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Protection of the Female Reproductive Tract in the Prevention of HIV

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PROTECTION OF THE FEMALE REPRODUCTIVE TRACT
IN THE PREVENTION OF HIV

by

CAMILA DIAZ

A thesis submitted in partial fulfillment of the requirements
for the Honors in the Major Program in Biomedical Sciences
in The College of Medicine
and in The Burnett Honors College
at the University of Central Florida
Orlando, Florida

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Thesis Chair: Dr. Alexander M. Cole

ABSTRACT

Worldwide, more than half of all HIV-infected individuals are women. Since mucosal surfaces are the primary gateway for HIV entry, maintaining the integrity of the female reproductive tract (FRT) is essential for preventing infection. The FRT employs many immune mechanisms that serve as the first line of defense against HIV transmission. Among these are vaginal fluid secretions rich in antimicrobial peptides, and commensal bacteria that colonize the vagina and prevent infections. We sought to study vaginal fluid as an innate immune component of the FRT in the prevention of HIV infection. Additionally, we investigated the anti-HIV microbicide candidate RC-101 as a possible treatment against pathogenic bacteria that disrupt the healthy microbiota of the FRT and create a suboptimal immune state that increases host susceptibility to viruses, such as HIV.

Here we report that vaginal fluid collected from healthy females inhibits HIV infection. Moreover, our studies reveal that vaginal fluid collected from Black and White women exhibit disparate anti-HIV activity, possibly rendering Black women more susceptible to HIV infection. In addition, we show that RC-101, which is active against HIV, can also inhibit pathogenic bacteria that compromise FRT innate immunity, providing a dual mechanism of protection against HIV acquisition.

Overall, these findings show that vaginal fluid is an important part of female innate immunity that protects the host from heterosexual HIV acquisition. Furthermore, the microbicide RC-101 may prevent HIV infection by both directly preventing viral entry, and by restricting the growth of pathogenic bacteria that disrupt the protective commensal vaginal flora. Together,

innate mechanisms and bolstered protection present a multifaceted approach to maintaining effective host immunity.

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CHAPTER ONE: INTRODUCTION

Globally, there are 33 million people living with human immunodeficiency virus (HIV) (1). Although HIV constitutes a worldwide epidemic, Africa bears most of the burden in the total number of infections. In fact, sub-Saharan Africa has the highest prevalence accounting for as much as 68% of all HIV positive individuals (1). Importantly, nearly 60% of those infected in sub-Saharan Africa are women, a trend that carries over to the global landscape, where women also constitute more than half of all HIV infections (1,2). This observed gender discrepancy warrants a closer look at the possible molecular mechanisms behind increased susceptibility of women to HIV acquisition.

The primary sites of HIV infection are mucosal tissues, such as vaginal and rectal mucosa, where the virus can breach the surface epithelium and gain access to more susceptible cells in the underlying tissue (2-4). Given that heterosexual HIV transmission constitutes the main mode of infection for women, the female reproductive tract (FRT) represents the primary gateway of entry for HIV (1,2,5,6). Here, vaginal and cervical epithelia are bathed in mucosal fluid secretions from endometrial and oviductal fluids, cervical vestibular glands, and plasma transudate (7). This fluid provides multiple modes of innate defense in the FRT. The vagina regularly sloughs off the fluid and epithelial cells, which serves to remove invading microbes (8). Moreover, the contents of these secretions are pivotal contributors to the innate immunity of the FRT. Vaginal fluid is comprised of a complex mixture of antimicrobial peptides and other proteins that together actively fight pathogens threatening to disrupt the integrity of the vaginal environment (5,9,10).

Generally, most antimicrobial peptides and proteins present in vaginal fluid act as microbicides targeted towards bacteria, fungi, and viruses. These antimicrobial agents include lactoferrin, calprotectin, lysozyme, secretory leukocyte inhibitor (SLPI), human neutrophil peptides (HNP1-3), and human beta defensins (HBDs) (9,10). Only recently have studies shown that several of these peptides confer anti-HIV protection in the human host (8). For instance, HBDs have been shown to regulate the CXCR4 co-receptor in T-cells, thereby halting viral entry. HNPs have also been shown to inhibit HIV replication via two mechanisms: in the absence of serum, HNPs inhibit replication before viral integration in T-cells; in the presence of serum, they prevent nuclear translocation of the viral genome and block genomic transcription of the virus (11-13). Although each cationic antimicrobial peptide functions by distinct mechanisms to inhibit HIV entry and replication, no single peptide is responsible for conferring full antiviral immunity to the host. Rather, the antiviral activity of these antimicrobial peptides is dependent on their synergistic activity in vaginal fluid, where they work in concert to prevent HIV infection (8,9). Antimicrobial peptides, therefore, act as effector molecules of FRT innate immunity that serve to protect the mucosal integrity of the genital tract (5). Yet, what remains elusive is whether these vaginal antimicrobial peptides are capable of exerting antiviral properties against all HIV strains. Interestingly, there is a link between distinct HIV subtypes and geographical regions, with different clades being more prevalent in particular locations around the world (14). Elucidating the ability of vaginal fluid to protect the host from diverse HIV isolates is critical in establishing the versatility of FRT innate immunity. Furthermore, the possibility that immunological differences among hosts determine HIV susceptibility and subsequent subtype distribution has yet to be studied. In support of this hypothesis, additional studies have

characterized differences in the expression of antimicrobial peptides among ethnic groups that may lead to differential HIV susceptibility (15). Elucidating FRT innate immune differences among distinct racial groups is essential in identifying ethnic backgrounds that are intrinsically more susceptible to HIV infection.

In addition to the presence of antimicrobial peptides, the vagina is host to a myriad of microbes that play a role in maintaining the FRT free of pathogenic organisms (16,17). The normal vaginal flora is comprised of lactobacillus species that secrete lactic acid and hydrogen peroxide, maintaining an acidic milieu inhospitable to many pathogens (17,18). Certain vaginal infections can disrupt the healthy vaginal microbiota and give rise to numerous disease states. Bacterial vaginosis (BV) is the most prevalent vaginal infection that compromises the innate host defenses and renders the FRT susceptible to foreign invaders (16,17). BV is a transient condition that affects up to 30% of females in the United States and is also the primary reason women seek gynecological care. BV is characterized by colonization of mixed anaerobic bacteria, such as *Atopobium vaginae*, *Mobiluncus curtisii*, *Gardnerella vaginalis*, and *Prevotella bivia*, that come into contact with epithelial cells of the FRT and disrupt commensal microflora (5,16,17). BV is associated with a higher susceptibility to acquisition of sexually transmitted diseases, such as HIV, and an increased rate of medical complications including pre-term birth and pelvic inflammatory disease (17,19). Yet, the interaction of BV bacteria with the FRT innate immune system is not well understood, and therefore the mechanism by which BV induces its pathological effects remains elusive. Previous studies performed by Valore and colleagues have shown that women experiencing BV have vaginal fluid deficient in key antimicrobial peptides (9). Compared to healthy women, vaginal lavage fluid of women with BV had decreased

concentrations of HNPs, SLPI, and HBDs, which are essential cationic peptides that help maintain innate immune competence in the FRT (9,10). As a result, finding effective treatments to combat BV is an essential component in the attempt to maintain an intact FRT equipped to mount an immune response to potentially harmful microbes.

A promising candidate, RC-101, has emerged as a possible treatment for BV. RC-101 is a circular cationic retrocyclin analog composed of 18 residues and three cysteine disulfide bonds (20). Retrocyclins are host defense peptides encoded in the human genome, expressed as transcripts, but not transcribed due to a premature stop codon. Solid phase synthesis has allowed us to recreate and study these lost defense peptides, which exhibit antimicrobial activity (20-23). RC-101 synthesis results in a peptide highly similar to the intact retrocyclin gene with the exception of a single amino acid substitution, which replaces an arginine residue with lysine (21). Currently, RC-101 is being developed as a topical microbicide for the prevention of heterosexual HIV acquisition. This antimicrobial peptide inhibits HIV entry by blocking the 6 helix bundle formation of gp41, an envelope glycoprotein that assists fusion of the virus to host cells (20,22). Importantly, previous studies have demonstrated that RC-101 is non-cytotoxic, non-immunostimulatory, and above all remains stable in the harsh conditions of the vagina (20,23). Although recent studies have focused on the antiviral activity of RC-101, our findings suggest that RC-101 may also act as a bactericidal agent against pathogenic bacteria common in BV, while leaving the commensal vaginal microflora intact. A microbicide that helps maintain a healthy vaginal microbiota, while simultaneously preventing HIV infection represents an optimal prophylactic treatment against pathogens that threaten the genital mucosa.

In the current study, we sought to elucidate the mechanisms by which the FRT maintains immune competence against potential pathogens. We reveal that vaginal fluid confers antiviral properties against diverse clinical HIV strains. Notably, vaginal fluid from Black females is less effective at inhibiting HIV infection compared to vaginal fluid from White females.

Additionally, we show that RC-101 inhibits the growth of BV-associated bacteria that disrupt the healthy microbiota of the FRT. Collectively, these studies suggest that vaginal fluid, an essential innate immune component, confers disparate protection to Black and White women against diverse strains of HIV. Furthermore, the microbicide candidate RC-101 can inhibit BV-associated bacteria, effectively supplementing FRT innate immunity and bolstering host defenses against potential pathogens.

CHAPTER TWO: MATERIALS AND METHODS

Collection and Processing of Vaginal Fluid

Vaginal fluid was collected from postmenarcheal, but premenopausal, female donors according to the guidelines of the Institutional Review Board of the University of Central Florida (8). Female donors afflicted with recent or current vaginal infections or under antibiotic treatment were excluded from the study. Participating donors were asked to identify their race and ethnicity as part of an optional accompanying survey. Whole vaginal fluid samples were homogenized by sonication using a microtip ultrasound probe. Pools of vaginal fluid were created by combining equal volumes of vaginal fluid from between 5-12 donors, vortexing to mix thoroughly, and aliquoting for subsequent use. Vaginal fluid used for antiviral assays was acid-extracted and neutralized. 100 µl of whole vaginal fluid was acid-extracted in 500 µl of 10% acetic acid. The sample was vigorously vortexed for 20 minutes at room temperature, then clarified by spinning at 13,000 rpm for 10 minutes at 4°C. The clarified supernatant was then transferred to a new tube and dried. The acid-extracted vaginal fluid was neutralized twice with water, each time diluting the acid ten-fold, before drying it down to a final volume of 50 µl. The sample was then resuspended in 100 mM sodium phosphate, pH 7.4.

Cell Lines and Viruses

TZM-bl cells, HeLa cell derivatives, were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program. TZM-bl cells stably express CD4, CXCR4, and CCR5 receptors needed for HIV infection. This cell line also possesses a luciferase reporter gene under the control of HIV long terminal repeat region, which allows for accurate quantification of HIV infection. TZM-bl cells were grown in Dulbecco's modified Eagle's medium supplemented

with 100 units/ml penicillin, 100 µg/ml streptomycin, and 10% fetal bovine serum. The HIV strains BaL, 92UG037, 92US712, 93MW960, 92RW008, 91US005, 92UG037, and 94UG114, all R5 strains, were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program. The 33015 clinical strain was a generous gift of Dr. Phalguni Gupta, University of Pittsburg, multicenter AIDS cohort study.

Antiviral and Cytotoxicity Assays

TZM-bl cells were seeded at 5,000 cells/well in black 96-well microtiter plate with optically clear well bottoms. After 24 hrs, cells were treated with 50 µl of culture medium containing acid-extracted vaginal fluid (neutralized with sodium hydroxide) at different dilutions (5%, 2.5%, and 1.25% final dilution) or a vehicle control (1X PBS). Immediately after treating the cells with vaginal fluid or vehicle 25 µl of DEAE dextran was added to the cells (final concentration of 20 µg/ml) along with 25 µl of virus, diluted appropriately. After a 4 hr incubation, treatments were removed and replaced with 50 µl of culture media and 50 µl of acid-extracted vaginal fluid at the appropriate dilutions. The cells were incubated for an additional 20 hrs at 37°C/5% CO₂. The cells were lysed and the amount of luciferase was quantified with Promega Bright-Glo reagent using an LMax luminometer. In parallel viability experiments, cell viability was determined by trypan blue exclusion assay, where the number of live cells treated with vaginal fluid and DEAE dextran was compared to PBS-treated cells with DEAE dextran.

Bacterial Cultures

Mobiluncus curtisii, *Atopobium vaginae*, *Prevotella bivia*, and *Gardnerella vaginalis* were obtained from ATCC. All bacteria were cultured in tryptic soy broth with 5% rabbit's

blood. Snap cultures of *Mobiluncus curtisii*, *Atopobium vaginae*, and *Prevotella bivia* were prepared and subsequently used for experiments. *Gardnerella vaginalis* was incubated aerobically in 37°C/5% CO₂ until use in experiments.

Bacterial Inhibition Assay

From snap cultures, bacteria were spun at 13,000 g for 5 minutes and resuspended in brain heart infusion media. Replicate cultures were mixed with different concentrations of RC-101, also diluted in brain heart infusion media. Cultures were transferred to a Terasaki tray under 3 µl liquid wax to prevent evaporation and incubated in anaerobic chambers at 37°C for 0, 8, 16, or 24 hrs. At each time point, the cultures were diluted in trypticase soy broth as necessary and plated on trypticase soy agar with 5% rabbit's blood. The plates were incubated in anaerobic chambers for up to 1 week. The resulting colonies were manually counted to calculate culture density at each time and condition.

Detection of RC-101 by Tris-Tricine Gel Electrophoresis

RC-101 (5 µg/ml) was incubated at 37°C/5% CO₂ with *Mobiluncus curtisii*, *Atopobium vaginae*, *Prevotella bivia*, and *Gardnerella vaginalis* for 24 hrs. Samples were acid-extracted with 5 volumes of 10% acetic acid and vigorously vortexed for 20 minutes. The samples were then spun at 13,000 rpm and the supernatant was transferred to new tubes. Samples were neutralized with water and dried down to near completion. All samples were then resuspended in sample buffer with 0.5 M dithiothreitol. A portion of each extract was run in a Tris-Tricine gel. Samples were then transferred to a polyvinylidene fluoride membrane and anti-RC-101 rabbit antiserum at 1:1000 was used to detect the presence of RC-101.

CHAPTER THREE: RESULTS

Vaginal Fluid Inhibits Infection by Diverse Clinical Strains of HIV

Previous studies have shown that vaginal fluid inhibits HIV infection of lab adapted HIV strains (17). However, studies investigating the antiviral properties of vaginal fluid against diverse clinical strains of HIV have not been reported. Here we examined the ability of vaginal fluid to inhibit 7 different clinical strains of HIV (see **Table 1**).

| Strain | Subtype | Location |
|---------|---------|---------------|
| 93MW960 | C | Malawi |
| 92RW008 | A | Rwanda |
| 92UG037 | A | Uganda |
| 94UG114 | D | Uganda |
| 92US712 | B | United States |
| 91US005 | B | United States |
| 33015 | B | United States |

Table 1. HIV-1 Primary Clinical Isolates Tested to Demonstrate Clade Diversity.

TZM-bl cells were treated with acid-extracted vaginal fluid at 3 different final concentrations along with DEAE dextran and diluted viral inoculum. After 4 hrs of incubation, treatments were removed and vaginal fluid only was reapplied to the appropriate wells. After 24 hrs infection levels were quantified by the amount of luciferase expressed in each condition.

Figure 1 shows viral inhibition by vaginal fluid pooled from equal populations of Black and White donors. These mixed pools demonstrated inhibition of all HIV clinical strains at >80% by 5% vaginal fluid. Furthermore, the mixed pools maintained at least 50% inhibition of all HIV strains at a vaginal fluid concentration of 2.5%.

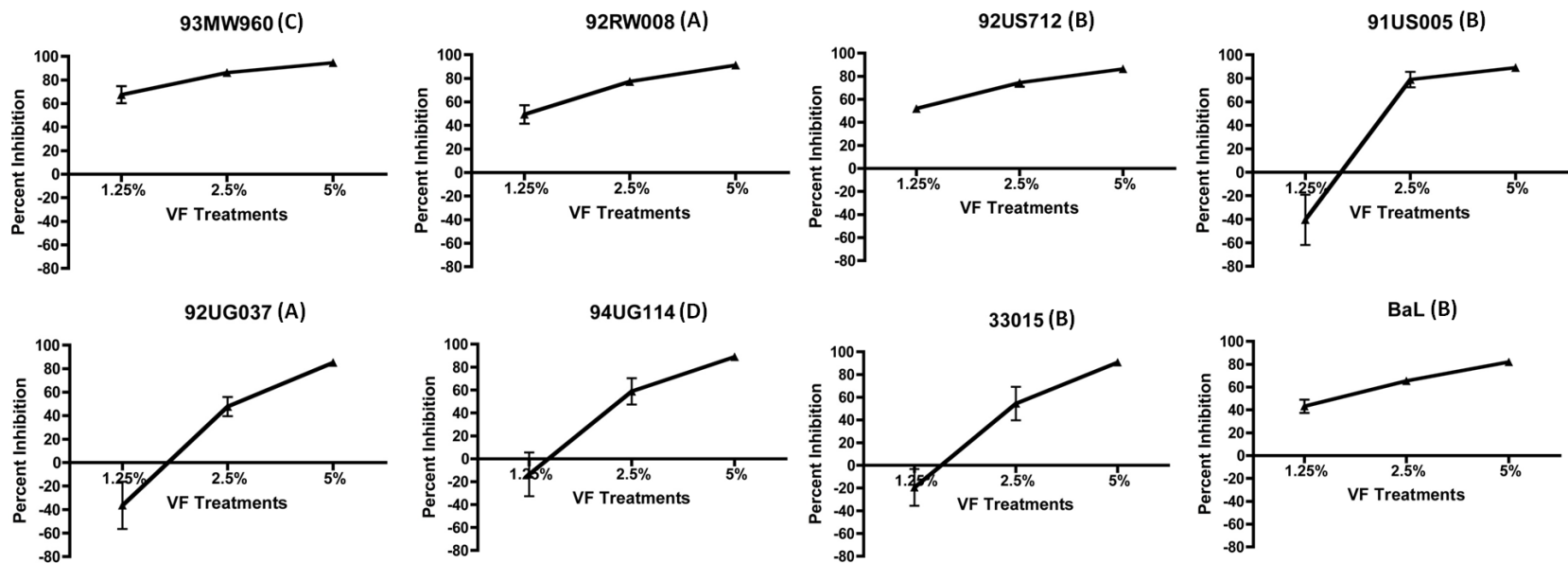


Figure 1. Vaginal Fluid Pools Inhibit HIV Infection by Diverse Clinical Strains of HIV.

Mixed vaginal fluid pools from both Black and White donors were assayed for their ability to inhibit HIV infection. Infection was quantified by measuring luciferase expression in each condition. Values were normalized to a media-only control and expressed as percent inhibition (n=3). The clade of each clinical isolate is given in parentheses. Error bars represent the SEM.

Vaginal Fluid from Black and White Females Confer Differential HIV Susceptibility

Additionally, we explored differences in infection by these clinical strains arising from intrinsic variability of vaginal fluid collected from Black and White females. We pooled vaginal fluid from 6 Black and 6 White females and compared their antiviral effects against the 7 clinical strains of HIV previously shown to be inhibited by the mixed pools of vaginal fluid. **Figure 2** shows a notable difference in the activity of vaginal fluid from Black and White females in viral inhibition. Although both pools inhibit infection at >80% for the highest vaginal fluid concentration, vaginal fluid pooled from White females consistently shows greater inhibition of all clinical strains compared to vaginal fluid pooled from Black women. The difference in antiviral activity of 5% vaginal fluid was significant for 3 out of the 7 clinical HIV isolates tested. Interestingly, 2 out of these 3 clinical isolates were A clade viruses, while the remaining clinical isolate was a B clade virus.

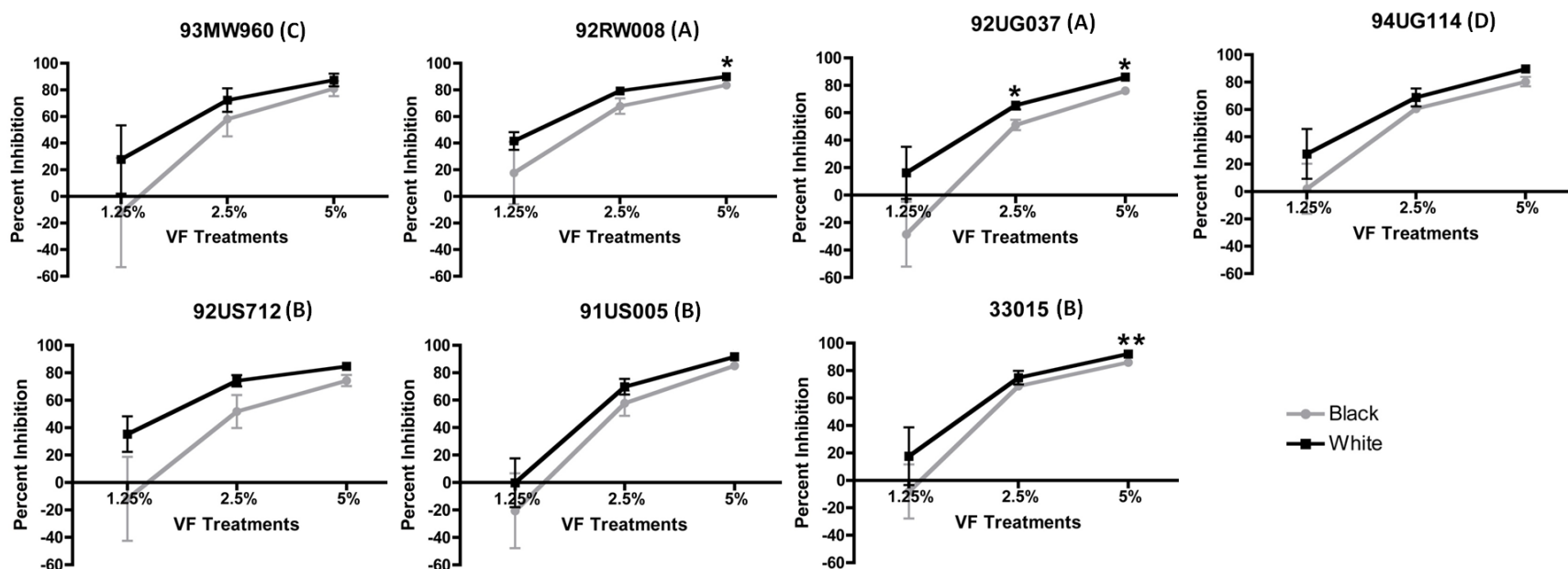


Figure 2. Vaginal Fluid From White Females Shows Greater HIV Inhibition than Vaginal Fluid from Black Females.

Vaginal fluid pooled from either White or Black donors was assayed for antiviral activity. Infection was quantified by measuring luciferase expression in each condition. Values were normalized to a media-only control and expressed as percent inhibition (n=3). The clade of each clinical strain is given in parentheses. Error bars represent the SEM. Significant differences in inhibition between Black and White vaginal fluid pools in a two-tailed t-test are shown by *= $p < 0.05$; **= $p < 0.01$

Tests for viability were performed in parallel to ensure that vaginal fluid did not exhibit cytotoxic effects on TZM-bl cells. Cell viability was established by trypan blue exclusion assay where the number of live cells were counted for each condition that received vaginal fluid and normalized to a media-only control. **Figure 3** reveals that vaginal fluid did not induce any cytotoxic effects on the cells.

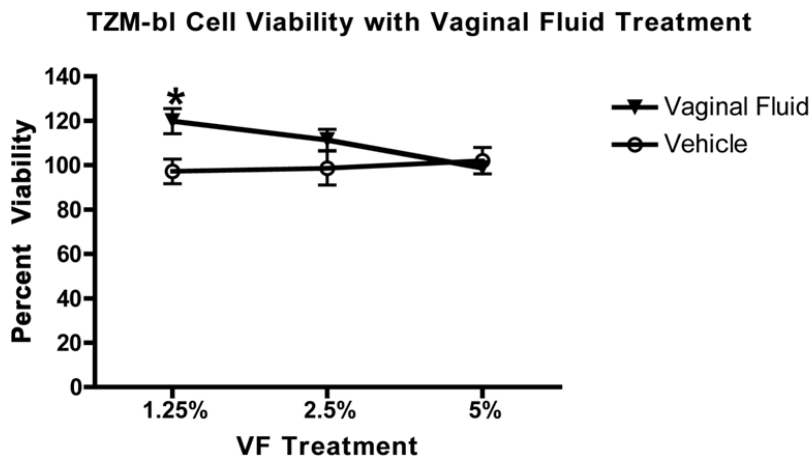


Figure 3. Acid-extracted Vaginal Fluid Is Non-cytotoxic to TZM-bl Cells.

Cells were treated in parallel to antiviral experiments, without addition of virus. Cell viability was determined by trypan blue exclusion assay. The number of live cells in each condition was normalized to a media-only control and values were expressed as percent viability (n=3). Error bars represent the SEM. Significant differences by a two-tailed t-test between vaginal fluid and PBS (vehicle) treatments are shown by *=p<0.05

Interestingly, vaginal fluid at a concentration of 1.25% appeared to stimulate cell growth compared to the PBS treatment. Factors responsible for this proliferative effect have yet to be identified. Together, these results indicate that vaginal fluid confers protection against HIV

infection to the female host; however, the antiviral activity of vaginal fluid from Black women is less effective at inhibiting HIV infection compared to vaginal fluid from White women.

These results emphasize that vaginal fluid, rich in antimicrobial peptides, represents an essential immune component that protects the mucosal surfaces of the vagina from HIV infection. Another equally important element that helps maintain the integrity of the FRT is the commensal bacterial flora that colonize and protect the vagina.

RC-101 Remains Intact in the Presence of BV-associated Bacteria

BV is a prevalent condition in females that can disrupt the healthy vaginal microbiota and increase host susceptibility to HIV acquisition. Previous studies have shown that women with BV have vaginal fluid lacking in important antimicrobial peptides compared to healthy women (18). This establishes a possible mechanism by which BV compromises innate defenses and increases host susceptibility to HIV infection. Thus, supplementing vaginal fluid with antimicrobial peptides may reestablish full innate immune competence in the FRT. To assess the potential of RC-101 as a therapy for women with BV, we first determined the ability of the peptide to remain intact in the presence of common BV-associated bacteria. BV-associated bacteria, represented by *M. curtisii*, *A. vaginae*, *P. bivia*, and *G. vaginalis* were incubated for 24 hrs with RC-101; samples were subsequently acid-extracted and western blotted to detect the presence of RC-101. **Figure 4** shows the peptide was recovered from all the culture conditions incubated with RC-101 and that the peptide migrated along side the RC-101 standard. These results suggest that RC-101 remains intact in the presence of pathogenic bacteria.

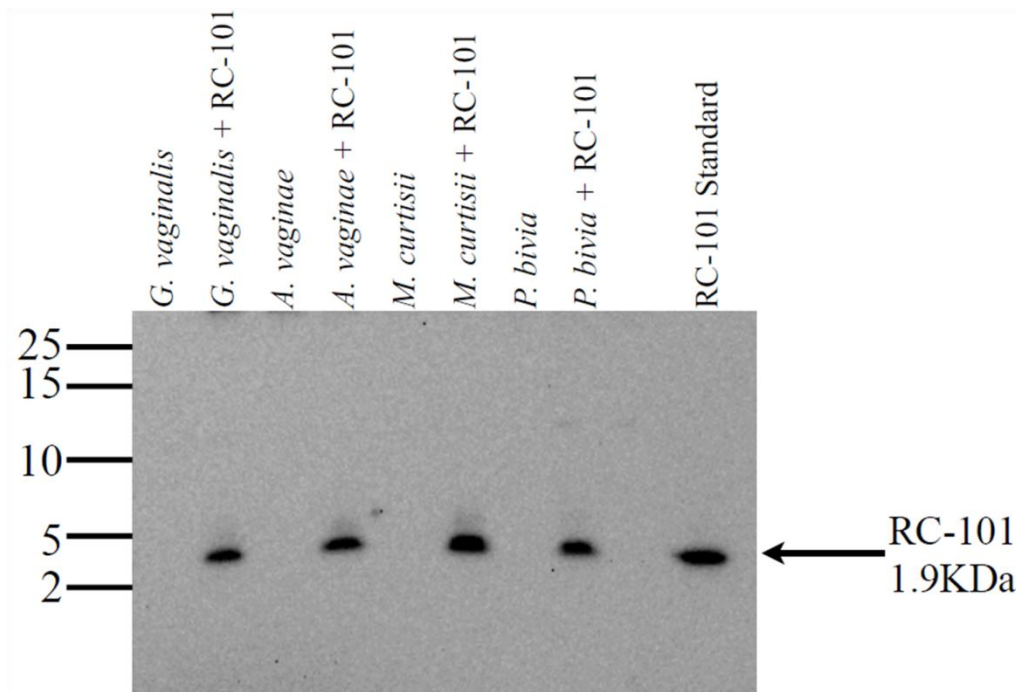


Figure 4. RC-101 Remains Intact in the Presence of Pathogenic Bacteria.

RC-101 was incubated in cultures of BV-associated bacteria. Cultures were acid-extracted and analyzed by western blot alongside an equivalent RC-101 standard. The blot shown is one representative of three independent experiments.

RC-101 Inhibits the Growth of Anaerobic Pathogenic Bacteria

After establishing that RC-101 is not degraded by BV-associated bacteria, we next tested the effectiveness of the peptide in inhibiting the growth of pathogenic bacteria. *M. curtisii*, *A. vaginae*, and *P. bivia* were incubated with different RC-101 concentrations for up to 24 hrs. These bacteria were also incubated with 0.5 µg/ml of clindamycin, the standard antibiotic used to treat BV. Culture density was determined for all conditions and the results were reported as percent inhibition compared to untreated culture controls. **Figure 5** shows that RC-101 effectively restricts the growth of all three bacteria at 24 hrs.

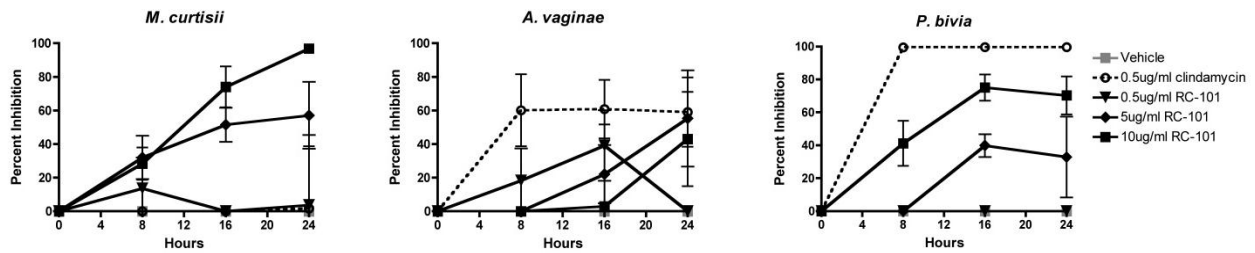


Figure 5. RC-101 Inhibits the Growth of Pathogenic Bacteria.

BV-associated bacteria *M. curtisii*, *A. vaginae*, and *P. bivia* were incubated with RC-101 or clindamycin for up to 24 hrs and plated to assess culture growth. Results are shown as percent inhibition compared to untreated cultures. Percent inhibition values lower than zero were plotted as zero percent inhibition.

RC-101 inhibits the growth of all pathogenic bacteria tested. At 24 hrs, 10 µg/ml RC-101 achieved >40% inhibition of all three bacteria. Additionally, RC-101 at 5 µg/ml and 10 µg/ml exhibited significantly greater inhibition of *M. curtisii* than clindamycin. Importantly, other data from our group (C.R.E., not shown) revealed that RC-101 at the same concentrations tested above does not inhibit the growth of 6 *Lactobacillus spp*, commensal bacteria found in the vagina. Collectively, these data suggest that RC-101 can act as a selective bactericidal agent, effectively inhibiting the growth of common pathogenic bacteria while leaving the protective microflora of the female vagina intact.

CHAPTER FOUR: DISCUSSION

The innate immunity of the FRT plays an essential role in determining host susceptibility to HIV acquisition. Vaginal fluid represents one protective immune component that maintains the integrity of the FRT and strengthens host defenses against foreign invaders. Our studies suggest that vaginal fluid from both Black and White females is active against infection by diverse HIV clinical strains. Yet, we showed that vaginal fluid from both races does not possess equal antiviral activity against HIV infection. Vaginal fluid from Black females consistently exhibited lower inhibition of HIV compared to vaginal fluid from White females. This difference in antiviral activity of vaginal fluid has profound implications for Black women, who may be more susceptible to HIV acquisition compared to their White counterparts. In support of this, African Americans are the group most affected by new HIV infections in the United States. In fact, the rate of HIV acquisition for African American women is significantly greater compared to White women (21). Although socioeconomic factors cannot be discounted as contributing factors for the observed differences in HIV infections between women of different races, our findings suggest the possibility that variations in innate immune components of the genital mucosa among White and Black women may contribute to this discrepancy in HIV transmission. Moreover, such susceptibility differences may contribute to the strikingly high rate of HIV occurrence in Black women across the globe.

In addition to investigating vaginal fluid as an innate immune component of the FRT, we also explored the ability of the microbicide candidate RC-101 in maintaining the integrity of the FRT. Another aspect of female innate immunity that plays an essential role in HIV protection is the commensal resident microbiota of the vagina. Previous studies have shown that when the

healthy bacterial flora is disrupted, vaginal fluid lacks key antimicrobial peptides, which may lead to a higher risk of HIV infection (24). As a result, developing an effective treatment that combats BV and maintains the integrity of the FRT is an important step in decreasing female susceptibility to HIV acquisition. In our study, we assessed the ability of the anti-HIV microbicide RC-101 to inhibit the growth of pathogenic bacteria commonly associated with BV. Interestingly, at 24 hrs the highest concentrations of the peptide significantly inhibited the growth of *M. curtisii* compared to clindamycin, which is the preferred treatment for BV. Our studies further suggest that RC-101 remains intact in the presence of *M. curtisii*, *A. vaginae*, *P. bivia*, or *G. vaginalis*, all pathogenic bacteria characteristically present in BV. These findings make RC-101 a potent prophylactic agent which may possess a dual mechanism of protection against HIV infection by both directly preventing viral entry, and by restricting the growth of pathogenic bacteria that disrupt protective commensal vaginal flora. Collectively, these findings elucidate novel mechanisms of HIV protection that warrant further investigation.

The observed difference in anti-HIV activity of vaginal fluid from Black and White women sets the stage for future studies that will characterize protein differences in vaginal fluid from these two races. Such disparities in protein content may contribute to a higher risk of HIV infection in Black females. In addition, future studies on RC-101 will determine the ability of this microbicide to inhibit the growth of BV-associated bacteria in the presence of vaginal fluid and explore possible synergistic effects with endogenous host defense peptides. Overall, these studies will contribute to a greater understanding of host-HIV interactions that elucidate promising targets for the prevention of HIV transmission.

APPENDIX: RACIAL AND ETHNIC COMPOSITION OF VAGINAL FLUID POOLS

| Pool | Race | | | Ethnicity | | |
|--------------|----------|----------|---------|-----------|--------------|---------|
| | White | Black | Unknown | Hispanic | Not Hispanic | Unknown |
| Mix 1 | 6 (50%) | 6 (50%) | 0 (0%) | 1 (8%) | 5 (42%) | 6 (50%) |
| Mix 2 | 6 (50%) | 6 (50%) | 0 (0%) | 2 (16%) | 5 (42%) | 5 (42%) |
| Mix 3 | 5 (42%) | 6 (50%) | 1 (8%) | 1 (8%) | 5 (42%) | 6 (50%) |
| B1 | 0 (0%) | 6 (100%) | 0 (0%) | 0 (0%) | 4 (67%) | 2 (33%) |
| W1 | 6 (100%) | 0 (0%) | 0 (0%) | 2 (33%) | 1 (17%) | 3 (50%) |
| B2 | 0 (0%) | 6 (100%) | 0 (0%) | 0 (0%) | 4 (67%) | 2 (33%) |
| W2 | 6 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | 4 (67%) | 2 (33%) |
| B3 | 0 (0%) | 6 (100%) | 0 (0%) | 0 (0%) | 5 (83%) | 1 (17%) |
| W3 | 6 (100%) | 0 (0%) | 0 (0%) | 1 (17%) | 2 (33%) | 3 (50%) |

Table 2. Racial and Ethnic Composition of Vaginal Fluid Pools.

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