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Johnson Mosoko Moliki is a PhD student and Carnegie Fellow in the Department of Molecular and Cell Biology, University of Cape Town. His doctoral research addresses the main question of whether glucocorticoids and hormonal contraceptives regulate mucosal barrier and immune functions in the female genital tract and how this regulation influence HIV infection and transmission.

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ISID Fellowship Report

Skills development and technology transfer to investigate the molecular mechanisms of regulation of primary epithelial cell tight junctions of the female genital tract by contraceptives, hormones and HIV-1

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Background

The female genital tract (FGT) is the main route of HIV-1 infection and transmission. Although the FGT is lined by a protective barrier of epithelial cells, evidence in the literature suggest that HIV-1 can disruption this barrier to establish infection. How the virus does this is not clearly understood, but mechanisms such as the dysregulation of tight junctions responsible for maintaining the epithelial barrier (1) and transcytosis (2) have been suggested. Tight junctions are a complex of interconnecting transmembrane proteins that seal-off the paracellular spaces between epithelial cells and in the FGT, HIV-1 has been shown to up-regulated the production of tumour necrosis factor (TNF)-alpha to disrupt these junctions (1). This suggest that the virus is capable of harnessing host factors to its deleterious end. But apart from TNF-alpha, the role of other factors that could regulate epithelial barrier function in the FGT is not known and how these factors modulate HIV-1 mucosal infection is only speculative. Glucocorticoids are potent regulator of epithelial barrier function (3) and HIV-1 pathogenesis (4). They mediate their actions through the glucocorticoid receptor (GR). But this receptor is promiscuous and can be activated by structurally related compounds such as medroxyprogesterone acetate (MPA), a synthetic steroid widely used in hormonal contraceptives (5). Recent observational studies suggest MPA might increase the risk to HIV-1 acquisition and transmission (6). Could MPA acting via the GR modulate HIV-1 entry in the FGT? Preliminary data from the Hapgood laboratory at the University of Cape Town suggest that MPA can down-regulate the expression of the tight junction protein claudin-4 in a cervical cell line (End 1 E6E7) in a manner consistent with GR activation. Given that End 1 E6E7 cells cannot form a functional barrier in vitro (7), it became necessary to verify this regulation of claudin-4 by MPA in primary epithelial cells and to assess its relevance in HIV-1 mucosal transmission.

The main objectives of this project were to learn and transfer methodology involving the isolation of primary female genital epithelial cells from hysterectomy tissue, to learn methods to assess tight junction integrity and HIV-1 transmigration, and to gain more insight into the theoretical and conceptual wealth of knowledge in the field of HIV-1 and female genital tract biology. In addition, to use these methods to assess the effects of dexamethasone and MPA on the HIV-1 induced disruption of female genital epithelial tight junctions.

Main Research Activities

Primary genital epithelial cells were isolated from the endometrial and endocervix using the protocol established by Kaushic et al (2011). Tissue samples were donated by women undergoing hysterectomy for benign conditions. Isolated cells were seeded onto Matrigel-coated 0.4μM PET transwell insert and grown to confluence as determined by daily transepithelial electrical resistance (TER) measurements. (TER is a measure of tight junction formation by epithelial

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cells). Only confluent monolayers with TER above 1000 ohms were used to assess the effect of hormones on the HIV-induced disruption of genital epithelial barrier function. Unless otherwise stated, cells were grown in primary culture media supplemented with non-heat inactivated defined serum. Confluent epithelial monolayers were pre-treated for 24 hours with 0.01% vehicle (EtOH), 100nM DEX and 100nM MPA in serum-free media. Thereafter, the monolayers were exposure to 105 infectious viral units of IIIB (X4 tropic) for 24 hours. Supernatants from the apical chamber were collected for TNF-alpha ELISA and the inserts were fixed and stained for the tight junction protein zonula occluden (ZO) – 1. The Two-way ANOVA with Bonferroni post-test was performed to test for statistical significance using the GraphPad Prism version 7 software.

Results and Discussion

In this project, we examined the effects of DEX and MPA on the HIV-mediated disruption of epithelial tight junctions of the endometrium and endocervix. We did not see evidence of HIV-1 impairing the barrier of both epithelial surfaces neither did we observe any regulatory effects by DEX and MPA on epithelial barrier function with or without HIV. Although this result contradicts early reports by Nazli et al (2010), it however demonstrates the importance of HIV-induced inflammation in barrier breakage. Nazli et al demonstrated that the induction of tumour necrosis factor (TNF)-alpha by HIV was responsible for the HIV disruption of genital epithelial barrier. We saw a weak to no TNF-alpha induction by the virus in our system (data not shown), which could explain why the barriers stayed intact after exposure to the virus for 24 hours. Several reasons could explain our result. The cells used in these experiments were isolated mainly from post-menopausal women and took between two to three weeks to established confluent monolayers contrary to 5 to 7 days as reported by Kaushic et al. It is thus possible that receptors necessary for both hormonal and viral regulation of epithelial barrier function could have been lost. In addition, the conditions under which the cells were grown differed slightly from those used by Nazli et al.

A **B**

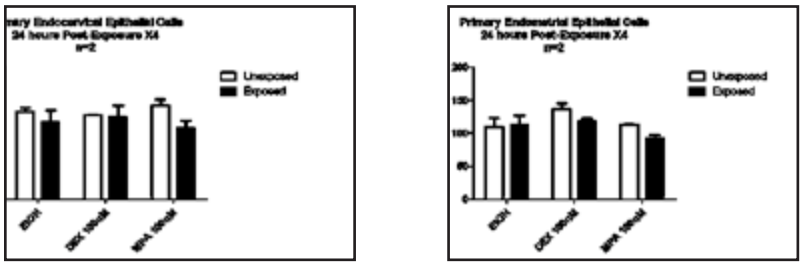

Figure 1: A. Effects of DEX and MPA on HIV-mediated loss of primary endocervical epithelial barrier function. Confluent primary cervical epithelial cells where pre-treated for 24 hours with vehicle (0.01% EtOH), 100nM DEX and 100nM MPA, and thereafter exposed to HIV-1 (X4-Tropic, 105 infectious viral units/mL) with or without hormones for another 24 hours. TER measurements were taken before and after exposure to HIV-1. Histograms represent two independent experiments (n = 2) performed in duplicates. B. Effects of DEX and MPA on HIV-mediated loss of primary endometrial epithelial barrier function. Confluent primary endometrial epithelial cells grown in charcoal-stripped serum supplemented media were pre-treated for 24 hours with vehicle (0.01% EtOH), 100nM DEX and 100nM MPA, and thereafter exposed to HIV-1 (X4-Tropic, 105 infectious viral units/mL) with or without hormones for another 24 hours. Vehicle, hormones and HIV were made up in serum-free

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Conclusions

In summary, our result demonstrates HIV fails to induce epithelial barrier breakage in the absence of inflammation. It also shows DEX and MPA on their own have no regulatory effects on epithelial tight junctions of the female genital tract.

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