Bacterial vaginosis associated microbes induce altered innate immune profiles in the female reproductive tract

Ryan K. Cheu[1,2], Avid Mohammadi[3], Tiffany Hensley-Mcbain[1,2], Jennifer Manuzak[1,2], Courtney Broedlow[1,2], Charlene Miller[1,2], Mark Yudin[4], Rupert Kaul[3], Nichole R. Klatt[1,2]

[1]*Department of Pharmaceutics, University of Washington, Seattle, WA, USA,* [2]*Washington National Primate Research Center, Seattle, WA, United States,* [3]Department of Immunology, University of Toronto, Toronto, Canada, [4]Core Obstetrics & Gynecology, University of Toronto, Toronto, Canada

Abstract

Background: Bacterial vaginosis (BV) is associated with inflammation and an increased transmission rate of HIV[1], however, the mechanism(s) underlying this are not clear. BV is characterized by an increased diverse anaerobic community, including *G. vaginalis*, versus a predominantly *Lactobacillus spp.* environment. Inflammatory cells such as neutrophils are critical for innate immune responses but can also contribute to barrier damage and inflammation. Currently, the role of neutrophils in HIV transmission and BV status is unknown.

Methods: In order to elucidate the mechanisms underlying inflammation in BV, we collected cervicovaginal cytobrushes from 11 women with BV (Nugent >7), and 10 without BV (Nugent <5). We used flow cytometry to assess phenotype and functionality of neutrophils and performed *in vitro* whole blood co-cultures with bacteria associated with BV (*G. vaginalis*), healthy commensals such as *L. iners* and *L. crispatus*, media alone (negative control) and lipopolysaccharide and peptidoglycan (positive controls).

Results: We demonstrated increased total neutrophils, increased neutrophil activation via CD62L downregulation ($p<0.0001$) and prolonged lifespan via decreased caspase-3 expression ($p<0.0001$) in the cytobrushes with women with BV. Similarly, in our co-culture experiments we observed more neutrophil activation ($p=0.0001$) and prolonged lifespan ($p=0.0018$) in cultures with *G. vaginalis* similar to our positive controls. Of interest, women without BV had reduced inflammatory immune responses similar to our *in vitro* experiments with cultures containing *Lactobacillus spp.* that maintained immune cell phenotypes similar to negative controls.

Rationale

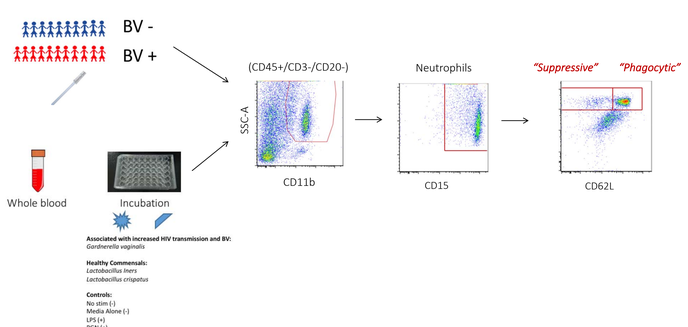
In the female genital tract (FGT), *Lactobacillus* species help to inhibit colonization by pathogenic bacteria and are associated with protection from HIV and other STIs. However, colonization by more diverse communities of anaerobic bacteria, notably *Gardnerella vaginalis*, is common and associated with the development of BV. A recent meta-analysis linked BV to a 60% increase in HIV acquisition rates.[1] Maintenance of the mucosal barrier is critical for preventing invading microorganisms, including HIV, and bacterial diversity in the FGT has been strongly associated with negative consequences for FGT mucosa.[2] Defining the mechanism(s) for how BV increases HIV acquisition rates will inform the rational design of better prevention for individuals who are most susceptible.

Hypothesis

BV-associated bacteria will induce neutrophil activation and prolonged life, promoting neutrophil accumulation in the female reproductive tract (FRT), thereby potentially promoting tissue damage

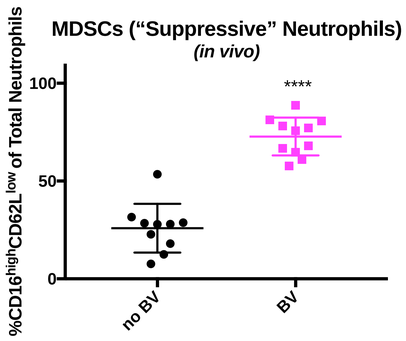
Methods

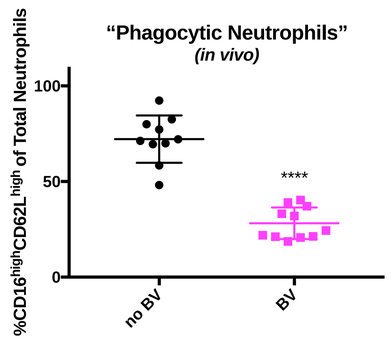
- Collect cervicovaginal cytobrushes from BV+/- women and assess phenotype and functionality of neutrophils
- Collect whole blood samples from HIV- individuals and incubate with BV associated bacteria

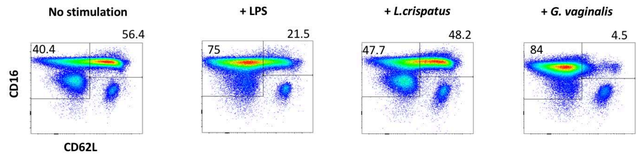


Results

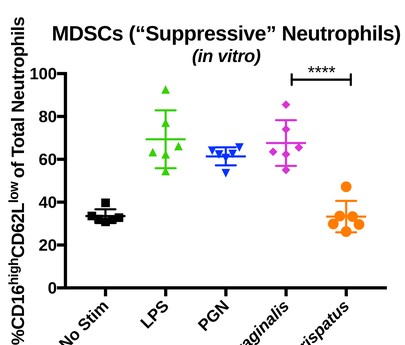
Cytobrushes from BV+ women demonstrate "suppressive" neutrophil phenotype, but not "phagocytic" neutrophils

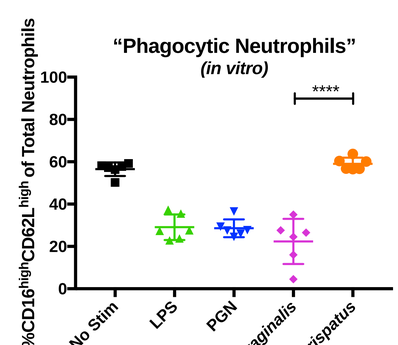






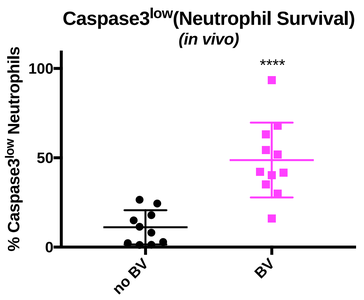
Microbiota associated with BV induce "suppressive" neutrophil phenotype, but not "phagocytic" neutrophils

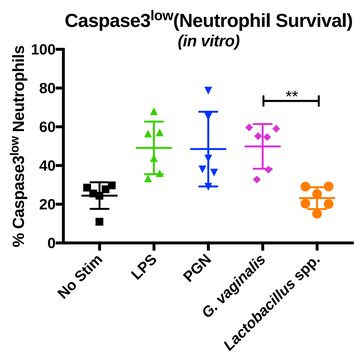


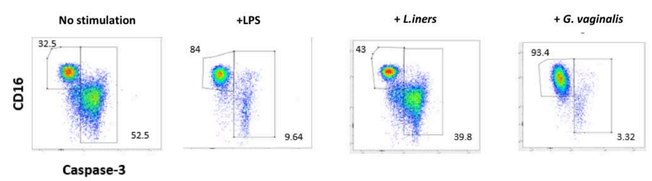


- Suppressive neutrophils may induce exhaustion of T cells
- Phagocytic neutrophils may help contain microbial translocation

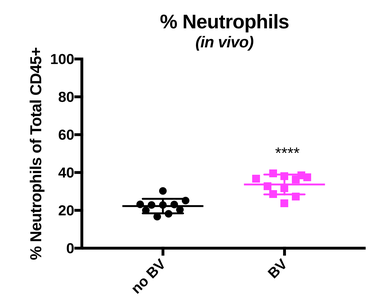
Neutrophil accumulation potentially due to enhanced survival as seen in cytobrushes from BV+ women and BV-associated bacteria

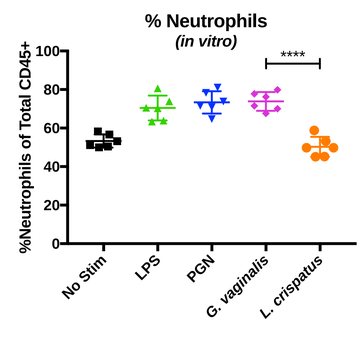






Cytobrushes from BV+ women and BV-associated bacteria demonstrate increases in total neutrophils





- Neutrophils are normally short-lived, preventing damage to tissues
- Neutrophils with prolonged survival may promote further epithelial damage

Conclusions

Here, we demonstrate that BV-associated bacteria induce neutrophil activation and delay apoptosis as a potential mechanism for promoting tissue damage. Women without BV, that have healthy vaginal mucosal bacterial communities (*Lactobacillus*) may support the balance between antimicrobial function of neutrophils and do not induce inflammation, whereas women with BV and dysbiotic vaginal communities (*G. vaginalis*) potentially lead to neutrophil accumulation and tissue damage. Thus, this study provides potential mechanistic insights into how BV may lead to FRT inflammation and increased HIV transmission.



Presenting Author

Ryan Cheu
rycheu@uw.edu
Graduate Student, University of Washington

Acknowledgements

We would like to thank the University of Washington Center for AIDS Research (CFAR) and Core Obstetrics & Gynecology from the University of Toronto for obtaining samples and start-up funds from the University of Washington Department of Pharmaceutics to NK.

References

[1]Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS. Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. AIDS (London, England). 2008 Jul 31;22(12):1493.

[2]Burgener A, McGowan I, Klatt NR. HIV and mucosal barrier interactions: consequences for transmission and pathogenesis. Current opinion in immunology. 2015 Oct 31;36:22-30.