SCFAs impact neutrophil response to HIV infection

Francisco J Carrillo-Salinas[1], Ragav S Parthasarathy[1,2], Laura Moreno de Lara[1,3], Anna Borchers[1], Christina Ochsenbauer[4], Alexander Panda[5,6], Marta Rodriguez-Garcia[1,2].

[1]Department of Immunology, Tufts University School of Medicine, Boston, MA
[2]Immunology Program, Tufts Graduate School of Biomedical Sciences, Boston, MA
[3]Immunology Unit, Biomedical Research Centre (CIBM), University of Granada, Granada, Spain
[4]Department of Medicine and UAB Center for AIDS Research, University of Alabama, Birmingham, AL
[5]Tufts Medical Center/Division of Pulmonary and Critical Care (PCCM), Boston, MA
[6]Tufts Clinical and Translational Science Institute (CTSI), Boston, MA

INTRODUCTION: Sexual transmission is the main route for human immunodeficiency virus (HIV) acquisition in women. Local innate immune mechanisms in the female reproductive tract (FRT), such us epithelial cell barrier, mucus and secreted immune mediators, contribute to the prevention of HIV infection. Neutrophils participate in mucosal protection against pathogens through phagocytosis, release of granule contents, and NETosis. NETosis is characterized by the release of Neutrophil Extracellular Traps (NETs), consisting of DNA associated with granular proteins with antimicrobial activity. Neutrophils are abundant in the FRT and can recognize, entrap, and inactivate HIV with NETs *in vitro*. Growing evidence indicates that bacterial vaginosis, alterations in vaginal microbiota mainly due to a reduction in relative abundance of *Lactobacillus* species, decreases the concentration of lactic acid and increases short-chain fatty acids (SCFAs) concentration, promoting colonization of harmful bacteria and increasing the risk of viral infections, such as HIV. Importantly, neutrophils highly express GPR43, the main SCFA receptor. In this context, we hypothesize that neutrophil-microbiota interactions impact innate immune protection in the FRT and the risk for HIV infection.

METHODS: Human purified neutrophils from blood were plated and stimulated with 25 mM of sodium acetate, sodium butyrate or sodium propionate, in the presence or absence of HIV-viral like particles (VLPs)-GFP labeled for at least 3h. NETosis was quantified as capture area (overlap of NETs and HIV-VLPs) using time-lapse microscopy Incucyte S3 system. Neutrophil migration was assessed stimulating neutrophils with 25 mM of the correspondent SCFA, using a transwell migration assay. Neutrophil phenotype was determined by flow cytometry using Cytek Aurora analyzer.

RESULTS: Pathological concentrations of SCFAs (butyrate or propionate) induced in neutrophils a sustained and stronger release of NETs in response to HIV-VLPs compared to untreated neutrophils. Although SCFAs can be chemoattractants for neutrophils, they migrated at the same ratio when stimulated with acetate and propionate, compared to controls; or significantly less in the presence of butyrate. Interestingly, only propionate-treated neutrophils significantly upregulated high levels of ICAM-1 after migration, a key molecule for transmigration into tissues. In addition, SCFAs significantly increased the proportion of $CD66b^+$ $CD16^+$ and $CD62L^+$ neutrophils. Finally, stimulation with SCFAs altered cytokine and chemokine secretion.

CONCLUSIONS: Our results demonstrate that increased concentrations of SCFAs trigger neutrophil activation and alter their response to HIV infection. Ongoing experiments will shed light about the role of SCFAs on modifying the function of tissue-derived neutrophils in protecting the FRT.