ORAL ABSTRACT SESSION 11: VIROLOGY AND IMMUNOLOGY OF TRANSMISSION

OA11.01

Identification of the first cells infected during vaginal transmission in the Depo-Provera rhesus macaque model

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Background: There is currently a significant effort to develop interventions to prevent the transmission of HIV. To facilitate these efforts, a better understanding of how the virus can breach mucosal epithelial barriers is required. We have previously explored the interaction of HIV with the female genital tract using human explants. This analysis revealed that HIV could penetrate both the columnar epithelium of the endocervix and the squamous epithelium of the vagina and ectocervix. Establishing whether the penetrating virus could lead to productive infection, however, remained to be elucidated.

Methods: To identify the first infected cells, we utilized a SIV-based vector system that would express the mCherry fluorescent protein. The vector was pseudotyped with the R5 tropic HIV envelope JRFL and concentrated by ultracentrifugation. Depo-Provera-treated female macaques were vaginally exposed to the inoculum. Four days later the animals were sacrificed, their genital tracts excised, and cryosections of the tissue were analyzed by fluorescent microscopy.

Results: Analysis revealed large but rare foci of red fluorescent cells. The expression of mCherry within these cells was confirmed by emission spectrum analysis. These rare foci of transduced cells were found in areas protected by both the columnar and squamous epithelium. The target cells are primarily CD4+ T cells that have infiltrated into the squamous epithelium or reside just below the columnar epithelium.

Conclusion: The clustering of transduced cells suggests that there are small regions within the female genital tract that are susceptible to infection. Understanding the mechanism that leads to this increased local susceptibility could lead to novel treatments that can reduce the male-to-female acquisition of HIV.

OA11.02

Susceptibility of Epithelial Cells from the Human Female Upper Reproductive Tract to Infection by Transmitted/Founder HIV-1

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Background: The mechanisms of HIV transmission in the female reproductive tract (FRT) remain poorly understood. In particular, upper FRT sites, especially uterus and fallopian tubes, are only recently being recognized as potentially vulnerable to HIV-1 infection. The endocrine-controlled immune functions of the FRT change profoundly throughout the menstrual cycle. This results in a "window of vulnerability" to HIV infection that has not yet been systematically explored.

Methods: We employed infectious molecular clones (IMC) of transmitted HIV-1, which we recently generated, to investigate the susceptibility of uterine epithelial cells (UEC) to infection. Since UECs can express HIV-1 coreceptors, they may represent first target cells for infection. Primary UEC were isolated from hysterectomy tissues and cultured as polarized monolayers. Cells were exposed to Renilla luciferase reporter viruses expressing transmitted or reference strain envelope (Env) genes (Env-IMC-LucR viruses) allowing sensitive detection of viral gene expression.

Results: Using a panel of 15 transmitted clade B Env from female and male subjects, and 4 reference Env, our findings indicate that UEC are susceptible to HIV-1 infection. Remarkably, in primary uterine cell cultures derived from multiple patients, we did not see evidence for infection/reporter gene expression after exposure to Env-IMCs of commonly used strains, BaL and SF162, while cultures challenged with transmitted Env-IMCs repeatedly exhibited low but detectable levels of luciferase expression. Moreover, when polarized cells were tested for route of infection, apical but not basolateral cell surface exposure led to UEC HIV infection.

Conclusion: To our knowledge, this is the first time HIV-1 susceptibility of uterine cells has been addressed with mucosally transmitted HIV-1. Our data suggest that uterine epithelial cells are possible targets of HIV-1 infection and transmission. Since the uterus is readily accessible to semen, the likely exposure of these tissues to HIV-1 is relevant to development of intervention strategies.

OA11.03

HIV ENV antibodies differentially modulate viral transport in cervical mucus

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Background: A better understanding of sexual transmission of HIV is essential for the development of viable vaccines and microbicides. Cervical mucus (CM) is one of the first obstacles HIV encounters during vaginal transmission. CM could potentially impede HIV transport by physically sieving the virions or through specific interactions of HIV virions with mucosal antibodies. Furthermore, the existence of repeatedly exposed but uninfected women – as in the case of discordant couples – could, in part, be explained by the protective function of CM and the antibodies it contains.

Methods: We use multi-color fluorescence video microscopy and computer aided particle tracking to follow the movement of individual HIV virions in CM. We add virions with wild-type GP120 envelope (mCherry-tagged) and control virions without GP120 (GFP-tagged) to each mucus sample. We image them simultaneously. We also image the two HIV phenotypes in the presence of various antiGP120, antiGP41, and antiHLA1 antibody isotypes. We characterize and quantify the motion of each virion by their mean squared displacement, and other standard measures of microscopic transport.

Results: We examine the effect of exogenous monoclonal antibodies, and of endogenous antibodies in HIV infected women on viral motion in CM. We find that the binding of antibodies modulates HIV transport in CM and that the strength of the modulation depends on the isotype used.

Conclusion: These studies lay a foundation for quantitatively studying the interaction of HIV with cervical mucus and the antibody response to HIV in infected women.

OA11.04

Low Dose Mucosal SIV Infection Restricts Early Replication Kinetics and Transmitted Virus Variants in Rhesus Monkeys

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Background: Defining the earliest virologic events following HIV transmission may be critical for the design of vaccine strategies aimed at blocking acquisition of HIV infection. Here we show that the dose of the virus inoculum impacts the length of the eclipse phase and the number of transmitted virus variants following intrarectal SIV infection of rhesus monkeys.

Methods: 24 adult rhesus monkeys (N=6/group) received a single intrarectal inoculation with virus doses of 109, 108, 107, or 106 SIV RNA copies of SIVmac251. Plasma SIV RNA levels were determined following infection. The number of transmitted/founder virus variants were determined by assessing the phylogenetic patterns of env diversity utilizing SGA and direct amplicon sequencing. Innate immune profiles were evaluated by quantifying serum cytokine levels, and PBMC phenotyping was determined by flow cytometry.

Results: For monkeys infected by 109, 108, 107, or 106 SIV RNA copies of virus, the eclipse phase was a median of 4, 4, 7, and 8.5 days, and the transmitted/founder virus variants were a median of >10, >10, 2 and 1, respectively. Compared with the higher dose groups, the lower dose groups also showed trends toward lower and delayed median peak cytokine levels, and less extensive depletion of central memory CD4 T cells.

Conclusion: Low dose SIV infection resulted in a lengthened eclipse phase, fewer transmitted virus variants, and decreased innate immune activation as compared with high dose SIV infection. These data suggest a mechanism by which it may be easier for a vaccine to protect against low-risk HIV transmission as compared with high-risk HIV transmission. These findings have major implications in the design and interpretation of HIV vaccine efficacy studies in humans.

OA11.05

Exploring HIV Transmission in the Male Genital Tract Using the Circumcised and Uncircumcised Macaque Model

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Background: The model of HIV sexual transmission in the male genital tract has not been well developed and this has hindered understanding of how male circumcision prevents against HIV infection. We aimed to create a more comprehensive model of HIV transmission in men.

Methods: Eight cadaveric penile explants (3 uncircumcised) were inoculated with photoactivatable(PA) GFP-Vpr HIVBal in culture. The genital tracts of seven uncircumcised male rhesus macaques (Macaca mulatta) from Yerkes and Tulane National Primate Research Centers were also inoculated with PA HIVBal in vivo, and penile tissue was removed during necropsy. Tissue cryosections were stained for Langerhans cells, CD4+ T-cells, keratin, and intercellular junctions. Images were obtained with DeltaVision RT systems and analyzed with SoftWorx software. Four uncircumcised male macaques were separately circumcised at Tulane. Trans-epithelial water loss and skin capacitance of the macaque penis before and after circumcision were measured using the Vapometer and MoistureMeter.

Results: HIV particles were predominantly visualized in the keratin of cadaveric penile epithelia, which tends to be thinner in the circumcised penis. 3-8% of virions were seen in deeper epithelial strata, up to 81 microns from the surface. In vivo inoculations of the macaque penis showed similar findings, with penetrating virions seen at depths of up to 60 microns from the surface and occasionally near target cells, thus validating the use of the human explant model.

The stratified squamous epithelium of the macaque penis was histologically very similar to that of the human penis. Non-invasive measurements of penile skin barrier function in the circumcised macaques demonstrated changes over time (correlating to a loss of barrier function) that may contribute to how circumcision affects HIV transmission.

Conclusion: These findings represent potential routes of HIV transmission in the male genital tract. Building a clearer model of transmission will aid in the development of future HIV prevention methods.

OA11.06

Differences In Glycosylation Patterns But Not Neutralization Sensitivity of HIV-1 Env in Acute and Chronic Infection From Heterosexual Transmission

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Background: Events surrounding the heterosexual transmission of HIV-1 remain an important area of research. Viral genetic variation offers a convenient source of information to explore questions surrounding the transmission event. We have examined patterns of sequence variation in the viral env gene and expressed these sequences to test their biological properties.

Methods: We used env gene sequences derived from subtype C HIV-1 in acutely infected subjects (n=69) and in contemporaneous chronically infected subjects (n=62) from Malawi and South Africa. In addition, HIV-1 env clones from a subset of subjects with acute and chronic infections were examined for neutralization sensitivity by generating pseudotyped viruses and testing them with panels of monoclonal antibodies and broadly neutralizing polyclonal sera.

Results: Env proteins from acute infection have fewer glycosylation sites than Env proteins from chronic infection. This difference is largely driven by the virus in acutely infected males (N=30). Two-thirds of the glycosylation count difference is located in the variable regions. In addition, five potential sites in the conserved regions are under-glycosylated in Env proteins from acute infection. There were no significant differences in neutralization patterns in viruses pseudotyped with Env proteins derived from viruses representing acute or chronic infections.

Conclusion: Our results indicate that heterosexual transmission specifically from females to males on average results in transmission of viruses with Env proteins that have 10% fewer glycosylation sites. We found no difference in the antibody sensitivity of viruses pseudotyped with Env proteins derived from viruses from acute infection compared to viruses derived from chronic infection. These data suggest that in women there is a restriction of virus that reaches or is able to leave the mucosal surface and the restriction is dependent on the extent of glycosylation on the virus. These results are currently being confirmed with an independent subtype C acute infection cohort.