

# Host and viral factors influencing heterosexual HIV transmission

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The World Health Organization estimates that 28–30 million people have been infected with human immunodeficiency virus (HIV). Although many important questions remain, much has been learned regarding the biology of heterosexual HIV transmission. For example, most sexual transmission of HIV is probably mediated by cell-free virus. Langerhans cells in the vaginal epithelium and dendritic cells or macrophages in the lamina propria are the most likely target cells in HIV transmission. Although there is restriction in the genotypes of viruses that are sexually transmitted, the common phenotypic characteristic of these viruses does not appear to be related to *in vitro* measures of tropism but rather to the relatively high *in vivo* replicative fitness of the transmitted viral variants. Clearly, host factors such as the amount of HIV-1 co-receptor expression on host cells and the presence of ulcerative sexually transmitted diseases affect the susceptibility of an individual to HIV infection. The growing body of information has increased the likelihood that a vaccine capable of preventing HIV-1 infection after exposure during sexual activity will be developed in the foreseeable future.

Twenty-eight to thirty million people have been infected with human immunodeficiency virus (HIV) worldwide, according to World Health Organization estimates. Most of these people were infected through heterosexual contact and this is particularly true in the urban centres of sub-Saharan Africa and Asia (Johnson, 1988; Piot *et al.*, 1988). Despite its importance, the biology of this mode of transmission is not fully understood. Many HIV-infected individuals who became infected by heterosexual contact may be unaware of their infection status. Because the mean incubation period from seroconversion to AIDS is about 8–10 years (Bacchetti and Moss, 1989), these individuals have the potential, through sexual contact, to disseminate HIV widely before they become aware that they are infectious. Thus, AIDS has the potential to become a far more serious global epidemic than is currently the case. It is likely that development of a vaccine capable of preventing heterosexual HIV transmission will be facilitated by a more complete understanding of the biology of heterosexual HIV transmission. Significant progress has been made in recent years, and much of this progress has been made by using the simian immunodeficiency virus (SIV)/rhesus monkey model of heterosexual HIV transmission (Miller *et al.*, 1989, 1990, 1994a; Miller, 1994; Lu *et al.*, 1996). Both HIV and SIV are retroviruses of the lentivirus family. They have a high degree of nucleotide sequence similarity and organization of viral genes is similar. We have shown that vaginal inoculation of SIV in rhesus macaques results in systemic infection.

The reproductive physiology, including a 28 day menstrual cycle, of rhesus macaques is remarkably similar to that of humans (Hendrickx and Cukierski, 1987). Furthermore, the gross and histological anatomy of the genital tract of women and female rhesus macaques is very similar. In both species, the mucosa of the vagina is composed of a nonkeratinized, stratified squamous epithelium and an underlying highly

vascularized lamina propria. The ectocervix has a similar architecture, and the endocervix (which is not normally exposed to material in the vaginal lumen) is composed of a simple columnar epithelium covering a highly vascularized lamina propria. Membranous cells (M cells) have not been demonstrated in the vagina or cervix; the intraepithelial antigen-presenting cell is the Langerhans cell. Langerhans cells are specialized members of the dendritic cell family that reside in stratified squamous epithelia of the body. In mice, antigen absorption in the vagina occurs via CD4<sup>+</sup> Langerhans cells (Parr and Parr, 1990). Langerhans cells are MHC II<sup>+</sup> and CD4<sup>+</sup> dendritic cells located within the ectocervical and vaginal squamous epithelium of humans (Edwards and Morris, 1985). These cells are also abundant in the vagina of the rhesus macaque and extend dendritic processes to the lumen of the vagina (Miller *et al.*, 1992a,b). Langerhans cells are common in the skin, where they have been shown, upon antigen stimulation, to migrate to the draining lymph node. In the draining lymph node, Langerhans cells transform into the interdigitating dendritic cells (IDCs) of the T cell-rich paracortex. Interdigitating dendritic cells present antigen to T helper cells in the paracortex, which in turn stimulate antigen-specific B cells. A resident population of monocyte-macrophages and T cells and B cells is present in the lamina propria of the vagina. These cells are specifically localized in the superficial lamina propria, just beneath the vaginal epithelium. Of particular significance is the presence of large numbers of CD8<sup>+</sup> T cells within the epithelium and superficial submucosa of the vagina and cervix in both rhesus macaques and humans (Edwards and Morris, 1985; Roncalli *et al.*, 1988; Miller *et al.*, 1992a). Other investigators have demonstrated the presence of plasma cells, predominantly IgA-secreting, in the human cervix. The identical cellular components of the human and rhesus macaque lower female reproductive tract suggest that the biology of the closely related SIV and HIV viruses is

likely to be very similar in their respective species. Thus, the rhesus macaque is an excellent animal model in which to study the biology of heterosexual HIV transmission and try to develop immunization strategies to prevent heterosexual transmission of HIV.

For development of vaccines, it is particularly urgent that several questions regarding the biology of sexual transmission of HIV be addressed. The relative importance of cell-associated and cell free virus in heterosexual transmission of HIV must be determined. The effect of endogenous and exogenous steroids on the reproductive tract and how this affects transmission of HIV needs to be understood. The extent to which specific viral variants are transmitted selectively by heterosexual intercourse, the target cells for viral transmission in the reproductive tract, and the route the virus takes to leave the reproductive tract and disseminate to the systemic lymphoid tissue need to be defined. In addition, an understanding of how this route of dissemination affects the kinetics of virus replication may have a significant impact on vaccine design.

### Host factors influencing transmission

In the USA, HIV appears to be transmitted more efficiently from men to women than from women to men (Padian *et al.*, 1987, 1991). Several cofactors have been identified that increase the risk of an individual acquiring HIV through heterosexual contact. Cervical ectopy, receptive anal intercourse, genital ulcer disease and infection with other sexually transmitted diseases are the most significant factors associated with HIV infection of women (Holmes and Kreiss, 1988; Holmberg *et al.*, 1989; Plummer *et al.*, 1991), while the presence of an intact foreskin and genital ulcer disease of the penis are the risk factors most often associated with HIV infection in men (Simonsen *et al.*, 1988; Cameron *et al.*, 1989).

It is estimated that for a single sexual contact, the infectivity of HIV is 0.3% (Padian *et al.*, 1987; Hearst and Hulley, 1988; Peterman *et al.*, 1988; Lawrence *et al.*, 1990). However, some individuals become infected after a single or a few sexual contacts (Staskewski *et al.*, 1987; Peterman *et al.*, 1988), while others remain uninfected despite hundreds of contacts (Lawrence *et al.*, 1990). There are three possible explanations for the variability in the sexual transmission of HIV. First, unique factors in the HIV-infected individual (stage of disease, immune response, presence of other sexually transmitted diseases) may influence infectivity. Second, unique factors in specific HIV strains may influence infectiousness. Some HIV strains may be inherently more likely than other strains to be shed in the secretions of infected persons or some strains may, by virtue of their cellular tropism or some other phenotypic characteristic, have an increased affinity for target cells in the reproductive tract. Third, unique factors (stage of menstrual cycle, nutritional status or the presence or absence of the risk factors listed above) may influence the susceptibility of individuals to HIV infection after exposure. One factor that may be associated with an increased risk of infection in women is the use of oral or injectable contraceptives (Plummer *et al.*, 1991; Daly *et al.*, 1994; Plourde and Plummer, 1994). A recent study in the SIV/rhesus monkey model of sexual transmission of HIV demonstrated that treatment of macaques with high doses of exogenous progesterone increases their susceptibility to infection

after vaginal inoculation of SIV (Marx *et al.*, 1996). However, similar effects have not been seen in women using progestin contraceptives (Duerr *et al.*, 1997). Some individuals may be highly susceptible to HIV infection, while others may be more resistant. In fact, several recent reports suggest that inherent resistance to infection is associated with the absence of a co-receptor for HIV on the cells of these individuals (Dean *et al.*, 1996; Liu *et al.*, 1996).

### Biology of the reproductive tract

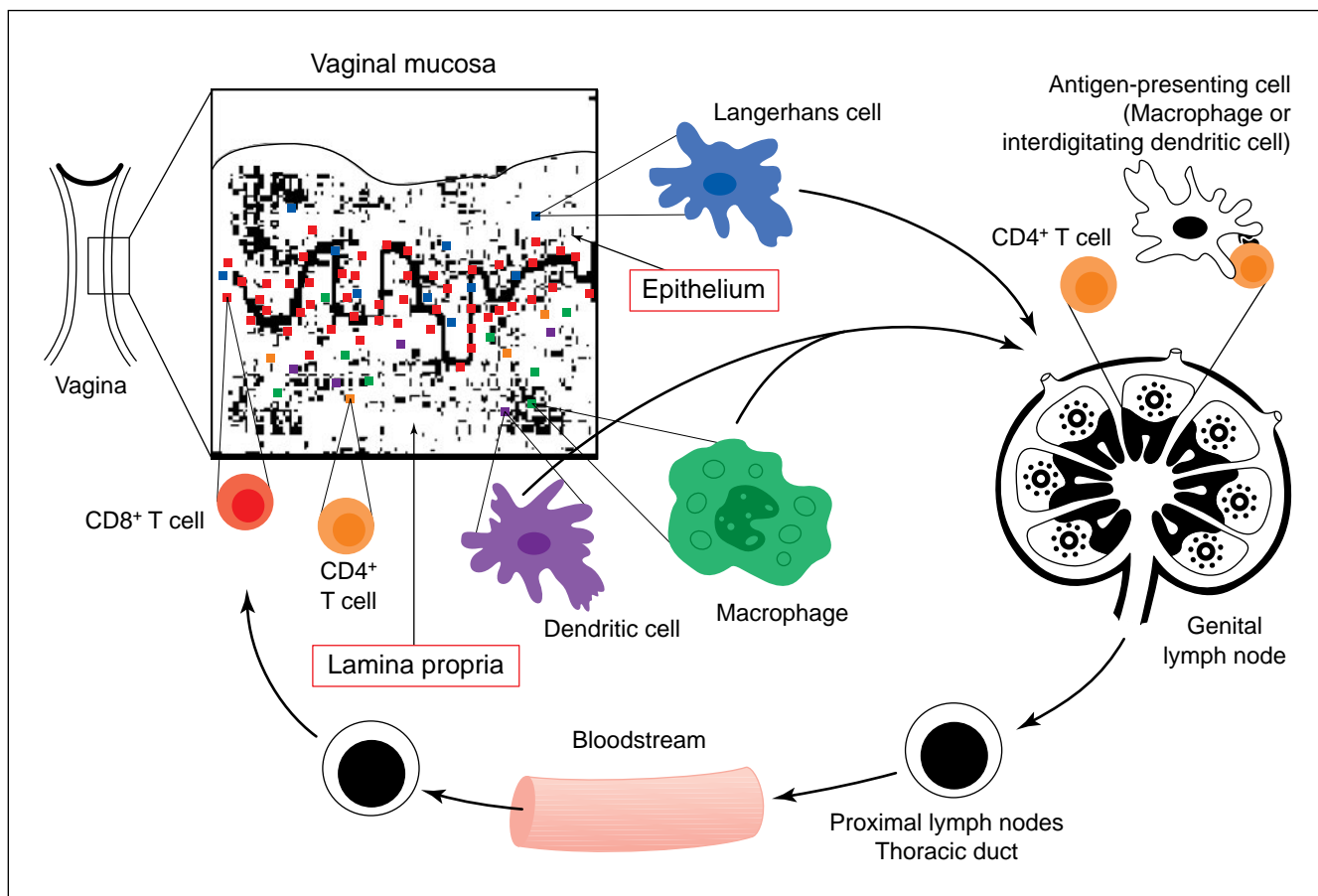
HIV and SIV infect CD4<sup>+</sup> cells of the immune system, including CD4<sup>+</sup> T lymphocytes, macrophages and dendritic cells. There have been several reports that HIV and SIV can infect epithelial cells *in vitro*, but this has never been demonstrated convincingly *in vivo*. Thus, the presumption must be that the CD4<sup>+</sup> cells in the reproductive tract are the target cells for HIV transmission. Understanding the biology of these viruses in any local anatomic site requires an understanding of the distribution and function of these cell types at the site. Significant progress has been made recently in characterizing the components of the genital immune system in males and females and several reviews have been published recently. This work is briefly summarized below.

#### *Distribution of immune cells in the female reproductive tract*

The vagina consists of a nonkeratinized, stratified squamous epithelium resting on an indistinct lamina propria and an underlying vascular submucosa (Fig. 1). The ectocervix has a similar architecture, while the endocervix consists of a simple columnar epithelium covering a vascular submucosa. The mucosal immune system in the genital tract of the female rhesus macaque consists of a resident population of monocyte-macrophages and T cells in the lamina propria of the vagina and cervix (Miller *et al.*, 1992a; Fig. 1). CD8<sup>+</sup> T cells are especially abundant in these tissues and are specifically localized in the deep layers of the vaginal epithelium or in the lamina propria, just beneath the vaginal epithelium. A similar population of lymphocytes and macrophages has been described in the submucosa of the human cervix, where they have been called mucosal associated lymphoreticular tissue (MALT; Edwards and Morris, 1985). MHC class II<sup>+</sup>, CD4<sup>+</sup> dendritic Langerhans cells are abundant in the vaginal and ectocervical mucosa of women (Hackemann *et al.*, 1968; Younes *et al.*, 1968; Bjercke *et al.*, 1983; Morris *et al.*, 1983; Edwards and Morris, 1985) and rhesus macaques (Miller *et al.*, 1992a) and dendritic processes from Langerhans cells can extend to the lumen of the vagina, presumably to sample antigens. In mice, absorption of soluble antigen from the lumen of the vagina occurs via Langerhans cells (Parr *et al.*, 1991).

#### *Distribution of immune cells in the male reproductive tract*

Pudney and Anderson (1993) provide an excellent description of the organization of immune cells in the male reproductive tract, which is briefly summarized below. There are numerous macrophages but no lymphocytes in the interstitium of the testis and no white blood cells are found in the seminiferous tubules of normal men. However, a variety of pathological conditions can result in greater numbers of T cells



**Fig. 1.** Distribution of lymphoid cells in the vaginal mucosa. The vaginal mucosa consists of a stratified squamous epithelium and an underlying vascular lamina propria. The vaginal mucosa contains all of the cellular components required to complete an antigen-specific immune response. Langerhans cells in the vaginal epithelium and dendritic cells and macrophages in the lamina propria are the antigen-presenting cells. These cells take up and process antigen and then migrate to the genital lymph node to interact with antigen-specific CD4<sup>+</sup> T cells, or they can interact with antigen-specific CD4<sup>+</sup> T cells in the lamina propria. These stimulated CD4<sup>+</sup> T cells orchestrate the development of antigen-specific B cells and CD8<sup>+</sup> T cells. Once differentiated, the CD8<sup>+</sup> T cells and B cells re-circulate to the vaginal mucosa, where they perform their individual effector functions. A striking finding is the large number of CD8<sup>+</sup> T cells in the vaginal mucosa. In SIV-infected monkeys (Lohman *et al.*, 1995) and HIV-infected women (Musey *et al.*, 1997), the CD8<sup>+</sup> cells from the vaginal or cervical mucosa have been shown to kill virus-infected cells *in vitro*, indicating that these cells may have an important anti-viral immune effector function in the female reproductive tract. Not shown is the fact that occasional lymphoid follicles can be found in the lamina propria and superficial submucosa. These nodules consist of large numbers of CD4<sup>+</sup> T cells and B cells and fewer macrophages. These structures may be the morphological manifestation of an ongoing secondary immune response in the vaginal mucosa.

within the testicles. In normal testicles from healthy men, CD8<sup>+</sup> T cells are located in the columnar epithelium of the rete testis and CD4<sup>+</sup> T cells are found in the connective tissue around the rete testis. T lymphocytes also occur in the epididymis, seminal vesicles and prostate of normal men and CD4<sup>+</sup> T cells are found in human epididymal epithelium. In addition to T cells, numerous macrophages are present in the interstitium of the excurrent ducts and accessory glands. The penile urethra also contains a complete complement of immune cells. Numerous CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes, dendritic cells and macrophages are present in the lamina propria and submucosa of the penile urethra (J. Pudney and D. Anderson, personal communication). In addition, numerous Langerhans cells are located in the foreskin of rhesus macaques (Miller *et al.*, 1994b) and men (C. Miller, unpublished).

#### HIV/SIV target cells in the genital tract

The identity and location of the cellular targets of HIV/SIV during genital transmission remain to be determined. However, significant progress has been made recently. Simple application of SIV onto the intact genital mucosa of mature and immature rhesus macaques results in virus transmission, and the disease induced by this route of inoculation is indistinguishable from that seen in animals inoculated intravenously (Miller *et al.*, 1989, 1990). SIV is transmitted efficiently to hysterectomized female macaques by inoculation of cell-free virus into blind vaginal pouches (Miller *et al.*, 1992c). Since there are no CD4<sup>+</sup> T cells in the vaginal epithelium and only a few CD4<sup>+</sup> T cells are present in the submucosa of the vagina (Miller *et al.*, 1992a), the most likely target cells in the vaginal mucosa are

macrophages or Langerhans cells (Miller *et al.*, 1992a,b). In chronically infected female rhesus macaques, SIV-infected cells are present in the uterus, cervix and vagina (Miller *et al.*, 1992b). The majority of the SIV-infected cells are located in the submucosa of the ectocervix and vagina and have a morphology consistent with T lymphocytes and monocyte-macrophages. Occasionally, SIV-infected cells are also found within the stratified squamous epithelium of the vagina. Some of the infected cells in this location have a dendritic morphology consistent with Langerhans cells (Miller *et al.*, 1992b). Using triple-label techniques, we have recently shown that CD1a<sup>+</sup>, p55<sup>+</sup> Langerhans cells in the vaginal epithelium contain mRNA encoding SIV (J. Hu and C. Miller, unpublished). This is the first unequivocal demonstration that Langerhans cells in the vaginal mucosa are infected with SIV. Langerhans cells of the skin can be infected with HIV in AIDS patients (Tschachler *et al.*, 1987; Zambruno *et al.*, 1991). The finding that cells in the vaginal epithelium are infected with SIV is the first indication that mucosal Langerhans cells might be infected with a lentivirus (Miller *et al.*, 1992b). In cervical biopsy material obtained from HIV-infected women, T cells and macrophages were determined to be the cell types infected with HIV (Pomerantz *et al.*, 1988; Nuovo *et al.*, 1993). These findings are consistent with the results in SIV-infected monkeys. All the cells that were found to be infected in the genital tract of both humans and rhesus monkeys presumably express the CD4 molecule. There is no evidence that nonCD4<sup>+</sup> cell types (epithelial cells) are infected in the reproductive tracts of either monkeys or humans.

The transmission of HIV/SIV to males may involve similar types of target cell. Studies to detect the location of HIV-infected cells in the penis of infected men are lacking, but the fact that CD4<sup>+</sup> Langerhans cells are located in the foreskin and the epidemiological evidence indicating that the presence of an intact foreskin is associated with an increased risk of HIV infection suggests that Langerhans cells in the foreskin may be target cells during genital transmission. In SIV-infected rhesus macaques, these Langerhans cells in the foreskin contain SIV nucleic acid (Miller *et al.*, 1994b). In addition, adult male rhesus macaques can be infected with SIV by placing cell-free virus onto the foreskin of the animals (Miller *et al.*, 1990).

These findings suggest that Langerhans cells have a role as target cells in the sexual transmission of HIV and SIV. These antigen-presenting cells are potentially efficient disseminators of virus from the genital mucosa to draining lymph nodes. These cells function to transport antigen from the stratified squamous epithelium to the draining lymph node (Shelley and Juhlin, 1976; Silberberg-Sinakin *et al.*, 1976; Shelley and Juhlin, 1977; Hoefsmi *et al.*, 1982; Kraal *et al.*, 1986; Kupiec-Weglinski *et al.*, 1988; Kripke *et al.*, 1990; Steinman, 1991). Blood dendritic cells (of which Langerhans cell precursors are a subset) can be infected with HIV (Patterson and Knight, 1987; Macatonia *et al.*, 1990; Langhoff *et al.*, 1991; Patterson *et al.*, 1991). Furthermore, when infected *in vitro*, these cells produce much higher concentrations of virus than do T cells, but they do not exhibit the usual cytopathic effects associated with HIV infection (Langhoff *et al.*, 1991). In addition, dendritic cell-T cell conjugates produce especially high concentrations of HIV *in vitro* (Pope *et al.*, 1995). Thus, Langerhans cells in the male and female genital tract (Hackemann *et al.*, 1968; Younes *et al.*, 1968; Bjerkke *et al.*, 1983; Morris *et al.*, 1983; Edwards and Morris,

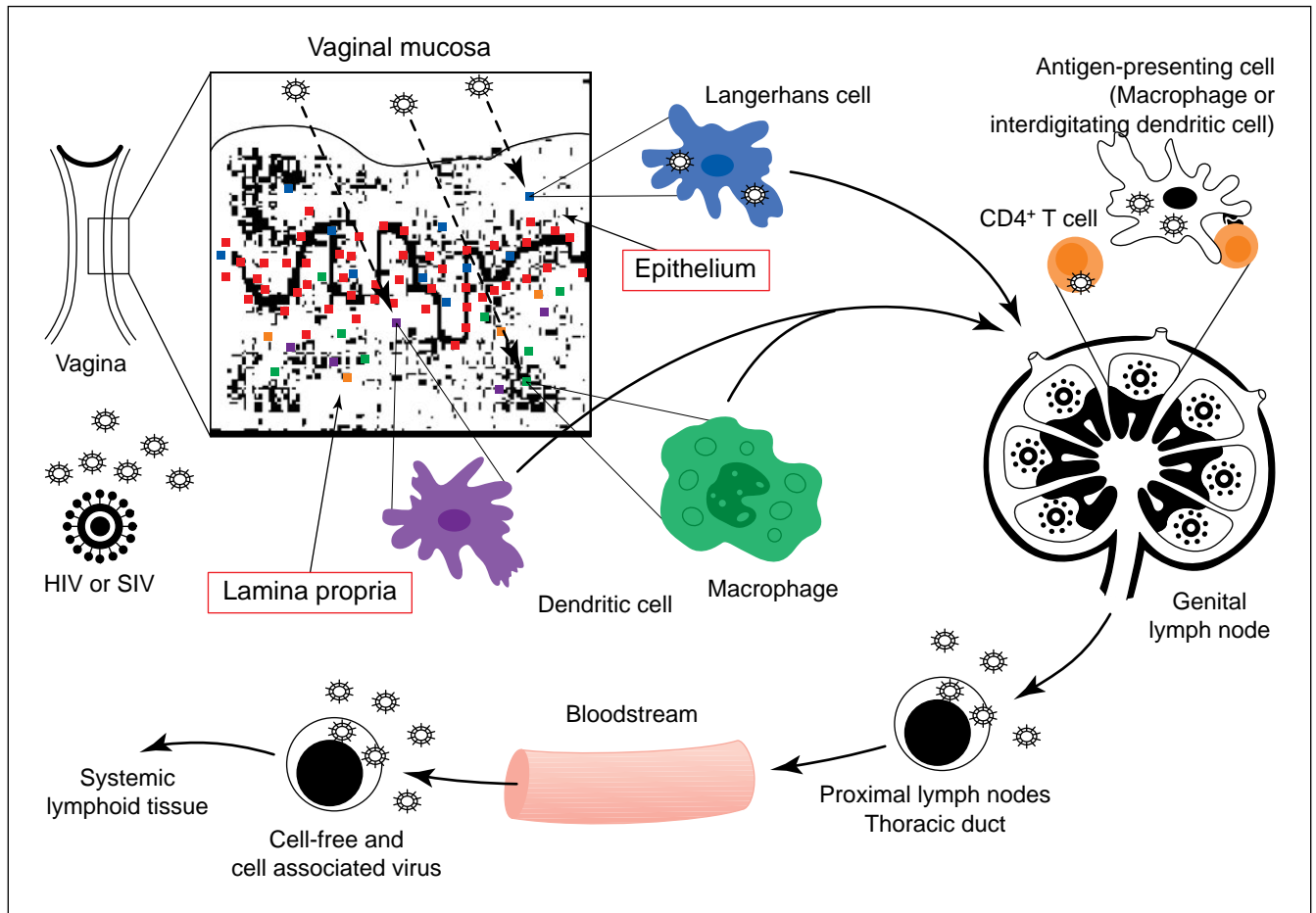
1985) may be particularly well suited as target cells for the sexual transmission of HIV and SIV (Miller *et al.*, 1992b,c). On the basis of these observations, we have proposed a model to explain the cellular events in the genital transmission of HIV and SIV (Miller *et al.*, 1992c,d; Fig. 2).

It has been suggested that trauma to tissues during intercourse may be necessary for heterosexual transmission of HIV and that virus can gain direct access to the bloodstream through these wounds; however, this is very unlikely. The haemodynamic pressures in the peripheral vasculature do not permit the movement of particles (cells or viruses) from tissues directly into blood vessels. Blood cells (including CD4<sup>+</sup> T cells) that escape from the peripheral vasculature do not re-enter the bloodstream but travel through lymphatics to reach the draining lymph nodes. This is the same pathway that vaginal macrophages and Langerhans cells would take after infection with HIV (Fig. 2; Miller *et al.*, 1992c). It is reasonable to assume that trauma would increase the number of viruses that cross the mucosa and that the haemorrhage associated with the trauma would increase the number of CD4<sup>+</sup> target cells in the submucosa of the vagina. The increased number of virions and target cells might increase the efficiency of HIV transmission but probably would not affect the route of virus dissemination from the genital tract to systemic lymphoid tissue (Miller *et al.*, 1992c). In heterosexual HIV transmission, the initial virus-host cell interaction occurs in the genital mucosa or submucosa and the early events in the dissemination of HIV occur in the draining lymph node.

## Viral factors in sexual transmission of HIV

### *Heterosexual transmission of cell-free or cell-associated HIV*

It is of interest to know which form of HIV, cell-free virions or virus infected cells, is more important for sexual transmission of HIV. Both cell-associated and cell-free HIV are present in genital secretions and it is not clear if one or both forms of virus are involved in sexual transmission (for review see Alexander, 1990). Cell-free and cell-associated HIV and SIV can be isolated from cervico-vaginal secretions at any stage of the menstrual cycle (Vogt *et al.*, 1986, 1987; Wofsy *et al.*, 1986; Miller *et al.*, 1990). HIV is present in semen in both cell-free and cell-associated form and can be isolated from asymptomatic individuals and AIDS patients (Zagury *et al.*, 1984; Anderson *et al.*, 1990, 1992; Kreiger *et al.*, 1991). Studies using PCR have produced widely divergent results on the prevalence of HIV nucleic acid in semen. In one study, the HIV genome was detected in the nonspermatozoal mononuclear cells in 74% (17/23) and in the seminal fluid of 65% (15/23) of the semen samples tested (Mermin *et al.*, 1991). In another study, HIV DNA was detected in nonspermatozoal mononuclear cells in 1 of 25 semen samples tested (VanVoorhis *et al.*, 1991). HIV can be isolated from the ejaculate of vasectomized seropositive men (Anderson *et al.*, 1991). Cell-free and cell-associated SIV can be isolated from the semen of infected rhesus macaques (Miller *et al.*, 1989). SIV can be isolated from the semen of intravenously inoculated animals before seroconversion, and the virus is present in both cell-free and cell-associated form (C. J. Miller, unpublished). We have recently shown that rhesus macaques inoculated intravaginally with up to 10<sup>4</sup> SIV-infected



**Fig. 2.** A model of HIV heterosexual transmission and subsequent virus dissemination. Cell-free HIV infects CD4<sup>+</sup> antigen-presenting cells in the epithelium (Langerhans cells) or in the lamina propria (macrophages or dendritic cells) of the vaginal mucosa. These HIV-infected antigen-presenting cells migrate to the iliac and obturator lymph nodes which drain the genital tract. In rhesus macaques, India ink injected into the vagina localizes in these lymph nodes within 72 h (C. J. Miller, unpublished). The infected antigen-presenting cells interact with CD4<sup>+</sup> T cells in the paracortex of the lymph node, spreading the virus infection. Once the virus is replicating in T cells, it is passed by cell-cell contact and as cell-free virions to infect numerous cells (CD4<sup>+</sup> T cells, interdigitating dendritic cells, macrophages) in the lymph node. Infected CD4<sup>+</sup> T cells and virions move through the efferent lymphatics up the para-aortic lymph node chain, disseminating the infection. Eventually, HIV-infected CD4<sup>+</sup> T cells and virions reach the bloodstream and disseminate the infection to distant lymphoid tissues. There appears to be a fundamental difference in the kinetics of virus replication between animals inoculated intravenously and animals inoculated intravaginally with a very pathogenic SIV/HIV (SHIV) chimaeric virus (C. J. Miller, unpublished). In the animals inoculated intravenously, the peak of antigenaemia occurred 7 days after inoculation. In contrast, in animals that became infected by intravaginal inoculation of the pathogenic SHIV 89.6-PD, peak antigenaemia occurred 14 days after inoculation. This 1 week delay in viral replication and dissemination is consistent with the model of stepwise dissemination of HIV from the mucosal surface to the draining lymph nodes and, subsequently, to the bloodstream. (Modified from Miller *et al.*, 1992c.)

peripheral blood mononuclear cells do not become infected with SIV, while intravenous inoculation with as few as ten SIV-infected cells produces systemic infection (Miller, 1994). These experiments are being repeated but, based on these preliminary studies, it seems that cell-free virus is more likely to be responsible for transmission during sexual contact.

#### Preferential heterosexual transmission of viral variants

If 'selection' for specific HIV variants by sexual intercourse occurs, vaccines should be designed to prevent transmission of these specific viruses. HIV-1 variants can be defined in at least two ways: viruses that differ on the basis of nucleotide sequence

are genotypic variants, whereas viruses that differ on the basis of biological behaviour are phenotypic variants. A few studies have been undertaken in humans recently infected with HIV-1. Most of these studies have concluded that, based on envelope sequence, there is selection for a limited number of genotypes in acute HIV infection (Wolinsky *et al.*, 1992; Zhang *et al.*, 1993; Zhu *et al.*, 1993). However, this restriction on genotype does not extend to other regions of the viral genome (Zhang *et al.*, 1993; Zhu *et al.*, 1993; Albert *et al.*, 1994). It is also not clear that this restriction on the viral genotype in acute infection is related to the route of HIV transmission.

For the purposes of this discussion, vaginal transmission of SIV is defined as the ability to detect virus in peripheral blood

**Table 1.** Relationship of simian immunodeficiency virus (SIV) and SHIV phenotype to vaginal transmission in rhesus macaques

Virus isolate/clone	<i>In vitro</i> phenotype			<i>In vivo</i> phenotype <sup>a</sup>		
	Replication in T-cell lines	Replication in macrophages	Replication kinetics	Plasma antigenaemia after i.v. injection	PBMC load after i.v. inoculation	Vaginal transmission
SIVmac251 <sup>b</sup>	+	+	Rapid	+	High	Yes
SHIV89.6PD <sup>b</sup>	+	+	Intermediate	+	High	Yes
SIVmac1A11/239	+	+	Delayed	+	Intermediate	Yes
SHIV89.6	+	-	Delayed	+	Intermediate	Yes
SIVmac239	+	-	Delayed	+	High	Yes
SIVmac1A11	+	+	Rapid	-	Low	No
SHIVHXBc2	+	+	Intermediate	-	Intermediate	No

<sup>a</sup>Data generated by characterizing the virologic parameters in rhesus macaques that were infected with each virus clone or isolate by intravenous (i.v.) inoculation.

<sup>b</sup>Virus stocks derived from uncloned viral isolates. All other virus stocks were derived from viral clones.

PBMC, peripheral blood mononuclear cells.

mononuclear cells, by virus isolation or PCR, after vaginal inoculation. This definition does not rule out the possibility that a virus can cross the mucosa but cannot disseminate systemically. SHIVHXBc2 and SHIV 89.6 are SHIV/HIV chimaeras between SIVmac239 and HIV-1 molecular clones (Li *et al.*, 1992; Lu *et al.*, 1996; Reimann *et al.*, 1996a). Both SIV/HIV chimaeric (SHIV) viruses reliably produce infection in macaques inoculated intravenously (Reimann *et al.*, 1996a). We have reported (Lu *et al.*, 1996) that four intravaginal inoculations of SHIVHXBc2 fail to produce a systemic infection in rhesus macaques. In contrast, as few as three intravaginal inoculations of SHIV89.6 consistently result in viraemia in rhesus macaques (Lu *et al.*, 1996). One experiment in this study demonstrated that, after intravaginally inoculating animals with a mixed inoculum containing both viruses, only the SHIV89.6 genome could be detected in the peripheral blood mononuclear cells of the animals. The only difference in the genotype of these two viruses is that the gp120 and gp41 coding regions are derived from different parental HIV-1 clones. This is clear evidence that, in the SHIV/rhesus macaque system, the coding sequence of envelope influences the ability of a virus to produce systemic infection after intravaginal inoculation. In addition, we have systemically infected rhesus macaques with a single intravaginal application of SHIV89.6-PD. SHIV89.6-PD is a pathogenic variant of SHIV89.6 that was derived from the plasma of a rhesus macaque that developed AIDS after intravenous inoculation with blood from another rhesus macaque infected with SHIV89.6 (Reimann *et al.*, 1996b). The genetic changes that distinguish SHIV89.6PD from the parental SHIV 89.6 have not been defined, but are the subject of intense investigation.

A second study involved the use of three molecular clones of SIV to assess transmission of viral variants during vaginal inoculation: SIVmac239, SIVmac 1A11 and a chimaeric virus that consisted of the regions encoding the gp41 and LTRs of SIVmac 239 in the background of SIVmac1A11 (SIVmac1A11/239). Intravenous inoculation of all three of these viruses reliably produces infection in rhesus macaques (Marthas *et al.*, 1993). However, animals inoculated intravaginally with SIVmac239 and SIVmac1A11/239 reliably become infected after a single intravaginal inoculation, while animals inoculated intravaginally

with SIVmac1A11 become infected only rarely. The only difference in the genotype of SIVmac1A11 and SIVmac1A11/239 is that the gp41 and LTRs of the latter virus are derived from SIVmac239 (Marthas *et al.*, 1993). Thus, the nucleotide sequences of gp41 or LTR, or both, can influence the ability of a virus to produce systemic infection after intravaginal inoculation.

We have shown that not all the SIV or SHIV virus genotypes that produce systemic infection by intravenous inoculation are capable of producing systemic infection by intravaginal inoculation. These results clearly demonstrate that the genotypic determinants that permit SIV or SHIV to produce systemic infection differ depending on the route of virus inoculation. However, the phenotypic characteristics that may be common to the genotypes that produce systemic infection after vaginal inoculation remain undefined. A summary of the *in vitro* phenotypes of the viruses used in this study is provided (Table 1). As can be seen, the ability of a virus to grow in rhesus macaque monocyte-derived macrophages *in vitro* does not predict the outcome of intravaginal inoculation. SIVmac1A11 and SHIVHXBc2 replicate efficiently in rhesus macaque macrophages but do not transmit vaginally, while SIVmac239 and SHIV89.6 do not replicate in macrophages but do transmit vaginally.

Because all of the viruses used in this study systemically infect animals after intravenous inoculation, we next sought to determine whether viruses that transmit vaginally share a common *in vivo* replication phenotype in animals inoculated intravenously. We assessed two parameters: (1) ability of a virus to produce plasma antigenaemia (viral antigens in blood) or plasma viral RNA concentrations; and (2) the cell-associated virus load in animals infected with a particular virus. A summary of this analysis is presented (Table 1). These *in vivo* studies demonstrate that viruses that produce a plasma antigenaemia after intravenous inoculation are uniformly capable of producing systemic infection after intravaginal inoculation. Not shown is the data that demonstrated that relative concentrations of viral RNA in plasma after intravenous inoculation paralleled the results of the plasma antigenaemia analysis. Thus, animals inoculated intravenously with SHIV 89.6, SHIV 89.6-PD, SIVmac239 and SIVmac1A11/239 have detectable

plasma antigen and relatively high plasma concentrations of viral RNA, whereas animals inoculated intravenously with SHIVHXBc2 and SIVmac1A11 do not have a plasma antigenaemia and have relatively low plasma viral RNA concentrations. The data on plasma antigenaemia in animals inoculated intravenously with SHIVHXBc2 and SHIV 89.6 were published by Reimann *et al.*, (1996a). Thus, the *in vivo* replicative capacities of the SHIV and SIV clones or isolates used in these studies predict the ability of each virus to produce systemic infection after intravaginal inoculation.

The findings in the SHIV and SIV studies summarized here are the first clear demonstration that there is selection, or exclusion, of specific genotypes during vaginal transmission. Previous studies demonstrated that a limited number of genotypes, as characterized by the nucleotide sequence in specific regions of the envelope gene, are found in humans infected recently with HIV-1. In one study, it was shown that two individuals, infected by heterosexual contact, had viral variants with a gp120 sequence that was found in only a minor fraction of the proviral population in the blood of the transmitter (Zhu *et al.*, 1993). A more recent study characterized viral variants in a single transmitter-recipient heterosexual pair and concluded that, while the envelope V3 loop sequences in the major variant of the donor and recipient were similar, the sequence of the envelope V1-V2 region sequences in the recipient were found only in a minor variant of the donor (Zhu *et al.*, 1996). The authors speculate that this was because these variants had a selective advantage at penetrating the mucosal barrier during sexual contact. A study involving a relatively large number of acute seroconverters found that, when the nucleotide sequence of viruses in each individual were compared, there was little sequence heterogeneity in two normally hypervariable regions of the *env* gene (Zhang *et al.*, 1993). However, this result was not limited to individuals who had been infected by sexual contact but was also found in individuals infected parentally (Zhang *et al.*, 1993). This finding does not support the hypothesis that viruses with certain envelope nucleotide sequences are transmitted by sexual contact because they can penetrate the vaginal mucosa more efficiently, but rather supports the idea that only a limited number of the viral genetic variants in a donor have the fitness to initiate an infection in a naive recipient, regardless of the route of transmission. In these studies, the sequence homogeneity of the envelope did not extend to the *gag* gene (Zhang *et al.*, 1993; Zhu *et al.*, 1993). Thus, the acute seroconverters were apparently infected with multiple virus variants that were homogeneous in envelope nucleotide sequence but heterogeneous in *gag* nucleotide sequence.

With regard to preferential sexual transmission of viruses with a particular phenotype, numerous studies have demonstrated that individuals acutely infected with HIV-1 have a viral variant that can grow in primary macrophages *in vitro* but not in T cell lines and does not cause syncytia in MT-2 cells. This is the macrophage tropic-nonsyncytium-inducing (NSI) phenotype. It has been widely presumed that these viral variants with a macrophage-tropic-NSI viral phenotype are selectively transmitted by sexual contact. This notion has been championed to explain the finding of limited genetic heterogeneity of HIV envelope in individuals infected by sexual contact. Thus, a virus with an envelope sequence that allows it to replicate in macrophages would be more likely to be transmitted by

sexual contact because the virus could infect the most likely target cells in the genital mucosa. However, as with envelope nucleotide homogeneity, this apparent restriction of viral phenotype in acutely infected people occurs regardless of the route of transmission. One study of a relatively large number of acute seroconverters infected by sexual contact found that the virus that was transmitted to the donor had the same phenotype as the major viral variant in the donor (Fiore *et al.*, 1994). In this study, all the HIV-1 isolates from 21 individuals with primary HIV-1 infection replicated in monocyte-derived macrophage cultures. Seven of these isolates also replicated in T-cell lines and were, thus, dual tropic. In addition, studies on ten pairs of individuals consisting of the index case and seroconverting sexual partner showed that, when the viral phenotypes in the two individuals forming a transmission pair were compared, the phenotype of the HIV-1 was the same in both individuals in nine of the ten transmission pairs. Furthermore, both of the individuals in five of the pairs were infected with an syncytium-inducing (SI) variant. Thus, this study found that there was no selection for macrophage-tropic-NSI viruses during sexual transmission (Fiore *et al.*, 1994). These authors point out that there are a number of case reports and smaller studies in which index cases with T cell-tropic-SI variants infected a sexual partner (Yoshiyama *et al.*, 1987; Clark *et al.*, 1991; Roos *et al.*, 1992; Nielsen *et al.*, 1993; Zhu *et al.*, 1993). In approximately half of these transmission events, the seroconverting partner became infected with a T cell-tropic-SI variant.

When all the published data is reviewed, it seems clear that there is no restriction on the sexual transmission of HIV-1 variants that can replicate in T-cell lines, but not macrophages, *in vitro*. The results of the SIV and SHIV *in vivo* studies described here support this conclusion. However, we have not determined whether *in vitro* tropism in dendritic cells is associated with increased vaginal transmission of SIV or SHIV. This may be an important omission. Soto-Ramirez *et al.* (1996) have shown that only some HIV-1 variants can replicate efficiently in Langerhans cells *in vitro*, and that these strains of HIV-1 are associated epidemiologically with outbreaks of sexually transmitted HIV. This finding also provides indirect support for the hypothesis that Langerhans cells are the initial target cell for HIV infection in the genital tract (Fig. 2).

We have now demonstrated experimentally that only some viral (SIV or SHIV) genotypes can produce a systemic infection after vaginal inoculation. This supports the conclusion that there is selection for viral genotypes during sexual transmission of HIV. However, the common phenotype, if any, of the selected genotypes is not apparent from *in vitro* studies of viral phenotype. We did find that all the viruses capable of transmission by vaginal inoculation had a common *in vivo* phenotype. After intravenous inoculation of rhesus macaques, all the transmitting viruses produced plasma antigenaemia and high concentrations of plasma viral RNA. In contrast, although the nontransmitting viruses infect rhesus macaques after intravenous inoculation, the infection that occurs after intravenous inoculation is characterized by a lack of viral antigen in plasma and the low concentrations of plasma viral RNA. On the basis of these results, it is likely that viruses that are adapted to replicate to high concentrations *in vivo* are capable of being transmitted by vaginal inoculation. This principle is probably applicable to the heterosexual transmission of HIV in humans.

## Conclusions

Although brief and incomplete, this overview does highlight the fact that much has been learned regarding the biology of heterosexual HIV transmission. Although many questions remain, unifying themes have emerged from studies conducted by a number of different approaches. On the basis of experimental findings in the SIV/rhesus macaque model, it seems likely that much of HIV sexual transmission is mediated by cell-free virus, and that dendritic cells or macrophages in the vaginal mucosa are the likely target cells for HIV transmission. Although there is restriction in the genotypes of viruses transmitted through sexual contact, the common phenotypic characteristic of these viruses does not appear to be related to *in vitro* measures of tropism but rather to the relatively high replicative fitness of the transmitted viral variants *in vivo*. It is clear that host factors, such as the expression of HIV-1 co-receptor on host cells, the use of exogenous progesterone and the presence of ulcerative sexually transmitted diseases may affect the susceptibility of an individual to HIV infection.

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