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Soluble Immune Mediators and Vaginal Bacteria Impact Innate Genital Mucosal Antimicrobial Activity in Young Women

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Abstract

Introduction—Innate activity against *Escherichia coli* in female genital secretions may represent contributions from vaginal bacteria and host soluble immune mediators. We analyzed the relationship between *E. coli* inhibitory activity, soluble immune mediators, and vaginal bacteria in participants in MTN-004, a placebo-controlled trial of VivaGel[®], a candidate product for topical HIV pre-exposure prophylaxis.

Methods—*Escherichia coli* inhibitory activity was quantified by colony reduction assay. Endocervical concentrations of interleukin (IL)-1 β , IL-6, IL-12p40, macrophage inflammatory protein (MIP)-1 α , granulocyte–macrophage colony-stimulating factor (GM-CSF), lactoferrin, and secretory leukocyte protease inhibitor (SLPI) were quantified to generate a cumulative mediator score. Vaginal bacteria were characterized by quantitative cultures.

Results—In the two placebo arms, higher soluble immune mediator score was associated with greater *E. coli* inhibitory activity ($\beta = 17.49$, 95% CI [12.77, 22.21] and $\beta = 13.28$, 95% CI [4.76, 21.80]). However, in the VivaGel arm, higher concentrations of *E. coli* ($\beta = -3.80$, 95% CI [-6.36,

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Mean concentrations of soluble immune mediators.

Table S2. Pearson correlation coefficients for immune mediator concentrations and cumulative soluble immune mediator score.

–1.25]) and group B *Streptococcus* ($\beta = -3.91$, 95% CI [–6.21, –1.60]) were associated with reduced *E. coli* inhibitory activity.

Conclusions—Both host mediators and vaginal bacteria impact *E. coli* inhibition in genital secretions. The relative contributions of host mediators and bacteria varied between women who used VivaGel vs placebos.

Keywords

Escherichia coli; genital innate immunity; *Lactobacillus*

Introduction

Studies of endogenous inhibitory activity against *Escherichia coli* in female genital secretions suggest that it represents a cumulative measure of the complex interactions of vaginal bacteria and host soluble immune mediators.^{1–6} However, the key determinants and biological relevance of this activity remain unclear and likely vary between groups of women. In U.S. women who did not have bacterial vaginosis (BV) or sexually transmitted infections (STI), high inhibitory activity (>90% inhibition of *E. coli*) in cervicovaginal lavage (CVL) samples was associated with vaginal *Lactobacillus crispatus* colonization⁷ and with the presence of surface proteins specific to *L. crispatus* and *L. jensenii*.¹ Higher *E. coli* inhibitory activity was also inversely correlated with genital *E. coli* colonization in U.S. pregnant women.² Thus a high degree of *E. coli* inhibition may represent primarily antimicrobial contributions from H₂O₂-producing vaginal lactobacilli and could indicate the more favorable reproductive health outcomes associated with these species.^{8–11}

In contrast, the factors that contribute to the more modest *E. coli* inhibition observed in genital tract secretions collected from women who have low levels of vaginal H₂O₂-producing lactobacilli are poorly defined. In this population, *E. coli* inhibitory activity may instead reflect contributions from host soluble immune mediators and may provide a biomarker of mucosal inflammation and HIV-1 seroconversion risk.⁵ Sexually active U.S. adolescents 15–18 years of age with a paucity of vaginal *L. crispatus* and *L. jensenii* had significantly lower median *E. coli* inhibitory activity in CVL relative to adult women (48% inhibition vs 66.5%, respectively) but higher concentrations of interleukin (IL)-6 and IL-1 α .³ Samples were collected at only one time point, so temporal relationships between shifts in vaginal bacteria, host mucosal soluble immune mediators, and *E. coli* inhibitory activity were not determined. In women from Malawi, South Africa, and Zimbabwe who participated in a randomized, placebo-controlled clinical trial of vaginal BufferGel and PRO 2000 gel for the prevention of HIV, overall *E. coli* inhibitory activity was low (mean $\pm$ standard deviation [SD]; 23.7 $\pm$ 34.2%), prevalence of BV was at least 30% across study arms, and a modest increase in *E. coli* inhibitory activity and detectable levels of human beta defensin (H β D)-2 were associated with an increased risk of HIV-1 seroconversion.⁵ Similarly, in a subset of CAPRISA 002 participants with a high prevalence of BV, average *E. coli* inhibitory activity was low (mean $\pm$ SD; 34.1 $\pm$ 17.9% in non-seroconverters), and modestly increased *E. coli* inhibitory activity and H β D-1 levels were independently associated with an increased risk of HIV-1 seroconversion (4).

We investigated the mechanisms underlying *E. coli* inhibitory activity from healthy participants in MTN-004, a Phase 1 randomized, double-blinded, placebo-controlled trial of the safety and acceptability of vaginal SPL7013 gel (VivaGel®) for the prevention of HIV. VivaGel is a dendrimer-based topical microbicide with *in vitro* activity against HIV and HSV-2.^{12,13} Initial results from the Phase 1 trial indicated that VivaGel use was associated with modest but statistically significant alterations of vaginal bacteria populations.¹⁴ Thus, genital tract samples collected at multiple time points during this study provided the opportunity to investigate how concentrations of vaginal bacteria and soluble immune mediators at one study visit impact subsequent activity against *E. coli* at the following study visit.

We hypothesized that higher concentrations of H₂O₂-producing lactobacilli and lower concentrations of BV-associated bacteria (anaerobes, *G. vaginalis*) and bacteria that may translocate from the rectum to vagina in the setting of sexual activity (*Enterococcus*, *E. coli*, group B *Streptococcus* [GBS]) would be associated with higher *E. coli* inhibitory activity. We also hypothesized that higher levels of specific soluble immune mediators at the genital mucosa, including IL-1β, IL-6, IL-12p40, macrophage inflammatory protein (MIP)-1α, granulocyte–macrophage colony-stimulating factor (GM-CSF), secretory leukocyte protease inhibitor (SLPI), and lactoferrin, would be associated with an increase in *E. coli* inhibitory activity. These mediators were selected for their association with mucosal inflammation and/or *E. coli* inhibitory activity in prior studies or for their potential antibacterial activity (SLPI and lactoferrin).^{15–22}

Methods

Study Participants and Procedures

Healthy, non-pregnant, sexually active, HIV uninfected women between the ages of 18 and 24 years were enrolled at three sites: San Juan, Puerto Rico; Tampa, Florida; and Pittsburgh, Pennsylvania.¹⁴ Participants were initially randomized in a 1:1 ratio to receive twice daily vaginal VivaGel or VivaGel matched placebo gel. An additional hydroxyethyl-cellulose (HEC) placebo gel arm was added to the study after an interim data review was conducted to investigate a higher than anticipated number of low-grade genital adverse events. Subsequent participants were then randomized in a 1:1:1 ratio to the three study arms (i.e., VivaGel, VivaGel placebo, and HEC gel). Participants were asked to administer study product intravaginally every 12 hr for a total of 14 days. Adherence to study product was assessed by self-report and by collection and counting of used product applicators at study visits. Participants were evaluated and genital tract samples were collected at four study visits: prior to study drug use (day 0, enrollment) and at 7 (range 6–8), 14 (range 13–15), and 21 (range 20–24) days after initiation of study product use.

BV was determined by Gram stain (Nugent's score) at each study visit in a single laboratory. A Nugent's score of 0–3 was considered normal, 4–6 was considered intermediate, and 7 was considered consistent with BV.²³ Samples for evaluation of soluble immune mediators and *E. coli* inhibitory activity were collected at each visit by inserting a Dacron® swab one centimeter into the cervical os, rotating the swab 360°, and then placing the swab into 400 µL of phosphate-buffered saline (PBS). Endocervical swabs

were stored at -70°C until they were processed.⁵ Quantitative vaginal culture samples were collected at each visit by rotating a Dacron swab across the lateral vaginal wall and then placing the swab into a culture transport tube for overnight delivery to the laboratory.

Laboratory Methods

Concentrations of IL-1 β , IL-6, IL-12p40, MIP-1 α , and GM-CSF were quantified from endocervical swab elutes by multiplex proteome array (Millipore Corporation, Billerica, MA, USA) using Luminex 100 (Luminex, Austin, TX, USA) and analyzed using StarStation software (Applied Cytometry Systems, Sacramento, CA, USA). Concentrations of lactoferrin and SLPI were quantified from cervical swab elutes by ELISA (lactoferrin: EMD Chemicals, Gibbstown, NJ, USA; SLPI: R&D Systems, Inc., Minneapolis, MN, USA).

Inhibitory activity of endocervical swab elutes against *E. coli* (ATCC strain 4382627) was quantified by colony-forming unit (cfu) reduction assay as previously described.³ *E. coli* (ATCC strain 4382627) was grown overnight to reach stationary phase. Three μL of *E. coli* was mixed with 27 μL of elute or control buffer and incubated for two hours at 37°C . The samples were diluted in buffer to yield 800–1000 colonies on control plates, then plated on agar with trypticase soy broth and incubated overnight at 37°C . Colonies were counted using ImageQuant TL v2005. All samples were tested in duplicates. *E. coli* inhibitory activity was reported as the percent reduction in number of cfu relative to control plates.

Quantitative cultures from vaginal swabs were performed for the following organisms: anaerobic Gram-negative rods, *Enterococcus* species, *E. coli*, *Gardnerella vaginalis*, GBS, H_2O_2 -producing and non- H_2O_2 -producing lactobacilli, *Staphylococcus aureus*, and *Candida* species.^{14,24–26} All lactobacilli were tested for production of H_2O_2 after anaerobic incubation on a tetramethylbenzidine agar plate. H_2O_2 produced by lactobacilli reacted with the horseradish peroxidase in the agar, resulting in oxidization of tetramethylbenzidine and blue discoloration of the H_2O_2 -producing colonies. The H_2O_2 -producing colonies were then counted and discriminated from the non-blue colonies that did not produce H_2O_2 .²⁷

Statistical Methods

Categorical variables at baseline were compared between groups by Fisher's exact or chi-square tests and continuous variables using ANOVA. Concentrations of IL-1 β , IL-6, IL-12p40, MIP-1 α , GM-CSF, lactoferrin, and SLPI were log-transformed to reduce skewness, and factor analysis with principal components extraction was used to generate a soluble immune mediator score from these variables for each participant at each visit. The factor analysis method allows for concentrations of multiple mediators to be combined into a single score that can then be used as a predictor variable, thus reducing the dimensionality of the data. For each woman and at each visit, a soluble immune mediator score was calculated that represented the combined levels of IL-1 β , IL-6, IL-12p40, MIP-1 α , GM-CSF, lactoferrin, and SLPI quantified from the visit sample. This score positively and significantly correlated with each of the individual soluble immune mediators, with Pearson correlation coefficients ranging from 0.82 (for IL-6) to 0.39 (for GM-CSF) (Table S2).

In addition, cumulative quantitative scores were generated by adding quantitative culture values for each participant at each visit to provide measures of specific vaginal bacterial populations, including H₂O₂-producing lactobacilli, BV-associated bacteria (anaerobes, *G. vaginalis*), and bacteria that typically colonize the rectum but may translocate to the vagina, especially in the setting of sexual activity (GBS, *Enterococcus* and *E. coli*).^{24,28–30} For each woman and at each visit, three vaginal bacterial scores were calculated: one to represent levels of H₂O₂-producing lactobacilli, one to represent levels of anaerobes and *G. vaginalis* (BV-associated bacteria), and one to represent levels of GBS, *Enterococcus*, and *E. coli*. Higher concentrations of bacteria resulted in a higher score, while lower concentrations of bacteria contributed to a lower score.

This study provided an opportunity to investigate how concentrations of soluble immune mediators and vaginal bacteria impact subsequent changes in *E. coli* inhibitory activity. To assess this temporal relationship, we used generalized estimating equations (logit [binary outcomes] or Gaussian [continuous outcomes] links with exchangeable correlation structure) controlling for product use to analyze the impact of soluble immune mediator score or vaginal bacteria score on *E. coli* inhibitory activity in the swab collected at the following weekly visit (7 days later). The objective was to determine whether a woman's soluble immune mediator or vaginal bacterial score at one visit was predictive of her endogenous activity against *E. coli* at the following visit. Use of generalized estimating equations allowed for assessment of these potential associations across multiple time points. A preliminary unstratified analysis (all participants analyzed as a single cohort) revealed that product use modified the effect of the predictor variables of interest on *E. coli* inhibitory activity. A stratified approach was therefore required, and multivariable analyses were conducted with participants stratified by study product arm.

Results

Sixty-one participants were enrolled and randomized to receive VivaGel ($n = 22$), VivaGel placebo ($n = 21$), or HEC gel ($n = 18$).¹⁴ Twenty participants were enrolled at the San Juan site, 30 at the Tampa site, and 11 at the Pittsburgh site. The average age of participants across the three study product arms was 21 years, and there were no significant differences in mean age between arms (Table I).¹⁴ Participants in this study are described in greater detail elsewhere.¹⁴

Prior to product exposure (baseline enrollment visit), the distribution of Nugent scores between study arms did not differ significantly (Table I). Fifty-nine percent of participants in the VivaGel arm, 57% of participants in the VivaGel placebo arm, and 61% of participants in the HEC gel arm had Nugent scores of 0–3 ($P = 0.97$) (Table I). The prevalence of participants with non-detectable H₂O₂-producing lactobacilli by quantitative culture at baseline was 24% in the VivaGel arm, 33% in the VivaGel placebo arm, and 25% in the HEC gel arm (Table I) ($P = 0.76$). The proportion of participants with Nugent score 7 did not differ significantly between groups prior to product exposure ($P = 0.3$) (Table I) and remained similar between groups at each visit (data not shown). Exposure to VivaGel was not associated with significant changes in the incidence of BV as assessed by Nugent

score.¹⁴ Details regarding exposure to VivaGel, VivaGel placebo, or HEC gel and shifts in vaginal bacteria are described elsewhere.¹⁴

Inhibitory activity against *E. coli* was quantified from genital tract samples collected prior to product exposure (baseline enrollment visit, Day 0), at Day 7 of product exposure, at Day 14 of product exposure, and at Day 21 (7 days after last product exposure). A total of 185 swabs were collected, including 66 swabs from participants in the VivaGel arm, 65 from the VivaGel placebo arm, and 54 from the HEC gel arm. Mean *E. coli* inhibitory activity was similar between study arms at the baseline enrollment visit and did not vary significantly between visits within each study arm (Table II). Mean soluble immune mediator scores did not differ significantly between groups at baseline and did not vary significantly over time within groups ($P > 0.05$ for all, Table II). The mean concentrations of individual soluble immune mediators are reported in Table S1. Pearson correlation coefficients for individual soluble immune mediators and the soluble immune mediator score are reported in Table S2. The mean score for *Enterococcus*, *E. coli*, and GBS increased significantly from baseline among women who received VivaGel at Day 7 (mean score 4.86, 95% CI [2.44, 7.28] vs 10.30, 95% [CI 7.29, 13.30]; $P = 0.006$) but did not differ significantly from baseline by Day 14 or 21 (Table III). Mean bacterial scores otherwise did not differ significantly between visits for any study arm ($P > 0.05$ for all).

The impact of vaginal bacteria and soluble immune mediator scores on subsequent *E. coli* inhibitory activity was analyzed with participants stratified by study product arm (Table IV). This stratified analysis utilized 145 available data points from 55 women and was performed to account for significant effect modifications of product use on the relationship between *E. coli* inhibitory activity and the predictor variables of interest that were observed in initial unstratified analyses. A higher soluble immune mediator score was associated with significantly higher *E. coli* inhibitory activity at the subsequent visit in participants receiving VivaGel placebo ($\beta = 17.49$, 95% CI [12.77, 22.21]) and HEC gel ($\beta = 13.28$, 95% CI [4.76, 21.80]) (Table IV). Thus, a one standard deviation increase in soluble immune mediator score was associated with a 17.5% and 13.3% increase at the subsequent visit in *E. coli* inhibitory activity in the VivaGel placebo and HEC gel groups, respectively. No significant associations were detected in the two placebo gel groups between *E. coli* inhibitory activity and vaginal bacteria scores, including H₂O₂-producing *Lactobacillus* and BV-associated bacteria scores (Table IV). No significant association was identified between *E. coli* inhibitory activity and concentration of lactobacilli when analyses were limited to women with higher levels of H₂O₂-producing *Lactobacillus* vaginal colonization (10^5 cfu/g vaginal fluid) (VivaGel placebo: $\beta = 0.42$, 95% CI [-9.45, 10.28]; HEC gel: $\beta = -5.42$, 95% CI [-16.69, 5.84]).

The effect of immune mediators on *E. coli* inhibitory activity at the subsequent visit was not observed in the VivaGel arm. For participants using VivaGel, higher concentrations of *E. coli*, GBS, and *Enterococcus* were significantly associated with subsequent diminished *E. coli* inhibitory activity ($\beta = -1.84$, 95% CI [-2.89, -0.80]) (Table IV). When these bacteria were analyzed separately, both *E. coli* ($\beta = -3.80$, 95% CI [-6.36, -1.25]) and GBS ($\beta = -3.91$, 95% CI [-6.21, -1.60]) concentrations were significantly associated with reduced *E. coli* inhibitory activity. *Enterococcus* concentrations alone were not significantly associated

with a change in subsequent *E. coli* inhibitory activity ($\beta = -2.72$, 95% CI $[-5.6, 0.16]$). Of note, H_2O_2 -producing *Lactobacillus* and BV-associated bacterial scores were not associated with significant changes in *E. coli* inhibitory activity (Table IV), and no significant association was identified between *E. coli* inhibitory activity and H_2O_2 -producing *Lactobacillus* score when the analysis was limited to women with 10^5 *Lactobacillus* cfu/g ($\beta = 0.42$, 95% CI $[-9.45, 10.28]$).

Discussion

We investigated the complex relationship between vaginal bacteria and host-derived soluble immune mediators and *E. coli* inhibitory activity in genital secretions collected from a cohort of young women who were sexually active and who had modest average *E. coli* inhibitory activity (12.5–50%). Of note, a significant proportion of the participants had nondetectable vaginal levels of H_2O_2 -producing lactobacilli by quantitative culture, and approximately 40% had Nugent scores of 4–10. Longitudinal sampling across visits allowed us to evaluate the temporal association between changes in concentrations of soluble immune mediators and/or bacteria and subsequent changes in *E. coli* inhibitory activity.

The observed association between higher *E. coli* inhibitory activity and higher soluble immune mediator score in genital secretions collected from participants in the two placebo study arms provides insight into the potential role of *E. coli* inhibitory activity in female genital secretions as a biomarker of mucosal inflammation in women with a paucity of vaginal H_2O_2 -producing lactobacilli.⁵ The cytokines IL-1 β , IL-6, IL-12p40, GM-CSF, and the chemokine MIP-1 α were included in the soluble immune mediator score because they could contribute to antibacterial activity by mediating activation, proliferation, and chemotaxis of immune cells, including phagocytes, neutrophils, natural killer cells, T lymphocytes, and antigen-presenting cells (APC). However, this immune activation could paradoxically increase the risk of HIV acquisition and replication by promoting target cell recruitment and by activating the NF- κ B pathway.^{31–33} Significantly, higher genital secretion concentrations of IL-1 β , IL-6, and MIP-1 α were observed in women with chlamydia (IL-1 β , IL-6, MIP-1 α), gonorrhea (IL- β , MIP-1 α), and BV (IL-1 β) relative to women without sexually transmitted infections or BV,^{19,34} suggesting a link between increased mucosal inflammation and active genital tract infection, which could impact HIV acquisition risk. Moreover, previously reported studies show that cervical levels of IL-12, IL-1 β , and IL-6, along with levels of other cytokines and chemokines, correlated with cervical cytobrush yield of APC (IL-12), CD24+ neutrophils (IL-1 β , IL-6), and CD3+ T lymphocyte (IL-1 β , IL-12) subsets.¹⁷ Thus, in women randomized to receive placebo gels (VivaGel placebo or HEC gel), higher *E. coli* inhibitory activity could represent higher cumulative levels of these mediators that promote immune activation and increased risk for HIV seroconversion.

Similarly, lactoferrin was included in the soluble immune mediator score because higher levels in genital secretions could contribute to microbial clearance and *in vitro* activity against *E. coli* but could also contribute to mucosal inflammation and increased HIV seroconversion risk. Lactoferrin exhibits potent antimicrobial activity by depriving bacteria of necessary iron stores, preventing bacterial adhesion to the mucosa, and through iron-

independent bactericidal mechanisms.³⁵ However, lactoferrin is the product of degranulating neutrophils and thus provides a surrogate marker of neutrophil influx. Lactoferrin may also play a role in neutrophil recruitment, proinflammatory cytokine upregulation, and APC activation.¹⁵

SLPI also exhibits antimicrobial properties and may exert bactericidal activity against *E. coli*.^{21,22} SLPI is thought to exert anti-inflammatory effects through inhibition of neutrophil elastase, blunting of macrophage activation in response to bacterial lipopolysaccharides (LPS), and inhibition of the NF- κ B pathway.¹⁶ However, higher levels of SLPI have been described in the setting of mucosal inflammation as part of a potential feedback mechanism.¹⁷

In women who were randomized to receive Viva- Gel, *E. coli* inhibitory activity was not predicted by the soluble immune mediator score. However, higher vaginal concentrations of GBS and *E. coli* were associated with modest but statistically significant decreases in *E. coli* inhibitory activity. These results are consistent with prior studies in which vaginal *E. coli* colonization was inversely correlated with *E. coli* inhibitory activity.² The association between vaginal GBS colonization and diminished *E. coli* inhibitory activity is intriguing, as vaginal GBS acquisition has been associated with more frequent sexual intercourse with more partners and may herald diminished vaginal colonization with H₂O₂-producing lactobacilli and subsequent BV.^{28–30,36} Thus, lower *E. coli* inhibitory activity suggested an increase in pathogenic bacteria that may promote genitourinary infection, adverse pregnancy outcomes, and increased risk of BV.

We did not identify a significant association between H₂O₂-producing *Lactobacillus* score and *E. coli* inhibitory activity, even among women with higher vaginal *Lactobacillus* concentrations. Prior studies have suggested that high levels of *E. coli* inhibition in genital secretions (>90% inhibition) are associated with H₂O₂-producing *Lactobacillus* vaginal colonization.⁷ Surface proteins specific to *L. crispatus* have been implicated in *E. coli* inhibition,¹ and *L. crispatus* isolates demonstrate strain-dependent bactericidal activity against *E. coli*.⁷ Our inability to identify an association between H₂O₂-producing *Lactobacillus* score and *E. coli* inhibitory activity may reflect the higher proportions of women in our study with Nugent scores indicative of intermediate or BV flora and low levels of vaginal H₂O₂-producing *Lactobacillus* by quantitative culture.

It is unclear why soluble immune mediator concentrations predicted *E. coli* inhibitory activity in the placebo study arms (VivaGel placebo and HEC gel arms), while specific bacterial species predicted *E. coli* inhibitory activity in the VivaGel arm. Although exposure to VivaGel was not associated with a significant change in incidence of BV in MTN-004 study participants, women in the VivaGel arm had a greater prevalence of vaginal *Enterococcus* colonization after 1–2 weeks of product use (OR 2.0; 95% CI 1.1, 3.5; $P = 0.01$) and had increased concentrations of vaginal *Enterococcus* (1.1 log₁₀ increase, $P = 0.002$), GBS (1.2 log₁₀, $P = 0.03$), and other coliform bacteria (1.2 log₁₀ increase, $P = 0.005$) relative to women using either placebo product.¹⁴ This is consistent with our observation at Day 7 (but not at Days 14 or 21) of a modest but significant increase from baseline in bacterial score for *Enterococcus*, *E. coli*, and GBS among women who received

VivaGel. Thus, these subtle changes in vaginal bacteria among women using VivaGel could have contributed to our finding that specific vaginal bacterial populations were predictive of *E. coli* inhibitory activity in the VivaGel arm.

Our study is limited by several factors. We did not utilize molecular methods, such as pyrosequencing, that could provide a more detailed representation of vaginal bacterial communities that may impact innate antimicrobial activity in genital secretions. However, some microbes, including *E. coli*, GBS, and *Enterococcus*, are generally not detected by pyrosequencing, as this method cannot detect microbial populations present in low abundance. Study participants were not required to be sexually abstinent during the study; we did not utilize a biomarker to assess for recent vaginal intercourse or for the presence of semen in samples,³⁷ which may significantly reduce endogenous activity against *E. coli* (unpublished data, Dr. Natasha Nakra and Dr. Betsy C. Herold). We also did not determine whether active SPL7013 could be recovered from samples,³⁸ although 3% SPL7013 has not been found to impact *E. coli* growth in a cfu reduction assay (unpublished data, Dr. Bernard Moncla, University of Pittsburgh). Other relevant behavioral variables, such as frequency of anal intercourse and douching, were not included in this analysis, nor did we adjust for study site.^{39–42} Moreover, we quantified only a narrow panel of soluble immune mediators and thus did not assess the relative contributions to *E. coli* inhibitory activity from other key antimicrobial peptides such as α - and β -defensins, which may also impact innate antimicrobial activity in genital secretions^{6,43} and HIV seroconversion risk.⁵ Prior studies investigating reduced activity against *E. coli* in the setting of BV have associated this reduction with a loss of human neutrophil peptides (α -defensins) and β -defensins.⁶ It is also important to note that the mediators that comprised the soluble immune mediator score were selected for their hypothetical roles in promoting mucosal inflammation or impacting HIV acquisition, and this theoretical framework requires validation in future larger studies.

Mechanistic and proteomic studies are needed to identify the precise host and microbial mediators of antimicrobial activity. The development of a cumulative score that can predict mucosal inflammation in diverse populations of women could be useful in identifying women at highest risk for HIV. In addition, elucidating the mediators of *E. coli* inhibitory activity in genital secretions could provide insight into the mucosal events that promote reproductive health or HIV acquisition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table I

Baseline characteristics of study participants

	VivaGel <i>n</i> = 22	VivaGel placebo <i>n</i> = 21	HEC gel <i>n</i> = 18	<i>P</i> value
Age (years), mean $\pm$ SD	20.8 $\pm$ 1.5	21.0 $\pm$ 1.6	20.8 $\pm$ 1.9	0.91
Nugent scores, No. of (%) participants				
Score 0–3	13 (59)	12 (57)	11 (61)	0.97
Score 4–6	6 (27)	2 (10)	2 (11)	0.22
Score >6	3 (14)	7 (33)	5 (28)	0.30
No. of participants (%) with non-detectable H ₂ O ₂ -producing lactobacilli	5 (24)	7 (33)	4 (25)	0.76

SD, standard deviation

Non-detectable H₂O₂-producing lactobacilli defined as $<10^2$ colony-forming units/gram by quantitative culture of vaginal fluid. Quantified lactobacilli data were available for 21 VivaGel recipients, 21 VivaGel placebo recipients, and 16 HEC gel recipients.

Table II

Escherichia coli inhibitory activity and soluble immune mediator scores for each study arm across visits

Study arm	Baseline	Day 7	Day 14	Day 21
Mean (95% CI) <i>E. coli</i> inhibitory activity				
VivaGel	53.3 (38.4, 68.3)	54.9 (43.1, 66.8)	37.8 (24.9, 50.8)	50.8 (35.0, 66.6)
VivaGel placebo	63.0 (47.7, 78.3)	65.6 (53.4, 77.9)	52.7 (34.7, 70.8)	66.7 (54.4, 78.9)
HEC gel	59.8 (42.5, 77.0)	68.4 (51.1, 85.6)	44.8 (28.3, 61.4)	64.5 (45.4, 83.5)
Mean (95% CI) soluble immune mediator scores				
VivaGel	0.21 (−0.30, 0.72)	0.03 (−0.50, 0.56)	0.05 (−0.26, 0.35)	0.11 (−0.25, 0.47)
VivaGel placebo	0.16 (−0.20, 0.52)	−0.67 (−1.21, −0.12)	−0.35 (−0.99, 0.30)	0.10 (−0.30, 0.51)
HEC gel	−0.02 (−0.63, 0.59)	0.38 (−0.01, 0.77)	−0.09 (−0.59, 0.41)	0.24 (−0.12, 0.59)

There was no significant difference in mean *E. coli* inhibitory activity between study arms at baseline, and activity did not vary significantly between visits within each study arm ($P > 0.05$ for all).

Concentrations of interleukin (IL)-1 β , IL-6, IL-12p40, macrophage inflammatory protein (MIP)-1 α , granulocyte-macrophage colony-stimulating factor (GM-CSF), lactoferrin, and secretory leukocyte protease inhibitor (SLPI) were log-transformed to reduce skewness, and principal components extraction was used to generate a cumulative soluble immune mediator score from these variables. There was no significant difference in mean soluble immune mediator score between study arms at baseline or between visits ($P > 0.05$ for all).

Table III

Mean bacterial scores for each study arm across visits

Arm	Baseline	Day 7	Day 14	Day 21
Mean H ₂ O ₂ + lactobacilli scores (95% CI)				
VivaGel	5.15 (3.66, 6.63)	5.05 (3.74, 6.36)	5.31 (4.04, 6.59)	5.54 (4.14, 6.93)
VivaGel placebo	4.67 (3.06, 6.29)	5.44 (4.06, 6.81)	5.66 (4.26, 7.05)	4.80 (3.25, 6.35)
HEC gel	4.79 (3.13, 6.45)	4.96 (3.30, 6.63)	4.54 (2.86, 6.23)	4.54 (2.76, 6.33)
Mean cumulative <i>Enterococcus</i> , <i>Escherichia coli</i> , GBS scores (95% CI)				
VivaGel	4.86 (2.44, 7.28)	10.30 ^a (7.29, 13.30)	8.06 (4.56, 11.56)	6.75 (4.66, 8.83)
VivaGel placebo	3.62 (1.78, 5.46)	4.03 (2.12, 5.94)	3.73 (1.66, 5.81)	4.15 (1.99, 6.32)
HEC gel	3.32 (1.02, 5.61)	2.92 (1.29, 4.55)	1.81 (0.29, 3.32)	3.97 (2.18, 5.76)
Mean BV-associated bacteria scores (95% CI)				
VivaGel	5.06 (2.07, 8.05)	2.11 (0.60, 3.62)	2.57 (0.86, 4.28)	6.24 (3.34, 9.14)
VivaGel placebo	7.42 (4.25, 10.59)	3.46 (1.17, 5.75)	3.96 (1.47, 6.45)	8.53 (4.82, 12.24)
HEC gel	7.16 (3.34, 10.98)	7.96 (3.64, 12.29)	6.79 (2.88, 10.71)	8.09 (4.41, 11.77)

GBS, group B *Streptococcus*; BV, bacterial vaginosis

Bacterial score groupings include cumulative vaginal swab levels of hydrogen peroxide-producing lactobacilli; cumulative levels of *Enterococcus*, *E. coli*, and group B *Streptococcus* (GBS); and cumulative levels of bacterial vaginosis (BV)-associated bacteria (*Gardnerella vaginalis* and anaerobes).

^aThe mean cumulative *Enterococcus*, *E. coli*, and GBS score increased significantly at Day 7 relative to baseline in the VivaGel group ($P = 0.006$), but mean scores at Day 14 and Day 21 did not differ significantly from baseline in this group. Otherwise there were no significant differences in mean scores between visits for any study arm.

Table IV

Multivariable relationship between *Escherichia coli* inhibitory activity, vaginal bacteria populations, and soluble immune mediator score

Covariate	β -Coefficient	95% CI	P value
VivaGel study arm (<i>n</i> = 18 participants)			
Immune mediator score	-1.98	-7.75, 3.79	0.5
H ₂ O ₂ + lactobacilli	1.14	-1.19, 3.47	0.3
<i>Enterococcus</i> , <i>E. coli</i> , GBS	-1.84	-2.89, -0.80	0.001
BV-associated bacteria	-0.90	-2.37, 0.58	0.2
VivaGel placebo study arm (<i>n</i> = 19 participants)			
Immune mediator score	17.49	12.77, 22.21	<0.001
H ₂ O ₂ + lactobacilli	-0.53	-2.40, 1.40	0.6
<i>Enterococcus</i> , <i>E. coli</i> , GBS	-0.02	-1.31, 1.28	1.0
BV-associated bacteria	1.11	-0.42, 2.64	0.2
HEC gel study arm (<i>n</i> = 18 participants)			
Immune mediator score	13.28	4.76, 21.80	0.002
H ₂ O ₂ + lactobacilli	-0.43	-3.35, 2.48	0.8
<i>Enterococcus</i> , <i>E. coli</i> , GBS	-0.20	-3.04, 2.63	0.9
BV-associated bacteria	-0.02	-1.01, 0.96	1.0

Bacterial score groupings include cumulative vaginal swab levels of hydrogen peroxide-producing lactobacilli; cumulative levels of *Enterococcus*, *E. coli*, and group B *Streptococcus* (GBS); and cumulative levels of bacterial vaginosis (BV)-associated bacteria (*Gardnerella vaginalis* and anaerobes).

Generalized estimating equations with a Gaussian link and exchangeable correlation structure were performed with participants stratified by study arm to investigate the effects of cumulative soluble immune mediator and vaginal bacteria scores on *E. coli* inhibitory activity at the subsequent study visit. A total of 145 data points, available from 55 participants, were used for these analyses.