

**Toll-Like Receptor Expression and Function in the Genital Tract of HIV-1  
Resistant Commercial Sex-Workers.**

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**A thesis submitted in partial fulfilment for the degree of master of science in molecular  
medicine in the Jomo Kenyatta University of Agriculture and Technology**

**2009**

## ABSTRACT

Toll-Like receptors (TLR) are pattern recognition receptors; a component of the innate immune system that are involved in the recognition of pathogen-associated molecular patterns (PAMPs). Recent studies have show that TLRs are able to recognize microbial components for pathogens causing sexual transmitted infections that increase the risk for acquiring HIV-1 virus. TLR7/8 was recently shown to recognize guanine and uridine rich ssRNA with sequence homology to ssRNA from the HIV-1 virus. This study tested the cytokine responses to TLR agonists by cervical mononuclear cells (CMCs) from the human female genital tract. The hypothesis for the study was that HIV-R women (HIV negative >3years) have different cytokine responses in CMCs after TLR stimulation compared to HIV susceptible (HIV negative <3 years) and HIV positive sex workers. The TLR agonists used were *Escherichia coli* Lipopolysaccharide (TLR4 ligand), ssRNA40/LyoVec (TLR 7/8 ligand) and Imiquimod (TLR7 ligand). The amount of cytokine produced was measured from culture supernatants using cytokine bead arrays and ELISA techniques. The main responses detected were TNF- $\alpha$ , IL-10, IFN- $\gamma$  and IFN- $\alpha$ , with minor levels of IL-6, IL-5, IL-4, and IL- 2 responses. HIV-R women had significantly lower TNF- $\alpha$  responses to *E. coli* LPS compared to HIV susceptible women p=0.0309, significantly lower TNF- $\alpha$  to ssRNA40/LyoVec compared to HIV-P women p=0.0043. A trend for lower TNF- $\alpha$  responses in the CMCs from HIV-P women compared to HIV-S women p=0.0752 was observed. Imiquimod stimulated significantly higher TNF- $\alpha$  responses in HIV-S compared to HIV-P women p=0.0226. HIV-R women had significantly lower IL-10 responses to *E. coli* LPS compared to both HIV-P p=0.0137 and HIV-S p=0.0196. HIV-R also had significantly lower IL-10 responses to ssRNA40/LyoVec compared to HIV-P p=0.0276 and lower IL-10 responses to Imiquimod compared to HIV-S p=0.0316. The IFN- $\gamma$  response in HIV-P women was

significantly higher than HIV-S following stimulation with ssRNA  $p=0.0179$  and Imiquimod  $p=0.0032$ , and higher than HIV-R after Imiquimod stimulation  $p=0.0446$ . Overall, CMCs from the genital tract of HIV-1 resistant women had lower proinflammatory responses to TLR ligands compared to HIV susceptible women. The two TLR7 signaling ligands ssRNA and Imiquimod stimulated different cytokine profiles, which indicate the possibility of use of different TLR signaling agents in immune-modulation to alter the cytokine profiles in the genital tract. These results help improve the understanding of the innate cytokine responses by CMCs in the genital mucosa, and may thus point to innate mechanisms that influence susceptibility to HIV-1 infection.