The Role of Inflammatory Cytokines and TLR Ligands in Modulating Genital Tract-derived Dendritic Cell Activation

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Background: Topically applied 1% Tenofovir gel showed a reduction in HIV incidence by 39% in South African women. Irrespective of gel use, women who became infected had higher genital levels of IL-1α, IL-1β, IL-8, MIP-1α, MIP-1β, and TNF-α compared with those who remained uninfected. We investigated the impact of these cytokines and TLR stimulation on whole blood and genital tract-derived dendritic cell function and activation.

Materials & Methods: Whole blood and fresh cervical tissues were incubated with cytokines cocktail (IL-1β, IL-8, MIP-1β and TNF-α) or ligands for TLR 2/1 (PAM3), TLR4 (LPS) or TLR7/8 (R848) in the presence or absence of IL-10.

Results: In blood, DC function (IL-6 expression) and activation (CD40 expression) were measured by flow cytometry.

In tissues, frequencies and activation (HLA-DR expression) of cells emigrating from tissues at 48 hours were measured by flow cytometry.

Results and conclusion: TLR ligands and cytokines induce IL-6 and or CD40 expression in whole blood DCs. Addition of IL-10 decreases IL-6 but not CD40 expression. Inflammatory cytokines and TLR ligands also induce activation of migrating DC in genital tract. However, there was no clear pattern on which condition induced more migration. LPS and cytokines seem to induce greater activation compared with PAM3 and R848. Increased levels of genital tract cytokines may increase risk of HIV acquisition by increasing DC migration and activation. This could possibly have an additional effect on mucosal strategies to reduce HIV transmission in the female genital tract.

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Figure 1: Whole blood DC identification and IL-6 expression after 6 hours of culture. A. Neutrophils (CD66c/CD15), and monocytes (CD14+) were excluded and myeloid and plasmacytoid DCs were identified as CD11c+ and CD123+, respectively. IL-6 expression was evaluated on myeloid DCs (B, C and D). Concentrations: IL-1β, IL-8 and MIP-1β at 1 μg/mL; TNF-α at 0.1 μg/mL; IL-10 at 0.1 μg/mL. Wilcoxon test was used for statistical analyses. B & C

Figure 2: Activation of whole blood DCs by TLR7/8 ligand and cytokines after 18 hours of culture. DCs were identified as in Figure 1A. Activation was determined by CD40 expression. Wilcoxon test was used for statistical analyses. Concentrations: IL-1β, IL-8 and MIP-1β at 1 μg/mL; TNF-α at 0.1 μg/mL; IL-10 at 0.1 μg/mL

Figure 3: Identification and activation of genital tract DCs after 48 hours of culture. A. After excluding dead and epithelial cells, DCs were identified as in Figure 1A. Frequencies of migrating cells (B) and upregulation of HLA-DR expression (C) were determined.

Figure 4: Migration and activation of genital tract DCs by cytokines and TLR ligands. Data presented as median with interquartile range. IL-1β, IL-8 and MIP-1β at 1 μg/mL; TNF-α at 0.1 μg/mL; R848: 1 μg/mL; LPS: 50 μg/mL; PAM3: 1 μg/mL. Wilcoxon test was used for statistical analyses. p<0.05, when compared with medium. M117F

Figure 5: Migration and activation of fresh cervical DCs by cytokines and TLR ligands. Data presented as median with interquartile range. IL-1β, IL-8 and MIP-1β at 1 μg/mL; TNF-α at 0.1 μg/mL; R848: 1 μg/mL; LPS: 50 μg/mL; PAM3: 1 μg/mL. Wilcoxon test was used for statistical analyses. p<0.05, when compared with medium. M117F.