Modeling Mucosal Cell-Associated HIV Type 1 Transmission in Vitro

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Human immunodeficiency virus type 1 (HIV-1) can efficiently spread by direct cell-to-cell contact, a mechanism termed cell-associated HIV transmission. By some estimates, cell-associated HIV transmission is 10–1000-fold more effective than cell-free HIV infection. Mucosal cell-associated HIV transmission may occur when HIV-bearing cells in mucosal secretions from an HIV-infected donor transfer virus directly to recipient target cells in or below the mucosal epithelium, or through HIV transcytosis across the mucosal epithelium of a non-infected host. This mechanism may play an important role in the sexual and vertical transmission of HIV-1, yet most in vitro tests of vaccine and microbicide efficacy assess cell-free virus transmission. This article reviews in vitro assays that have been used to model mucosal cell-associated transmission, including microscopy, immune cell cocultures, use of HIV-infected cells in epithelial cell transcytosis assays, and cell-associated infection of mucosal tissue explants. Assays that authentically simulate mucosal cell-associated HIV transmission could provide valuable insight into mechanisms and molecules that can potentially be targeted for HIV prevention, as well as critical models for testing novel HIV prevention strategies for efficacy against cell-associated HIV transmission.

Keywords. HIV-1; cell-associated transmission; vagina; mucosa; in vitro; prevention; genital tract; microbicides; vaccines.

Many viruses, including human immunodeficiency virus type 1 (HIV-1), can spread (1) as cell-free virions that bud from infected cells and encounter target cells via diffusion through the extracellular milieu or (2) by infected cells through direct cell-to-cell contact [1, 2]. Cell-to-cell HIV transmission, also known as cell-associated HIV transmission, has been shown to be 10-fold to >1000-fold more efficient than cell-free transmission in vitro [3, 4]. This striking difference in efficiency between these 2 modes of HIV transmission has been ascribed to a number of factors: (1) proximity of the cell-associated virus to its target, (2) receptor clustering at points of cell-to-cell contact, (3) increased multiplicity of infection due to the targeted budding of virus at synapses formed between cells, and (4) the relative resistance of cell-associated transmission to a number of factors that inhibit the infectiousness of cell-free virions, such as neutralizing antibodies, and host restriction factors, such as tetherin and TRIM 5-α [5]. Cell-to-cell HIV transmission among cells in lymphoid organs and possibly other sanctuary sites is thought to underlie HIV persistence in vivo [5].

There is mounting evidence that cell-associated HIV transmission could play a role in sexual and vertical transmission of HIV [6]. Since the mechanisms underlying cell-associated HIV transmission differ from those of cell-free transmission, cell-associated transmission could require different prevention strategies. For example, some broadly neutralizing antibodies and antiretroviral therapies are less effective at blocking cell-associated HIV transmission than cell-free transmission [7, 8]. On the other hand, strategies that block cell attachment and the formation of viral synapses may be particularly effective at blocking cell-associated transmission [9]. Through this review article I hope to bring attention to this understudied field by presenting the strengths

and weaknesses of in vitro cell-associated HIV transmission assays that have been used to date to assess mucosal cell-associated transmission and test HIV prevention strategies.

IN VITRO ASSAYS USED TO MODEL MUCOSAL CELL-ASSOCIATED HIV TRANSMISSION

Microscopy

Much of what we know about cell-associated HIV transmission has been learned from microscopy image analysis. Early evidence for virus transmission between cells was provided by electron microscopy images showing directional budding of virus at cell-cell contacts [10, 11]. Subsequently, use of sophisticated fluorescence microscopy imaging techniques enabled visualization of the recruitment of HIV and its receptors to intercellular junctions, the formation of virologic synapses [12], and the direct transfer of HIV via synapses from infected to uninfected cells [13]. Microscopy has also been used to identify components of virologic synapses that could be targeted to block cell-associated HIV transmission. HIV gp160 env, CD4, and chemokine coreceptors play crucial roles in synapse formation; ICAM-1/LFA-1 adhesion molecules stabilize cell-cell contacts; and actin and cytoskeleton proteins are remodeled during cell-associated HIV transmission [14]. The targeted use of antibodies and other antagonists to many of these structures inhibits cell-associated HIV transmission [15, 16]. Microscopy has also been used for testing the efficacy of HIV broadly neutralizing antibodies and microbicides in blocking cell-to-cell HIV transfer and provided the first evidence that cell-associated transmission may be resistant to patient serum and broadly neutralizing monoclonal antibodies [4, 17]. Although this approach is labor intensive and has been largely supplanted by other methods (described below), a related quantitative flow cytometry method using infectious green fluorescent protein–labeled HIV for assessment of cell-associated HIV transmission was recently published [18] and shows promise as a platform for screening vaccine-induced antibodies and microbicides.

Immune Cell Coculture Assays

Cell-to-cell HIV transmission is commonly demonstrated in cell suspensions containing infected T cells [19, 20], macrophages [21], or HIV-bearing dendritic cells [22] as virus donors and uninfected T cells or highly permissive reporter cell lines as target cells [1]. In these cocultures, cells form transient adhesive structures called virologic synapses through which virus is directionally transmitted to adjacent cells [4]. The efficiency of cell-associated transmission in coculture assays can vary depending on the types of infected and target cells used [7]. Cell-associated HIV transmission is difficult to quantify in coculture systems because of simultaneous infection of target cells with cell-free HIV. Various approaches have been used to reduce this possibility, including comparison of virus transmission in static versus gently shaken cultures, to differentiate cell-free from cell-associated transmission [21]; treatment of infected cells with mitomycin C or antiviral drugs such as saquinavir (protease inhibitor), to block new virus assembly [7, 23]; use of activated primary CD4$^+$ T cells as targets instead of engineered reporter cell lines (eg, TZM-bl) that are highly susceptible to cell-free infection [7]; and use of specialized reporter vectors [3].

An early hypothesis predicted that virus transmitted via the cell-associated pathway would be shielded from the effects of neutralizing antibodies [2, 24]. However, studies to determine the relative efficacy of HIV broadly neutralizing antibodies in cell-associated versus cell-free HIV transmission assays have produced mixed results (Table 1). Several of the major studies have concluded that antibodies directed against the CD4 binding site on HIV effectively neutralize cell-associated HIV transmission, whereas others have shown diminished neutralizing activity with CD4bs antibodies but effective neutralization with antibodies directed against the gp41 MPER region. Antibodies and other antagonists directed against cell HIV receptors (CD4 and CCR5) appear to also be effective [8, 25–28]. Discordance in these data may be due to differences in (1) timing of antibody treatment (eg, CD4bs antibodies can affect synapse formation but may show reduced efficiency once synapses are formed), (2) source and types of virus and antibodies, (3) effector:target cell systems, and (4) possibly other factors, such as background levels of cell-free HIV transmission [7]. It underscores the need for much more systematic research in this field to fully understand the effects of HIV-directed antibodies and other factors on cell-cell HIV transmission.

Coculture assays are currently being used to screen microbicide compounds for efficacy against cell-associated HIV transmission. The Southern Research Institute in the United States has developed a comprehensive HIV screening algorithm for the discovery and preclinical testing of topical microbicides

Table 1. Neutralization Activity of Broadly Neutralizing Human Immunodeficiency Virus (HIV) Antibodies in Cell-Associated HIV Transmission Assays

Reference	System	CD4bs (VRCO1, b12)	gp41 MPER (4 E10, 2F5)
Frankel et al [25]	DC-T	+	+
Abela et al [26]	PBMC-TZMbl	−	+
Malbec et al [27]	T-T	+	−
Sagar et al [22]	DC-T	-	+
Duncan et al [28]	MDM-T	+	−
Martin et al [29]	Jurkat/A301.R5	+	+
Zhong et al [30]	Jurkat/SupT	ND	+

Abbreviations: +, strong neutralization in both cell-free and cell-associated HIV transmission assays; −, poor neutralization activity in cell-associated HIV transmission assays.

that includes cell-associated HIV transmission assays [23]. For this purpose, they use HIV-infected H9 or MOLT cells, as well as GHOST target cells. Their algorithm calls for a pH transition to simulate vaginal conditions, as well as the addition of 12.5% seminal plasma. R. J. Shattock's laboratory in the United Kingdom uses HIV-infected PM1 cells and TZM-bl (ie, HeLa cervical carcinoma derived) reporter cells [31]. The Virology Unit at the Institute of Tropical Medicine in Antwerp, Belgium, routinely screens antiretrovirals and microbicides in a cell-associated HIV transmission assay consisting of HIV-infected leukocytes and T-cell lines (R5 and X4 MaRBLE) containing a firefly luciferase reporter gene [32].

It could be argued that coculture assays such as the ones described above simulate HIV transmission that potentially occurs between HIV-bearing immune cells in mucosal secretions and uninfected HIV target cells residing in the mucosal epithelium. HIV-infected T cells and macrophages have been isolated from semen obtained from HIV-positive men [33], and infected leukocytes have also been detected in cervicovaginal secretions and breast milk from HIV-infected women [34, 35]. Uninfected counterparts of these cells along with dendritic cells could serve as HIV target cells in genital and gastrointestinal epithelia, especially under inflammatory conditions [36]. HIV-infected leukocytes from semen, cervicovaginal secretions, or breast milk could encounter uninfected target cells residing on the surface of the epithelium or within the stratum corneum, the superficial layer of the vaginal and penile epithelium that is devoid of tight junctions [37, 38], or they could transmigrate into the epithelium to encounter target cells within or below the mucosal layer [6]. However, it should be kept in mind that HIV-infected cells and target cells in mucosal tissues have distinct characteristics that distinguish them from PBMCs or cell lines that are normally used in coculture assays. T lymphocytes at mucosal sites are predominately differentiated memory T cells, and tissue macrophages usually have an M2 phenotype [36, 39]. In addition, the Langerhans cells and numerous dendritic cells in mucosal tissues could play a major role in cell-associated HIV transmission [22, 40]. Finally, cell-associated HIV transmission in vivo could be affected by the degree of allogenicity between donor and host.

Cell-Associated HIV Epithelial Transcytosis Assays

Various polarized epithelial cell monolayers have been used to study HIV mucosal transmission. It has been known for some time that contact between HIV-infected cells and epithelial cells results in a massive and rapid budding of HIV virions toward the epithelium [10, 11]. This is followed by the internalization of virions into endosome-like structures and their passage across the epithelial barrier via a characteristic epithelial transcellular vesicular pathway, a process termed transcytosis [41]. After their passage, the virions are capable of infecting target cells residing below the epithelium [41]. Efficient HIV

transcytosis has been demonstrated to occur across polarized monolayers of immortalized vaginal cells [42], as well as transformed intestinal [43], endometrial [44], and cervical epithelial cells [45, 46]. In most transcytosis models, HIV-infected leukocytes are more efficient than cell-free virus in producing infection of subepithelial target cells. Synapse formation between HIV-infected lymphocytes and epithelial cells has been demonstrated by electron microscopy [47, 48]. Whereas HIV transcytosis has been readily demonstrated with transformed epithelial cell models, the physiological relevance of this cell-associated HIV transmission mechanism is unclear; HIV transmission was inefficient when infected cells were added to the apical surface of polarized primary cultures of human ectocervical and endocervical epithelia [49, 50], ectocervical and endocervical epithelial sheets [49], and a reconstructed vaginal epithelial model [51].

Epithelial transcytosis assays have been used to test the efficacy of mucosal and monoclonal antibodies to prevent cell-associated HIV transmission. Mucosal HIV antibodies, especially immunoglobulin A, efficiently block HIV transcytosis from infected cells [52], whereas several neutralizing monoclonal antibodies against HIV have failed to effectively block cell-associated HIV transcytosis [53]. Cell-associated HIV transcytosis assays have also been used to test microbicide candidates [31, 54–56].

Cell-Associated HIV Infection of Mucosal Tissue Explants

Mucosal tissue explants, comprising an intact epithelium and resident mucosal HIV target cells, have also been used for studies of cell-associated HIV transmission. In one study [57], cell-free HIV or infected cells were placed on the luminal side of polarized ectocervical explant tissue, and viral transmission was detected by measuring the HIV load in the lower chamber at different time points. The addition of X4 cell-associated HIV and both X4 and R5 cell-free HIV resulted in transmission of the virus across the mucosa. In another study [58], labeled viable cells from semen were shown to bind to and penetrate the ectocervical epithelium but failed to bind to endocervical explants because of their entrapment in mucus secreted by these cells. Two studies have demonstrated efficient cell-associated HIV transmission across inner but not outer foreskin tissue in vitro [48, 59]; cell-free HIV was ineffective in this ex vivo foreskin model. Tugizov et al [60] recently demonstrated that HIV-infected macrophages but not T cells were able to transverse fetal oral and intestinal epithelia but not adult epithelia. In contrast, another group reported that HIV-infected CD4$^+$ T cells transmigrate across human colonic explant tissues to infect target cells below [61].

Mucosal tissue explants have the advantage of containing an intact epithelial layer, authentic mucosal HIV target cells, and other factors in the mucosal environment. However, they have several disadvantages, including (1) marked interdonor

variation in hormone status, inflammation, sexually transmitted disease history, and HIV target cell populations, making assay reproducibility difficult; and (2) rapid deterioration of tissue structure in vitro [62]. Nonetheless, mucosal tissue explants have been widely used to screen microbicide candidates for efficacy against cell-free HIV infection but rarely against cell-associated infection [31, 54, 63].

ADDITIONAL CONSIDERATIONS

For enhanced authenticity, cell-associated HIV transmission models should incorporate mucosal tissue-derived cells as infection sources and/or targets and should include other elements of the mucosal environment, such as semen, cervicovaginal secretions, a pH range, genital pathogens, and microflora [64]. This poses several challenges: few viable immune cells are normally recovered from genital secretions by noninvasive techniques, and these cells vary in phenotype, activation state, and function, depending on local conditions such as inflammation, infections/microflora, and hormone status [65, 66]. Furthermore, the molecular composition of genital secretions and the nature of tight junctions in genital epithelium may also be affected by these and other factors [67, 68]. Little research has been done to determine whether such factors affect cell-associated HIV transmission or the efficacy of antibodies and other compounds being tested in cell-associated transmission assays.

Another relevant topic is immune defense against infected cells that mediate cell-associated HIV transmission. Three principal effector mechanisms are known to target HIV-infected cells: cell-mediated immunity, carried out by CD8^{+} T cells; natural killer (NK) cell immunity, mediated by NK cells; and antibody dependent cellular cytotoxicity (ADCC), mediated by Fc-bearing immune cells and antibodies [69, 70]. Vaccine and microbicide candidates should also be tested in these assays because it should be determined that prevention strategies do not interfere with these important defense mechanisms. The ideal prevention strategy would potentiate these mechanisms. A few laboratories have begun to systematically test monoclonal antibodies and vaccine antisera to determine which antibody specificities, subclasses, and isotypes are most effective in mediating ADCC killing of HIV infected cells [71]. This work should continue.

CONCLUSIONS

Cell-associated HIV transmission is increasingly recognized as a potentially important mechanism underlying HIV transmission across mucosal surfaces. However, the focus for microbicide and vaccine screening remains cell-free HIV infection assays. Relatively little effort has been spent on developing authentic mucosal cell-associated HIV transmission assays, and as such much more work is needed to expand, refine, and validate these assays.

Notes

References

1. Mothes W, Sherer NM, Jin J, Zhong P. Virus cell-to-cell transmission. J Virol 2010; 84:8360–8.
2. Sattentau Q. Avoiding the void: cell-to-cell spread of human viruses. Nat Rev Microbiol 2008; 6:815–26.
3. Mazurov D, Ilinskaya A, Heidecker G, Lloyd P, Derse D. Quantitative comparison of HTLV-1 and HIV-1 cell-to-cell infection with new replication dependent vectors. PLoS Pathog 2010; 6:e1000788.
4. Chen P, Hubner W, Spinelli MA, Chen BK. Predominant mode of human immunodeficiency virus transfer between T cells is mediated by sustained Env-dependent neutralization-resistant virological synapses. J Virol 2007; 81:12582–95.
5. Kulpa DA, Brehm JH, Fromentin R, et al. The immunological synapse: the gateway to the HIV reservoir. Immunol Rev 2013; 254:305–25.
6. Anderson DJ, Politch JA, Nadolski AM, Blaskewicz CD, Pudney J, Mayer KH. Targeting Trojan Horse leukocytes for HIV prevention. AIDS 2010; 24:163–87.
7. Zhong P, Agosto LM, Ilinskaya A, et al. Cell-to-cell transmission can overcome multiple donor and target cell barriers imposed on cell-free HIV. PloS One 2013; 8:e53138.
8. Sagar M, Akiyama H, Etemad B, Ramirez N, Freitas I, Gummuluru S. Transmembrane domain membrane proximal external region but not surface unit-directed broadly neutralizing HIV-1 antibodies can restrict dendritic cell-mediated HIV-1 trans-infection. J Infect Dis 2012; 205:1248–57.
9. Bridges CG, Brennan TM, Taylor DL, McPherson M, Tyms AS. The prevention of cell adhesion and the cell-to-cell spread of HIV-1 in vitro by the alpha-glucosidase 1 inhibitor, 6-O-butanoyl castanospermine (MDL 28574). Antiviral Res 1994; 25:169–75.
10. Sato H, Takeshita H, Furukawa M, Seiki M. Epstein-Barr virus BZLF1 transactivator is a negative regulator of Jun. J Virol 1992; 66:4732–6.
11. Phillips DM. The role of cell-to-cell transmission in HIV infection. AIDS 1994; 8:719–31.
12. McDonald D, Wu L, Bohks SM, KewalRamani VN, Unutmaz D, Hope TJ. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. Science 2003; 300:1295–7.
13. Sherer NM, Lehmann MJ, Jimenez-Soto LF, Horensavitz C, Pypaert M, Mothes W. Retroviruses can establish filopodial bridges for efficient cell-to-cell transmission. Nat Cell Biol 2007; 9:310–5.
14. Jolly C, Booth NJ, Neil SJ. Cell-cell spread of human immunodeficiency virus type 1 overcomes tetherin/BST-2-mediated restriction in T cells. J Virol 2010; 84:12185–99.
15. Felts RL, Narayan K, Estes JD, et al. 3D visualization of HIV transfer at the virological synapse between dendritic cells and T cells. Proc Natl Acad Sci U S A 2010; 107:13336–41.
16. Wang JH, Kwas C, Wu L. Intercellular adhesion molecule 1 (ICAM-1), but not ICAM-2 and -3, is important for dendritic cell-mediated human immunodeficiency virus type 1 transmission. J Virol 2009; 83:4195–204.
17. Hubner W, McNerney GP, Chen P, et al. Quantitative 3D video microscopy of HIV transfer across T cell virological synapses. Science 2009; 323:1743–7.
18. Dale BM, McNerney GP, Hubner W, Huser TR, Chen BK. Tracking and quantitation of fluorescent HIV during cell-to-cell transmission. Methods 2011; 53:20–6.
19. Sigal A, Kim JT, Balazs AB, et al. Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. Nature 2011; 477:95–8.

20. Del Portillo A, Tripodi J, Najfeld V, Wodarz D, Levy DN, Chen BK. Multiploid inheritance of HIV-1 during cell-to-cell infection. J Virol 2011; 85:7169–76.

21. Duncan CJ, Russell RA, Sattentau QJ. High multiplicity HIV-1 cell-to-cell transmission from macrophages to CD4+ T cells limits antiretroviral efficacy. AIDS 2013; 27:2201–6.

22. Puryear WB, Akiyama H, Geer SD, et al. Interferon-inducible mechanism of dendritic cell-mediated HIV-1 dissemination is dependent on Siglec-1/CD169. PLoS Pathog 2013; 9:e1003291.

23. Lackman-Smith C, Osterling C, Luckenbaugh K, et al. Development of a comprehensive human immunodeficiency virus type 1 screening algorithm for discovery and preclinical testing of topical microbicides. Antimicrob Agents Chemother 2008; 52:1768–81.

24. Anderson DJ, Yunis EJ. "Trojan Horse" leukocytes in AIDS. N Engl J Med 1983; 309:984–5.

25. Frankel SS, Steinman RM, Michael NL, et al. Neutralizing monoclonal antibodies block human immunodeficiency virus type 1 infection of dendritic cells and transmission to T cells. J Virol 1998; 72:9788–94.

26. Abela IA, Berlinger L, Schanz M, et al. Cell-cell transmission enables HIV-1 to evade inhibition by potent CD4bs directed antibodies. PLoS Pathog 2012; 8:e1002634.

27. Malbec M, Porrot F, Rua R, et al. Broadly neutralizing antibodies that inhibit HIV-1 cell to cell transmission. J Exp Med 2013; 210:2813–21.

28. Duncan CJ, Williams JP, Schiffner T, et al. High-multiplicity HIV-1 infection and neutralizing antibody evasion mediated by the macrophage-T cell virological synapse. J Virol 2014; 88:2025–34.

29. Martin N, Welsch S, Jolly C, Briggs JA, Vaux D, Sattentau QJ. Virological synapse-mediated spread of human immunodeficiency virus type 1 between T cells is sensitive to entry inhibition. J Virol 2010; 84:3516–27.

30. Zhong P, Agosto LM, Munro JB, Mothes W. Cell-to-cell transmission of viruses. Curr Opin Virol 2013; 3:44–50.

31. Buffa V, Stieh D, Mamhood N, Hu Q, Fletcher P, Shattock RJ. Cyanovirin-N potently inhibits human immunodeficiency virus type 1 infection in cellular and cervical explant models. J Gen Virol 2009; 90:234–43.

32. Selhorst P, Grupping K, Bourlet T, Delezay O, Arien KK, Vanham G. In vitro activities of candidate microbicides against cell-associated HIV. Antimicrob Agents Chemother 2012; 56:805–15.

33. Quayle AJ, Xu C, Mayer KH, Anderson DJ. T lymphocytes and macrophages, but not motile spermatozoa, are a significant source of human immunodeficiency virus in semen. J Infect Dis 1997; 176:960–8.

34. Tuomala RE, O'Driscoll PT, Bremer JW, et al. Cell-associated genital tract virus and vertical transmission of human immunodeficiency virus type 1 in antiretroviral-experienced women. J Infect Dis 2003; 187:375–84.

35. Koulinska IN, Villamor E, Chaplin B, et al. Transmission of cell-free and cell-associated HIV-1 through breast-feeding. J Acquir Immune Defic Syndr 2006; 41:93–9.

36. Pudney J, Quayle AJ, Anderson DJ. Immunological microenvironments in the human vagina and cervix: mediators of cellular immunity are concentrated in the cervical transformation zone. Biol Reprod 2005; 73:1253–63.

37. Blaskewicz CD, Pudney J, Anderson DJ. Structure and function of intercellular junctions in human cervical and vaginal mucosal epithelia. Biol Reprod 2011; 85:97–104.

38. Dinh MH, Okocha EA, Koons A, Veazey RS, Hope TJ. Expression of structural proteins in human female and male genital epithelia and implications for sexually transmitted infections. Biol Reprod 2012; 86:32.

39. Rodriguez-Garcia M, Barr FD, Crist SG, Fahey JV, Wira CR. Phenotype and susceptibility to HIV infection of CD4+ TH17 cells in the human female reproductive tract. Mucosal Immunol 2014.

40. Harman AN, Kim M, Nasr N, Sandgren KJ, Cameron PU. Tissue dendritic cells as portals for HIV entry. Rev Med Virol 2013; 23:319–33.

41. Bomsel M. Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. Nat Med 1997; 3:42–7.

42. Kinlock BL, Wang Y, Turner TM, Wang C, Liu B. Transcytosis of HIV-1 through vaginal epithelial cells is dependent on trafficking to the endocytic recycling pathway. PloS One 2014; 9:e96760.

43. Meng G, Wei X, Wu X, et al. Primary intestinal epithelial cells selectively transfer R5 HIV-1 to CCR5+ cells. Nat Med 2002; 8:150–6.

44. Carreno MP, Krieff C, Irinopoulou T, Kazatchkine MD, Belec L. Enhanced transcytosis of R5-tropic human immunodeficiency virus across tight monolayer of polarized human endometrial cells under pro-inflammatory conditions. Cytokine 2002; 20:289–94.

45. Chancey CJ, Khanna KV, Seegers JF, et al. Lactobacilli-expressed single-chain variable fragment (scFv) specific for intercellular adhesion molecule 1 (ICAM-1) blocks cell-associated HIV-1 transmission across a cervical epithelial monolayer. J Immunol 2006; 176:5627–36.

46. Van Herrewege Y, Michiels J, Waeytens A, et al. A dual chamber model of female cervical mucosa for the study of HIV transmission and for the evaluation of candidate HIV microbicides. Antiviral Res 2007; 74:111–24.

47. Alfsen A, Yu H, Magerus-Chatinet A, Schmitt A, Bomsel M. HIV-1-infected blood mononuclear cells form an integrin- and agrin-dependent viral synapse to induce efficient HIV-1 transcytosis across epithelial cell monolayer. Mol Biol Cell 2005; 16:4267–79.

48. Ganor Y, Zhou Z, Tudor D, et al. Within 1 h, HIV-1 uses viral synapses to enter efficiently the inner, but not outer, foreskin mucosa and engages Langerhans-T cell conjugates. Mucosal Immunol 2010; 3:506–22.

49. Greenhead P, Hayes P, Watts PS, Laing KG, Griffin GE, Shattock RJ. Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal virucides. J Virol 2000; 74:5577–86.

50. Bobardt MD, Chatterji U, Selvarajah S, et al. Cell-free human immunodeficiency virus type 1 transcytosis through primary genital epithelial cells. J Virol 2007; 81:395–405.

51. Bouschbacher M, Bomsel M, Verronese E, et al. Early events in HIV transmission through a human reconstructed vaginal mucosa. AIDS 2008; 22:1257–66.

52. Hocini H, Belec L, Iscaki S, et al. High-level ability of secretory IgA to block HIV type 1 transcytosis: contrasting secretory IgA and IgG responses to glycoprotein 160. AIDS Res Hum Retroviruses 1997; 13:1179–85.

53. Chomont N, Hocini H, Gody JC, et al. Neutralizing monoclonal antibodies to human immunodeficiency virus type 1 do not inhibit viral transcytosis through mucosal epithelial cells. Virology 2008; 370:246–54.

54. Lara HH, Ixtepan-Turrent L, Garza-Trevino EN, Rodriguez-Padilla C. PVP-coated silver nanoparticles block the transmission of cell-free and cell-associated HIV-1 in human cervical culture. J Nanobiotechnology 2010; 8:15.

55. Saidi H, Nasreddine N, Jenabian MA, et al. Differential in vitro inhibitory activity against HIV-1 of alpha-(1–3)- and alpha-(1–6)-D-mannose specific plant lectins: implication for microbicide development. J Transl Med 2007; 5:28.

56. Lorin C, Saidi H, Belaid A, et al. The antimicrobial peptide dermaseptin S4 inhibits HIV-1 infectivity in vitro. Virology 2005; 334:264–75.

57. Collins KB, Patterson BK, Naus GJ, Landers DV, Gupta P. Development of an in vitro organ culture model to study transmission of HIV-1 in the female genital tract. Nat Med 2000; 6:475–9.

58. Maher D, Wu X, Schacker T, Horbul J, Southern P. HIV binding, penetration, and primary infection in human cervicovaginal tissue. Proc Natl Acad Sci U S A 2005; 102:11504–9.

59. Zhou Z, Barry de Longchamps N, Schmitt A, et al. HIV-1 efficient entry in inner foreskin is mediated by elevated CCL5/RANTES that recruits T cells and fuels conjugate formation with Langerhans cells. PLoS Pathog 2011; 7:e1002100.

60. Tugizov SM, Herrera R, Veluppillai P, et al. Differential transmission of HIV traversing fetal oral/intestinal epithelia and adult oral epithelia. J Virol 2012; 86:2556–70.

61. Kolodkin-Gal D, Hulot SL, Korioth-Schmitz B, et al. Efficiency of cell-free and cell-associated virus in mucosal transmission of human immunodeficiency virus type 1 and simian immunodeficiency virus. J Virol 2013; 87:13589–97.

62. Anderson DJ, Pudney J, Schust DJ. Caveats associated with the use of human cervical tissue for HIV and microbicide research. AIDS 2010; 24:1–4.

63. Cummins JE Jr, Guarner J, Flowers L, et al. Preclinical testing of candidate topical microbicides for anti-human immunodeficiency virus type 1 activity and tissue toxicity in a human cervical explant culture. Antimicrob Agents Chemother **2007**; 51:1770–9.

64. Gupta P, Lackman-Smith C, Snyder B, et al. Antiviral activity of retrocyclin RC-101, a candidate microbicide against cell-associated HIV-1. AIDS Res Hum Retroviruses **2013**; 29:391–6.

65. McKinnon LR, Hughes SM, De Rosa SC, et al. Optimizing viable leukocyte sampling from the female genital tract for clinical trials: an international multi-site study. PloS One **2014**; 9:e85675.

66. Poltich J, Marathe J, Anderson DJ. Characteristics and Quantities of HIV Host Cells in Human Genital Tract Secretions. J Infect Dis **2014**.

67. Anderson DJ, Marathe J, Pudney J. The structure of the human vaginal stratum corneum and its role in immune defense. Am J Reprod Immunol **2014**; 71:618–23.

68. Cone RA. Vaginal factors that may influence transmission of cell-associated HIV. J Infect Dis **2014**.

69. Ackerman ME, Alter G. Opportunities to exploit non-neutralizing HIV-specific antibody activity. Curr HIV Res **2013**; 11:365–77.

70. Streeck H, Lu R, Beckwith N, et al. Emergence of individual HIV-specific CD8 T cell responses during primary HIV-1 infection can determine long-term disease outcome. J Virol **2014**.

71. Forthal D, Hope TJ, Alter G. New paradigms for functional HIV-specific nonneutralizing antibodies. Curr Opin HIV AIDS **2013**; 8:393–401.