

Title: Gene Expression Profile of Vaginal Epithelial Cells Exposed to Proinflammatory Agents—Identification of Novel Biomarkers of Mucosal Safety

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Background: Susceptibility to HIV infection may be increased by cervicovaginal mucosal inflammation and immune activation. Therefore, it is important to develop biomarkers that identify these conditions and can be used in the selection of safe and efficacious anti-HIV microbicide candidates. The purpose of this study was to analyze gene expression profiles of human vaginal cells exposed to proinflammatory and noninflammatory compounds in order to identify these biomarkers.

Methods: VK2/E6E7 cells were treated with noninflammatory (NIC) and proinflammatory (PIC) compounds. NIC (class 1) included hydroxyethyl and carboxymethyl cellulose. PIC included cytotoxic surfactant compounds, nonoxynol-9, C31G and benzalkonium chloride (class 2), TLR ligands, PAM3CSK4, MALP-2 and imiquimod, and the proinflammatory cytokine, TNF- α (class 3). Gene expression was evaluated by cDNA microarray technique using Affymetrix oligonucleotide GeneChip Human Genome U133 Plus 2.0. Genes showing statistically significant different expression between groups were identified using ANOVA or t-test with false discovery rate set to 0.05.

Results: Comparison between PIC and NIC groups identified around 500 differentially expressed genes in class 2 and about 100 genes in class 3 with at least 2-fold upregulation compared to class 1. Among them, about 80 genes were upregulated in both PIC groups reflecting common cellular responses. Gene set enrichment analysis revealed that the NF- κ B pathway (genes activated by NF- κ B) was upregulated in most PIC treatments. Eleven genes were selected for further analysis. Most of these genes are involved in immune and/or inflammatory responses. A custom-made heatmap allowed for easy identification of PIC and NIC. Microarray results for these genes were confirmed by qPCR. Protein expression of four selected genes, CCL20, PTGS2, IL8 and IL13RA2 was detected by immunoblot, flow cytometry, or ELISA.

Conclusions: Gene expression profiling revealed a group of genes that is significantly upregulated in human vaginal cells in response to immunoinflammatory stimuli, and therefore, can serve as biomarkers of genital mucosal inflammation and immune activation.