners, 36.6% had begun antiretroviral therapy (ART), and the last $\log_{10}$ plasma HIV-1 RNA level before providing the sample averaged 3.1. Recent condomless sex was reported by 12.1% of the women. PrEP, placebo, and post-PrEP recipients had comparable ages (34.8 versus 35.1 years for PrEP versus placebo, respectively [$P = 0.58$], and 34.8 versus 33.7 years for PrEP versus post-PrEP, respectively [$P = 0.20$]) and HIV-1 exposure scores (mean score, 0.03 versus 0.36 for those on PrEP versus placebo, respectively [$P = 0.39$] and 0.03 versus 0.13 for PrEP versus post-PrEP, respectively [$P = 0.38$]). All samples collected from women on PrEP were sampled at the study drug stop, which occurred typically after 36 months of drug use. Most women were highly adherent by self-report and pill count measures (1). Importantly, TFV was not detected in genital eluates (data not shown), and therefore HIV-1-neutralizing effect is not attributable to residual TFV.

Women on PrEP had a statistically higher average percentage of IgA neutralization, at 33% compared to 7% among those on placebo ($P = 0.008$) (Table 1). Two months after discontinuing PrEP, the percentage of IgA neutralization was 26% ($P = 0.29$ compared to those on PrEP [Table 1], and $P = 0.04$ compared to those on placebo). When using stringent criteria of replicate assay wells having ≥90% reduction in p24 antigen (IgA-90), 19% of women on PrEP met the cutoff compared to 0% on placebo ($P = 0.09$) (Table 1). Two months after discontinuing PrEP, the proportion of women with an IgA-90 was 8%, which is not statistically significant from the 19% on PrEP sampling ($P = 0.13$), nor was it statistically significant from the 0% of women on placebo ($P = 0.56$). In analyses limited to the women contributing samples both before and after discontinuing PrEP, estimates were very similar for both percentage of IgA neutralization and IgA-90 compared to those found in the larger group.

The HIV-1 exposure score and mean percentage of IgA neutralization are shown in Fig. 1 for each of the three treatment groups. Increased HIV exposure was found to have a trend toward higher mean percentage of IgA neutralization, with an overall 1-unit increase in the exposure associated with a 5% point increase in neutralization level ($P = 0.06$) (Table 2). Consistent with this, the probability of observing IgA-90 was found to be higher in women with higher exposure scores ($P = 0.04$).

We also examined whether there was an interaction between PrEP use and the effect of HIV-1 exposure. In the context of PrEP use, the HIV exposure effect appears stronger, with each 1-unit increase in exposure score resulting in an 8% increase in percentage of IgA neutralization compared to a 2% increase at 2 months post-PrEP and a 2% decrease for placebo (Fig. 1). However, when we tested the interaction term to see whether the observed variation in the effect of exposure was statistically significant, we found that it was not ($P = 0.19$ for PrEP versus placebo, and $P = 0.24$ for PrEP versus post-PrEP). Thus, our data do not provide evidence that the effects of PrEP and exposure score on percentage of IgA neutralized modify one another rather than acting independently,

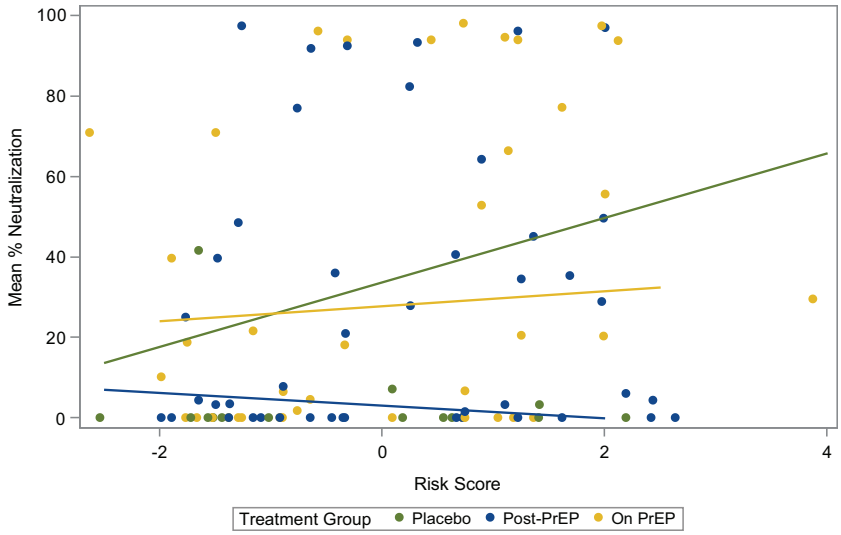

FIG 1 Exposure score and percentage of IgA neutralization, by PrEP use. The percentage of IgA neutralization is the mean level of HIV-1 neutralization in duplicate wells. In linear mixed model analysis where outcome represents the percentage of IgA and predictor represents the exposure score, and incorporating a random intercept for each participant to adjust standard errors for within-person correlation, we found that while on PrEP or placebo or 2 months post-PrEP, the percentage of IgA neutralization increased an average of 8%, −2%, or 2%, respectively, for each 1-unit increase in exposure score. These results were not statistically different from one another ($P = 0.19$ for PrEP versus placebo, and $P = 0.24$ for PrEP versus post-PrEP). Overall, a 1-unit increase in the exposure was associated with a 5% point increase in neutralization level ($P = 0.06$).

although the sample was likely underpowered to detect such interactions.

DISCUSSION

Clinical trials have reported efficacy of oral PrEP against sexual HIV-1 transmission (1–4), and nonhuman primate studies have shown that PrEP can elevate anti-SIV/SHIV immune responses in the absence of productive infection (5–7). Here we extend these observations by evaluating how oral PrEP treatment affects mucosal immune responses in women sexually exposed to HIV-1. Genital HIV-1-neutralizing IgA responses were higher in women in HIV-1 serodiscordant relationships using oral PrEP than in placebo-treated subjects (33% versus 7%). Furthermore, the proportion of these HIV-1-exposed women with a stringent ≥90% neutralizing IgA capacity tended to be more prevalent in those using oral PrEP compared to placebo (19% versus 0%; $P = 0.09$). Finally, a higher HIV-1 exposure score was associated with higher HIV-neutralizing IgA capacity ($P = 0.06$ for percentage of IgA neutralization, and $P = 0.04$ for IgA-90). Similar to our findings, a recent study using samples from the CAPRISA 004 microbicide trial to determine if topical antiretroviral usage had an impact on mucosal and systemic antibody responses found that HIV-specific IgA and IgG responses are increased in the genital tract of individuals who acquire HIV infection in the presence of tenofovir gel (27).

Since all women were HIV-1 seronegative at the time of sample collection, our findings suggest that a local IgA-mediated immune response to HIV-1 may be elicited through sexual HIV-1 exposure without resulting in a systemic anti-HIV-1 IgG response. Although not repeated here, we have previously analyzed whether HESN women may have local genital HIV-1-specific IgG antibodies in addition to the IgA response, but this was not the case (28).

TABLE 2 Associations between exposure scores and HIV-1-neutralizing IgA

	Result for[b]:				
Exposure score (n)[a]	IgA-90, % (n)	Without IgA-90, % (n)	P value	% IgA neutralization, mean % (SD)	P value
Total (99)	Mean, 0.70; SD, 1.11	Mean, −0.08; SD, 1.44	0.04[c]	Slope, 5; 95% CI, 0–10[d]	0.06[d]
By category					
<−1 (31)	3 (1)	97 (30)		16 (31)	
≤−1 to <0 (19)	16 (3)	84 (16)		29 (40)	
≥0 to <1 (19)	11 (2)	90 (17)		30 (41)	
≥1 (30)	20 (6)	80 (24)		35 (41)	

[a] Exposure score as a predictor was used continuously in the analysis but is presented in separate categories here to illustrate any trends.
[b] Results for IgA-90 (i.e., ≥90% neutralization in both wells) and "Without IgA-90" are presented as percentage (n), except as noted. Results for "% IgA neutralization" are presented as mean (SD) percentage, except as noted.
[c] The P value shown is for association between exposure score and IgA-90 estimated from the generalized estimating equation (GEE) model with logistic link, adjusting standard errors for correlation in outcomes from the same participant.
[d] The estimated slope represents the mean increase in percentage of IgA neutralization per 1-unit increase in exposure score. The slope, 95% confidence interval (CI), and P value were estimated from the linear mixed model to adjust for correlation in outcomes from the same participant.

IgA-depleted fractions of cervicovaginal secretions do, however, contain antimicrobial peptides and other components with antiviral activity that can contribute to the HIV-neutralizing activity (29).

While prior studies have documented that PrEP use reduces the likelihood of HIV-1 infection after sexual HIV-1 exposure, our findings support the concept that PrEP may also enhance the HIV-1-neutralizing IgA response, although whether oral PrEP plays a direct or indirect immunomodulatory role in the mucosal IgA response requires further study. However, at this time, it is unclear if this mucosal anti-HIV-1 IgA could also mediate enhanced HIV-1 neutralization after PrEP is discontinued. The study design did not allow for mechanistic experiments to determine how PrEP usage directly results in enhanced HIV neutralization. We do hypothesize that HIV exposure in the context of PrEP allows for chemovaccination effects in the form of enhanced immune priming, perhaps leading to enhanced T-cell help and/or B-cell priming. Additionally, it is possible that serial local and abortive HIV-1 infections sustained by HESN on PrEP allow for the generation of organized lymphoid tissue structures within the genital tract tissues or the establishment of a pool of antibody-secreting cells that reside within the genital tract, thereby serving as first responders upon local exposure to virus encounter. In our previous studies of HESN enrolled in the Partners PrEP Study, we detected HIV-specific $CD4^+$ and $CD8^+$ peripheral blood T-cell responses in 10 to 20% of 247 subjects evaluated, and although the response rate and magnitude of T-cell responses did not vary significantly between those assigned PrEP versus placebo, it is possible that there is a difference in the tissue-resident response of these cells that could have consequences for the local IgA response, particularly in terms of providing T-cell help.

HIV-1-neutralizing IgA antibodies were previously reported in 23% of HIV-1-exposed seronegative women in HIV-1 serodiscordant relationships in the context of a different Kenyan cohort and using a comparable assay format (23). Although the overall HIV-1 acquisition rate was higher in our present study than that in the other cohort, the empirical HIV-1 exposure scores (30) were relatively low overall, due in part to the fact that at the time that samples were collected from these women, their reported rate of condomless sex was low (12.1% for participants sampled in this study as discussed above in Results). Indeed, in contrast to the 19% of samples from women on-PrEP with IgA-90, a previous study evaluating female commercial sex workers at very high sexual HIV-1 exposure risk (but without use of PrEP) found 80% had genital HIV-1-neutralizing IgA (17, 18). While these data suggest that increased HIV-1 exposure and use of oral PrEP may both elicit a local humoral anti-HIV-1 response, the variation in IgA levels detected across cohorts further suggests that, in addition to methodological variations, distinct biological mechanisms may be selected for in different epidemiological contexts. For example, the context of highly exposed, persistently seronegative commercial sex workers may select for individuals with natural HIV-1 resistance (31) or may reflect individuals with maximally induced HIV-1-specific genital mucosal IgA. In contrast, the less extreme but quantifiable HIV-1 exposure exhibited by stable, heterosexual couples may have facilitated our ability to detect a direct relationship between HIV-1 exposure and the HIV-1-specific mucosal IgA response. Finally, other factors influencing genital immune responses, including age, sexual habits, genital coinfections, altered genital flora, and levels of sex hormones (32), may also be affecting these host responses.

To our knowledge, this is the first report of increased human genital HIV-1-neutralizing IgA response in the context of oral PrEP use. We further extended these findings by including genital samples collected 2 months posttreatment and showed IgA-90 in 8% of those samples. The observed somewhat lower frequency of genital HIV-1-neutralizing IgA posttreatment suggests there may be a short half-life of the mucosal antibodies in the current setting of relatively low HIV-1 risk, indicative of a weak local memory B-cell response. In comparison, genital fluids from individuals at the earliest stages of acute HIV-1 infection have been reported to have mucosal IgA antibodies, possibly induced by an initial T-cell-independent response, and these had a short half-life of about 3 days (33). Interestingly, HIV-1-specific IgA mucosal antibodies derived from cervical B cells of HESN women that have evolved slowly during repeated and long-term HIV-1 exposures could potentially accumulate high-affinity, antigen-binding somatic mutations. These antibodies could target both cell-free and cell-associated infectious virus and thereby block HIV-1 entry into mucosal tissues (22). In another study, women remaining in stable HIV-1 serodiscordant relationships also had HIV-1-neutralizing IgA activity persisting over time (23).

The strength of the present study is the use of a longitudinal HIV-1 risk score and the collection and functional assessment of samples representing the longitudinal host response in the genital tract mucosa, which may be a more relevant site than peripheral blood for observation of potentially protective immune events occurring in response to sexual HIV-1 exposure of various durations. The data further implicate a cross-clade HIV-1-neutralizing IgA response, since HIV-1 subtypes A and D predominate at the study site, while an HIV-1 subtype C viral isolate was used as a target in our neutralizing assay, and indeed, by using only a single virus isolate for assessment of HIV-neutralizing activity, we may have underestimated the presence of HIV-neutralizing activity, since IgA present in some samples not found to neutralize the ZA97009 isolate may have exhibited neutralization against other isolates. Furthermore, the detection of IgA-mediated HIV-neutralizing activity may have been underestimated due to the presence of endogenous proteases despite careful attention to immediate storage of the samples at −80°C and transportation between laboratories on dry ice without cold chain interruption. There is, however, no reason to believe that this underestimation would be unevenly distributed between the study groups. Even though the specificities of the IgA antibodies detected in the Partners PrEP Study participants are not yet known, previous studies have shown that mucosal HIV-1-neutralizing IgA antibodies were directed against HIV-1 gp120 and/or HIV-1 gp41 (34–36).

The present study has some limitations, including assessment of only IgA1 antibodies. IgA2 does not bind to jacalin used for IgA purification, and this subclass could have had complementary and synergistic antiviral activity. Our present data thus may have underestimated the functional activity of mucosal IgA. Furthermore, due to small sample volumes, we could not investigate additional antiviral, nonneutralizing, functional properties of mucosal IgA, which may contribute to a protective antiviral role (37–39). We also found no evidence for tenofovir being carried over in jacalin-agarose eluates, despite use of sensitive LC-MS/MS methods. Our study design did not allow us to determine whether the HIV-1-neutralizing responses have any antiviral significance *in vivo* in terms of protection or merely represent a "footprint" of virus exposure. Finally, our sample size, particularly for the women in the placebo arm, was limited given early termination of that arm due to study drug efficacy. An analysis using a larger sample size, especially of

participants naive to PrEP, would provide more power to discern differences in the magnitude of the local humoral response.

Locally produced or passively administered mucosal IgA antibodies can block entry and spread of experimental SIV/SHIV infection as shown in various nonhuman primate models (38–40). The finding that oral PrEP use is associated with elevated mucosal HIV-1-neutralizing IgA responses warrants further study in humans and nonhuman primate models to elucidate whether induced mucosal immune responses could augment oral PrEP efficacy and overall protection from HIV-1 acquisition.

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