Finally, a Macaque Model for Cell-Associated SIV/HIV Vaginal Transmission

Deborah J. Anderson
Departments of Obstetrics and Gynecology and Microbiology, Boston University School of Medicine, Boston Massachusetts

In the current issue of the Journal, Bettina Sallé and coworkers [1] describe infection of female macaques after atraumatic instillation of simian immunodeficiency virus (SIV)–infected cells into the vaginal cavity. This new SIV infection model represents a significant advance for human immunodeficiency virus (HIV) transmission and prevention research. Whereas HIV-infected cells in genital secretions (“Trojan Horse leukocytes”) may play an important role in the sexual transmission of HIV [2], they have been largely overlooked in recent studies on mechanisms of HIV transmission and in the design and testing of HIV vaccine and microbicide candidates. Current preclinical assays for the development of HIV prevention drugs and vaccines predominately use cell-free viral stocks, and the most popular macaque vaginal SIV transmission models used for vaccine and microbicide preclinical efficacy trials require superphysiological doses of cell-free virus and treatment with high doses of progestins to achieve high infection rates [3]. Because the molecular events underlying cell-associated transmission differ from those involved in cell-free virus transmission, many of the current vaccine and microbicide candidates shown to be effective against cell-free virus may not protect against cell-associated viral transmission. The failure of several recent vaccine and microbicide clinical trials to prevent HIV transmission [4] may be due, in part, to this oversight.

Cell-associated HIV transmission is an attractive theory because infected cells may transport virus across the mucosal epithelium while protecting it from the adverse effects of antiviral defense molecules in the genital environment, and because cell-to-cell HIV transfer through viral synapses is highly efficient. Several studies have conclusively shown that infected cells are much more effective than cell-free virus at infecting subepithelial target cells in polarized epithelial monolayer cultures [5–8], and effective cell-associated mucosal transmission has also been demonstrated in small animal models such as the feline immunodeficiency virus [9, 10] and hu-SCID mouse HIV infection models [11]. Furthermore, recent clinical studies provide evidence that human cell-associated HIV transmission may occur. In one study, unprotected heterosexual intercourse was associated with alloimmunization to partner’s HLA antigens [12] while condom-using couples had no response, indicating that seminal leukocytes may commonly infiltrate the vaginal epithelium after intercourse. In another study, genetic sequencing of HIV in blood from acutely infected individuals showed that the genotype of the infecting virus in 3 of 5 cases more closely matched that of HIV in semen cells than in free virus [13].

Only 4 nonhuman primate studies on cell-associated SIV and HIV transmission have been published to date. In 1998, one group reported that chimpanzees could be infected with HIV after placement of either cell-free virus or infected peripheral blood mononuclear cells (PBMCs) near the cervical os [14], whereas another group did not detect systemic infection in rhesus macaques after vaginal exposure to cryopreserved SIV-infected PBMCs [15]. More recently, scientists at the Primate Center in Madison, Wisconsin, demonstrated transvaginal infection of rhesus macaques after multiple low-dose exposures to fresh SIV-infected PBMCs in animals with chemically induced vaginal ulcers, as well as untreated intact animals that were used as controls [16, 17]. These preliminary studies suggest that HIV-infected and SIV-infected leukocytes can be infectious when delivered vaginally to nonhuman primates, but that results depend on the characteristics of the viral stock and the dosing protocol.

Why has cell-associated HIV transmis-
sion been largely overlooked in research on HIV transmission mechanisms and preclinical drug development? For one, it has been difficult to make reproducible stocks of highly infected cells for such studies, whereas well-characterized cell-free stocks are readily available, as are convenient commercial assays to quantify cell-free viral titers. In the current study, Sallé et al [1] used a novel approach to generate stocks of highly infectious cells; they harvested infected cells from spleens of SIVmac251-infected rhesus macaques at the peak of viremia (12 days after infection). Infected leukocytes were enriched on Ficoll-Hypaque and frozen in dimethyl sulfoxide at 10⁷ cells per ampoule. A representative batch of infected spleen cells from one donor contained 4.2 × 10⁸ viral DNA copies per 10⁶ cells and a 50% tissue culture infectious dose of 5576 cells. Central memory T cells, comprising 25% of the total spleen cell population, contained 2.17 × 10⁷ viral DNA copies and 7.92 × 10⁶ viral messenger RNA copies per 10⁶ cells. The monocyte/macrophage population comprised only 2% of the population and contained 5.37 × 10⁵ viral DNA copies and 1.64 × 10⁶ viral messenger RNA copies per 10⁶ cells.

Figure 1. Potential mechanisms underlying cell-associated HIV transmission. A, Columnar epithelium: (1) Infected cell migrates between epithelial cells to infect susceptible host cells in the lamina propria or draining lymph nodes. (2) HIV transcytosis through epithelial cells to infect susceptible target cells in lamina propria. B, Stratified squamous epithelium: (3) Transfer of HIV from infected leukocyte to epithelial cell, which transfers virus to intraepithelial or subepithelial target cells through (a) transcytosis or (b) attraction by means of release of chemokines. (4) Direct cell-to-cell transfer of HIV from infected leukocyte to intraepithelial target cell by means of viral synapses. (5) Transepithelial migration of infected leukocyte to infect intraepithelial target cells within the epithelium. (6) Transepithelial migration of infected cell to infect target cells in the subepithelium or draining lymph nodes. Originally published in Anderson et al [2].
To document transepithelial penetration of infected spleen leukocytes, cells were tracked in tissues and blood after vaginal application. Fluorescein-labeled spleen cells were detected in draining lymph nodes and peripheral blood, and SIV-infected cells were detected by in situ hybridization in the lamina propria of the vaginal epithelium and T cell areas of distal lymph nodes at 21 and 41 h after exposure. Four of five female animals that received single intravaginal doses of 10^7 spleen cells became systemically infected with SIV. The dose needed to infect 50% of females was determined to be 6.69 × 10^7 viral DNA copies, which corresponds to the number of HIV DNA proviral copies detected in semen cells from some HIV-infected men [2].

The new macaque model for cell-associated SIV transmission presented by Sallé et al [1] is promising, but several questions remain. One advantage to using spleen cells from infected animals is the availability of differentiated macrophages, a cell type that normally outnumbers CD^4^ T cells in semen from HIV-infected men [18] and is capable of infecting PBMC target cells in vitro [19]. However, the viral stocks in this study contained few mature macrophages, possibly because they were not retained on the Ficol-lhypaque gradients. Future studies should be conducted to determine the relative efficiency of SIV-infected T cells versus macrophages in cell-associated SIV transmission. Other questions pertain to the physiological relevance of the model. Is progestin treatment required to achieve reliable cell-associated HIV transmission? It would be preferable to avoid high-dose progestin treatment because it is immunosuppressive and blocks ovarian estrogen production, resulting in an artificially thinned vaginal epithelium. Does systemic infection occur after multiple low-dose vaginal exposures? A low-dose multiple exposure model could more closely represent natural conditions, where HIV infection occurs at a low frequency in healthy women.

This model could also be used to evaluate the contribution of risk factors such as specific sexually transmitted infections to cell-associated HIV transmission, and to further define molecular mechanisms underlying this mode of transmission. On the basis of limited experimental evidence, we recently proposed 3 potential cell-associated HIV transmission pathways [2].

1. HIV-infected leukocytes attach to the apical surface of epithelial cells and shed nascent virions toward the epithelial cell plasma membrane. These highly infectious viral particles may be sequestered by epithelial cells for subsequent transfer to HIV-susceptible host cells within the epithelium, or transferred through epithelial cell layers by transcytosis to target cells in the lamina propria. (2) HIV is directly transferred from infected leukocytes to target cells within the epithelium, possibly through the formation of an infectious synapse; it is possible that target cells are attracted to infected leukocytes by chemokines released either by the infected cell or by epithelial cells that are activated by contact with infected cells. (3) Infected leukocytes may migrate through the epithelium to infect target cells in the lamina propria or draining lymph nodes (Figure 1). Each of these pathways entails molecular interactions that could be targeted and tested in an authentic macaque model of cell-associated HIV transmission. If cell-associated HIV transmission proves to be a major infection mechanism, such research could very well lead to new HIV prevention strategies.

References
