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T Cell—Tropic Simian Immunodeficiency Virus (SIV) and Simian-Human Immunodeficiency Viruses Are Readily Transmitted by Vaginal Inoculation of Rhesus Macaques, and Langerhans' Cells of the Female Genital Tract Are Infected with SIV

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Abstract

Intravaginal inoculation with T cell–tropic molecular clones of simian immunodeficiency virus (SIV) or simian-human immunodeficiency virus (SHIV) or some dual-tropic strains of SIV or SHIV produced systemic infection in rhesus macaques. Vaginal inoculation with other dual-tropic molecular clones of SIV or SHIV did not infect rhesus macaques even after multiple inoculations. While in vitro measures of macrophage tropism do not predict which primate lentiviruses will produce systemic infection after intravaginal inoculation, the level to which a virus replicates in vivo after intravenous inoculation does predict the outcome of intravaginal inoculation. Another series of studies, using combined in situ hybridization and immunolabeling to simultaneously detect SIV RNA and identify the immunophenotype of infected cells, demonstrated that a large proportion (~40% in some animals) of the SIV-infected cells in the vagina and cervix were Langerhans' cells. This is the first in vivo demonstration that Langerhans' cells in the genital tract are infected with SIV and that dendritic cells are significant reservoirs for lentiviruses.

Human immunodeficiency virus (HIV) is transmitted primarily by sexual contact. An animal model, using the simian immunodeficiency virus (SIV)–rhesus macaque system, of HIV sexual transmission has been developed (reviewed in [1]). We have shown that cell-free SIVmac251 (reviewed in [1]) and some strains of SIV/HIV chimeric viruses (SHIV) [2] can cross the vaginal mucosa and initiate a systemic infection in rhesus macaques. Some studies of small numbers of individuals acutely infected with HIV through sexual contact suggest that the virus transmitted during such contact represents a variant present at low frequency in the transmitting partner's virus population and that the transmitted virus is macrophage-tropic and nonsyncytium-inducing (NSI) [3]. The capacity of a virus to infect and productively replicate in discrete populations of cells is defined as tropism, and HIV and SIV variants have been classified on the basis of their in vitro ability to replicate in macrophages (macrophage-tropic), T cell lines (T cell–tropic), or both (dual-tropic). We

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The California Regional Primate Research Center, University of California Davis, is committed to comply with the Principles for the Use of Animals, the Guide for the Care and Use of Laboratory Animals, the Provisions of the Animal Welfare Act, and all other applicable laws and regulations. Rhesus macaques at the California Regional Primate Research Center, University of California, Davis, are housed in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines. Prior to initiation, all work was approved by the Animal Care and Use Administrative Advisory Committee at the University of California, Davis.

hypothesized that the SIV or SHIV molecular clones that had a macrophage-tropic phenotype in vitro would be more likely to cross the vaginal mucosa and initiate a systemic infection than would be viral clones that were strictly T cell–tropic. We sought to test this hypothesis, using well-characterized clones of SIVmac, SHIV, and 2 uncloned viral stocks. Because a paper describing the details of this study has recently been published [4], only a summary and discussion of the work will be provided here.

Studies characterizing immune cell populations in the genital tract of women and female rhesus macaques have demonstrated that antigen-presenting cells, macrophages, and $CD1a^+$ Langerhans' cells (LC) are the most abundant $CD4^+$ cells in the cervicovaginal epithelium [5,6]. We previously showed that SIV-infected cells are located in the vaginal epithelium and lamina propria of chronically infected rhesus macaques [7]; and a recent study demonstrated that 24 h after intravaginal SIV inoculation, cells in the lamina propria of the rhesus macaque vagina contain proviral DNA [8]. However, those studies did not definitively identify the type of cell that was infected. In this study, we sought to immunophenotype SIV-infected cells in the reproductive tract of chronically infected rhesus macaques. We now report that $CD1a^+/p55^+$ LC make up a large proportion of the SIV-infected cells in the lower genital tract of some chronically infected female rhesus macaques. Because a paper describing the details of these studies has been recently published [9], only a summary and discussion of the work will be provided here.

Vaginal Transmission of SIV and SHIV Molecular Clones

For the purposes of this paper, vaginal transmission of SIV is considered to have occurred if virus could be detected in peripheral blood mononuclear cells by virus isolation or polymerase chain reaction after vaginal inoculation of SIV. This definition does not rule out the possibility that a virus can cross the mucosa but cannot disseminate systemically. SHIVHXBc2 and SHIV89.6 reliably produce infection in intravenously inoculated macaques [10]. We have reported [2] that four intravaginal inoculations of SHIVHXBc2 failed to produce a systemic infection in rhesus macaques. In contrast, as few as three intravaginal inoculations of SHIV89.6 consistently resulted in viremia in rhesus macaques [2]. After intravaginal inoculation with a mixed inoculum containing both viruses, we could detect the SHIV89.6 genome only in the peripheral blood mononuclear cells of animals. The only difference in the genotype of these 2 viruses is that the gp120 and gp41 coding regions are derived from different parental HIV-1 clones. This is clear evidence that, in the rhesus macaque system, the coding sequence of *env* can influence the ability of a virus to produce systemic infection after intravaginal inoculation. In addition, we have been able to reliably infect mature female rhesusmacaques with a single intravaginal application of SHIV89.6PD [11]. SHIV89.6PD is an uncloned virus stock generated by serial in vivo passage of SHIV89.6 [11].

We conducted additional studies, using 3 molecular clones of SIV during vaginal inoculation, to assess transmission of virus variants. SIVmac239, SIVmac1A11, and a chimeric virus that consists of the gp41 and 3′ long terminal repeat (LTR) coding regions of SIVmac239 in the background of SIVmac1A11 (SIVmac1A11/239). Intravenous inoculation of all 3 of these viruses reliably produces infection in rhesus macaques [12]. Animals inoculated intravaginally with SIVmac239 and SIVmac1A11/239 reliably become infected after one intravaginal inoculation, while animals inoculated intravaginally with SIVmac1A11 only rarely become infected. 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 [12]. Thus the coding sequences of gp41 or LTR (or both) can influence the ability of a virus to produce systemic infection after intravaginal inoculation [4].

The results above 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 following vaginal inoculation remain undefined. A summary of the in vitro phenotype of the viruses used in this study is provided in table 1. Note that 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 [4].

Because all 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 intravenously inoculated animals. We assessed 2 parameters: the ability of a virus to produce plasma antigenemia or plasma viral RNA levels and the cell-associated virus load in animals infected with a particular virus. A summary of this analysis is presented in table 1. These in vivo studies demonstrate that viruses that produce a plasma antigenemia after intravenous inoculation are uniformly capable of producing systemic infection after intravaginal inoculation. Not shown are the data that demonstrated that relative levels of viral RNA in plasma after intravenous inoculation paralleled the results of the plasma antigenemia analysis. Thus, animals inoculated intravenously with SHIV89.6, SHIV89.6PD, SIVmac239, and SIVmac1A11/239 have detectable plasma antigen and relatively high plasma levels of viral RNA, while animals inoculated intravenously with SHIVHXBc2 and SIVmac1A11 do not have a plasma antigenemia and have relatively low plasma viral RNA levels. The data on plasma antigenemia in animals inoculated intravenously with SHIVHXBc2 and SHIV89.6 were published by other investigators [10]. Thus, the in vivo replicative capacity of the SHIV and SIV clones and isolates used in these studies predicts the ability of each virus to produce systemic infection after intravaginal inoculation [4].

Localization and Immunophenotype of Infected Cells in the Lower Female Reproductive Tract

In situ hybridization with digoxigenin-labeled riboprobes combined with immunohistochemistry was used to assess the cell types that were SIV RNA positive in paraffin-embedded sections of vagina and cervix. Most of the SIV-infected cells in the reproductive tract were found in the lamina propria, but some infected cells were found in the stratified squamous epithelium of the vagina and ectocervix. Further, most of the SIV-infected cells were $CD3^+$ T cells and were found in the lamina propria. SIV-infected $CD3^+$ T cells were never found in the overlying epithelium. Although less common than infected T cells, SIV-infected $p55^+$ dendritic cells were relatively abundant in the lamina propria, and SIV-infected $p55^+$ dendritic cells were occasionally found in the stratified squamous epithelium. It is important to note that all of the SIV-infected cells in the epithelium were $p55^+$ and thus were dendritic cells. Mature dendritic cells [13] express p55, an intracellular F-actin bundling protein [14]. In the lamina propria of the vagina and cervix, SIV-infected macrophages were rare. In situ hybridization with digoxigenin-labeled riboprobes combined with immunofluorescent antibodies was used to immunophenotype SIV RNA–positive cells in cytospins of cells isolated from the vagina and cervix. Of the SIV-infected cells isolated from the lower genital tract, most were $CD1a^+/p55^+$ LC (figure 1) and T cells ($CD2^+$). Although most of the SIV-infected cells were T cells, we estimate that 40% of the infected cells were LC. $CD1a^-/ p55^+$ dendritic cells were also recognized.

Discussion

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 of specific regions of the envelope gene, are found in humans recently infected with HIV-1 [3,15,16]. However, this result is not limited to individuals that had been infected by sexual contact—it is also found in individuals infected parenterally [15]. The combined results of these studies and the work we have presented here do not support the hypothesis that viruses with certain envelope nucleotide sequences are transmitted by sexual contact because they can more efficiently penetrate the vaginal mucosa; instead, our results support the idea that only a limited number of the viral genetic variants in a donor have the fitness required to initiate an infection in a naive recipient by a particular route of transmission.

With regard to preferential sexual transmission of viruses with a particular phenotype, some studies have demonstrated that persons acutely infected with HIV-1 have a macrophagetropic NSI virus variant, and it was presumed that this viral phenotype was selectively transmitted by sexual contact. This notion has been championed to explain the finding of limited genetic heterogeneity of HIV envelope in persons infected via sexual contact. However, as with envelope nucleotide homogeneity, this apparent restriction of viral phenotype in acutely infected people occurs regardless of the route of transmission. A study by Fiore et al. [17] 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 virus variant in the donor. Studies on 10 pairs of individuals consisting of the index case and seroconverting sexual partner showed that when the viral phenotypes in the 2 individuals forming a transmission pair were compared, the phenotype of the HIV-1 was the same in both individuals in 9 of 10 transmission pairs. Further, both persons in 5 of the pairs were infected with a syncytium-inducing (SI) variant. Thus, Pope et al. [17] found that there was no selection for macrophage-tropic NSI viruses during sexual transmission of HIV. As the authors pointed out, there are a number of case reports and smaller studies in which index cases with T cell–tropic SI variants were transmitted to a sex partner.

Recently the significance of macrophage-tropic versus T cell–tropic HIV-1 phenotypes has been called into question. In a carefully controlled in vitro study, 6 of 8 macrophage-tropic HIV-1 isolates or clones productively infected ≥1 T cell lines producing high titers of viral p24 antigen and infectious progeny virus [18]. This demonstrates that most “macrophage-tropic” HIV isolates are actually dual-tropic. Further, most SI HIV primary isolates replicate in both macrophages and T cell lines and can use both CxCR4 and CCR5 as co-receptors [19].When all the published data are reviewed, it seems clear that there is no restriction on the sexual transmission of T cell–tropic SI HIV-1 variants. The results of the in vivo SIV and SHIV studies described here support that conclusion.

We have demonstrated that only some viral genotypes can produce a systemic infection after vaginal inoculation. This supports the conclusion that there is selection for virus genotypes during sexual transmission of HIV. However, the common phenotype, if any, of the selected genotypes is not apparent from in vitro studies of virus phenotype. We did find that all the viruses that were capable of transmission by vaginal inoculation had a common in vivo phenotype. After intravenous inoculation of rhesus macaques, all the transmitting viruses produced plasma antigenemia and high levels of plasma viral RNA. In contrast, although the nontransmitting viruses do infect rhesus macaques after intravenous inoculation, the infection that occurs after such inoculation is characterized by a lack of viral antigen in plasma and low levels of plasma viral RNA. On the basis of these results, we conclude that

viruses that are adapted to replicate to high levels in vivo can be transmitted by vaginal inoculation. This principle is also likely to apply to transmission of HIV in humans.

Using in situ hybridization, we found SIV-infected cells in the vagina and cervix of chronically infected rhesus macaques. Further, infected cells were found in both the lamina propria and stratified squamous epithelium of the lower reproductive tract. Using combined in situ hybridization and immunolabeling techniques on tissue sections and cytospin preparations of cell suspensions derived from the lower reproductive tract, we definitively identified the infected cells. We now report that a significant proportion of the SIV-infected cells in the reproductive tract are $CD1a^{+}/p55^{+}$ LC. This result unequivocally demonstrates that SIV infects dendritic cells and LC in vivo and that dendritic cells in general, and LC in particular, are important for HIV and SIV replication. This finding supports the hypothesis that intraepithelial LC are the first cells to be infected during vaginal transmission of HIV [20]. Further, this is the first evidence that dendritic cells are significant reservoirs for SIV in the genital tract.

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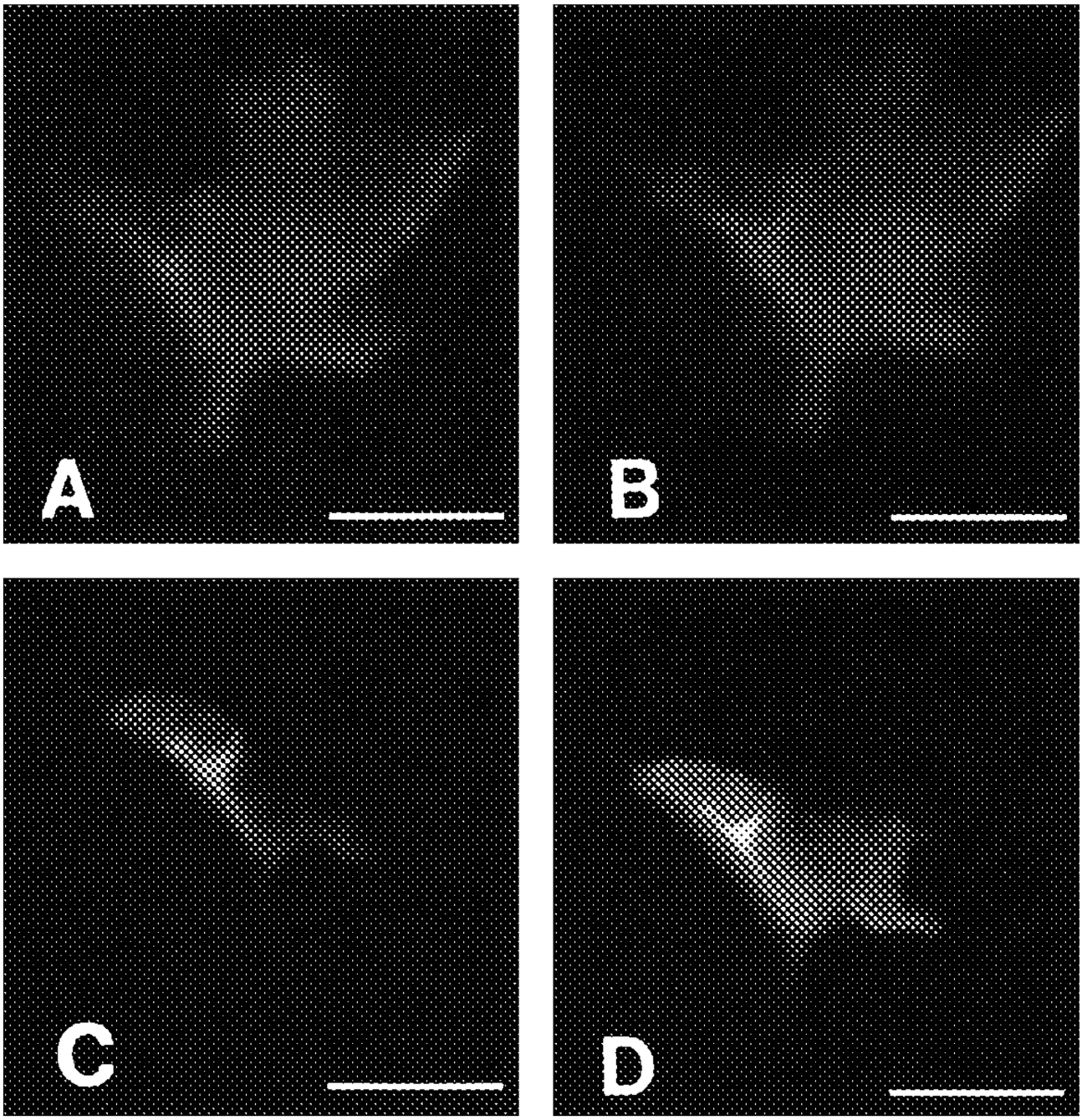

Figure 1.

Immunophenotypic characterization of SIV-infected Langerhans' cells (LC) in cytospin preparations of vagina. *A–D*, Photographs of single field from cytospin preparation from vagina of chronically infected rhesus macaque. *A*, Single p55 (actin-bundling protein)–positive dendritic cell (blue) is seen. *B*, Same dendritic cell also expresses CD1a (red) and, thus, is LC. *C*, Cell also contains SIV RNA (green), as detected by fluorescent in situ hybridization. *D*, Field viewed with a double filter simultaneously demonstrates that SIV RNA–positive cell expresses CD1a (yellow, red, and green). Figure provides clear evidence that SIV infects LC in vagina of rhesus macaques. Bar = 30 μm.

Table 1

Relationship of SIV and SHIV phenotype to vaginal transmission in rhesus macaques.

	In vitro phenotype			In vivo phenotype[a]		
Virus isolate or clone	**Replication in T cell lines**	**Replication in macrophages**	**Replication kinetics**	**Plasma antigenemia after intravenous inoculation**	**PBMC load after intravenous inoculation**	**Vaginal transmission**
SIVmac251[b]	+	+	Rapid	+	High	Yes
SHIV89.6PD[b]	+	+	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

NOTE. PBMC = peripheral blood mononuclear cell.

[a]Data were generated by characterizing virologic parameters in rhesus macaques that were infected with each virus clone or isolate by intravenous inoculation.

[b]Virus stocks were derived from uncloned viral isolates; all other virus stocks were derived from viral clones.