Published in final edited form as:
AIDS Res Hum Retroviruses. 1998 April ; 14(Suppl 1): S79–S82.

Does Viral Tropism Play a Role in Heterosexual Transmission of HIV? Findings in the SIV–Rhesus Macaque Model

CHRISTOPHER J. MILLER
Virology and Immunology Unit, California Regional Primate Research Center, and Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California-Davis, Davis, California 95616.

Abstract

Substantial effort is being directed toward generating vaccines that can prevent the heterosexual transmission of HIV-1. If "Selection" for specific variants during sexual intercourse occurs, then vaccines should be designed to prevent transmission of these specific viruses. Using the SIV–rhesus macaque model to test the hypothesis that specific HIV genotypes are more efficient at producing infection by sexual transmission, it was possible to demonstrate that the genotypic determinants that permit SIV or SHIV to produce systemic infection differ depending on the route of virus inoculation. This finding supports the conclusion that there is selection for viral genotypes during sexual transmission of HIV. However, the ability of a virus to grow in rhesus macaque monocyte-derived macrophages *in vitro* does not predict the outcome of intravaginal inoculation with that virus. We did find that after intravenous inoculation all the vaginally transmitting viruses produced plasma antigenemia and high levels 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 low levels of plasma viral RNA. On the basis of these results, it is clear that viruses which are adapted to replicate to high levels *in vivo* are transmitted by vaginal inoculation. This principle may also apply to the transmission of HIV in humans.

INTRODUCTION

HETEROSEXUAL INTERCOURSE REMAINS the most common means of HIV-1 transmission. Substantial effort is being directed toward generating vaccines that can prevent the transmission of HIV-1 by this route. Because empirical attempts at producing an HIV-1 vaccine have been unsuccessful, there is an increasing emphasis on understanding the biology of heterosexual transmission of HIV-1. It is hoped that a thorough understanding of the transmission and dissemination of HIV following sexual contact will provide the basis for rationale vaccine design and development.

In particular, it will be important to determine if a subset of viral variants is commonly transmitted by sexual contact. If "selection" for specific variants by sexual intercourse occurs, then 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 biologic behavior are phenotypic variants. A few studies have been undertaken in humans that were recently infected with HIV-1. Most of these studies have concluded that, on the basis of the envelope sequence, there is selection for a limited number of genotypes in acute

Address reprint requests to: C.J. miller Virology and Immunology Unit California Regional Primate Research Center University of California-Davis Davis, California 95616.

HIV infection.1–3 However, this restriction on genotype does not extend to other regions of the viral genome.1,2,4 It is also not clear that this restriction on the viral genotype in acute infection is related to the route of HIV transmission.

We used the SIV–rhesus macaque model to test the hypothesis that specific HIV genotypes are more efficient at producing infection by sexual transmission. For these studies three molecular clones of SIV, two molecular clones of SIV/HIV (SHIV) chimeric viruses, and uncloned stocks of SIV and SHIV were used for vaginal inoculation of rhesus macaques. The molecular clones are well characterized with regard to sequence. In addition, these molecular clones have well-defined *in vitro* tropism and replication phenotypes and using them allowed us to determine if mucosal transmission is associated with a specific *in vitro* virus phenotype. Because an article describing the details of these studies has been submitted,4a only a summary and discussion of the work are provided here.

METHODS AND RESULTS

For the purposes of this article, vaginal transmission of SIV is defined as the ability to detect virus in peripheral blood mononuclear cells (PBMCs), by virus isolation or polymerase chain reaction (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 SHIV89.6 are chimeras between SIVmac239 and HIV-1 molecular clones.5–7 Both viruses reliably produce infection in intravenously inoculated macaques.5 We have reported6 that four intravaginal inoculations of SHIVHXBc2 fail to produce a systemic infection in rhesus macaques. In contrast, as few as three intravaginal inoculations of SHIV 89.6 consistently result in viremia in rhesus macaques.6 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 PBMCs of the animals. The only difference in the genotype of these two viruses is that the gpl60-coding region is derived from different parental HIV-1 clones. Thus this is clear evidence that, in the rhesus macaque system, the coding sequence of gp160 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 SHIV89.6.7 A manuscript describing this stock in detail has been submitted.4a 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, SIVmaclAll, and a chimeric virus that consisted of the regions encoding gp41, some accessory genes, and the long terminal repeats (LTRs) of SIVmac239 in the background of SIVmaclAll (SIVmaclAll/239). Intravenous inoculation of all three of these viruses reliably produces infection in rhesus macaques.8 Animals inoculated intravaginally with SIVmac239 and SIVmaclAll/239 reliably become infected after a single intravaginal inoculation, whereas animals inoculated intravaginally with SIVmaclAll only rarely become infected. The only difference in the genotype of SIVmaclAll and SIVmaclAll/239 is that the gp41, some accessory genes, and LTRs of the latter virus are derived from SIVmac239.9 Thus the coding sequences of gp41 and/or the LTR can influence the ability of a virus to produce systemic infection after intravaginal inoculation.

We have shown that not all the 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 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 provide in Table 1. As can be clearly seen, the ability of a virus to grow in rhesus macaque monocyte-derived macrophages *in vitro* does not predict the outcome of intravaginal inoculation. SIVmaclAll and SHIVHXBc2 replicate efficiently in rhesus macaque macrophages but do not transmit vaginally, whereas SIVmac239 and SHIV89.6 do not replicate in macrophages but do transmit vaginally.

Because all the viruses used in this study systemically infect animals after intravenous inoculation, we next sought to determine if viruses that transmit vaginally share a common *in vivo* replication phenotype in intravenously inoculated animals. We assessed two parameters: (1) ability of a virus to produce plasma antigenemia or plasma viral RNA levels, and (2) the cell-associated virus load in animals infected with a particular virus. A summary of this analysis is presented in Table 2. 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, SHIV89.6-PD, SIVmac239, and SIVmaclAll/239 have detectable plasma antigen and relatively high plasma levels of viral RNA, whereas animals inoculated intravenously with SHIVHXBc2 and SIVmaclAll 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.5 Thus the *in vivo* replicative capacity of the SHIV and SIV clones and isolates used in these studies predict the ability of each virus to produce systemic infection after intravaginal inoculation.

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 in specific regions of the envelope gene, are found in humans recently infected 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.2 A more recent study by the same group characterized viral variants in a single transmitter–recipient heterosexual pair and concluded that whereas the V3 loop sequences in the major variant of the donor and recipient were similarly, the sequence of the V1–V2 env sequences in the recipient were found only in a minor variant of the donor.9 The authors speculate that this was because these variants had a selective advantage in penetrating the mucosal barrier during sexual contact. A study involving a relatively large number of acute seroconverters found that, when the nucleotide sequences of viruses in each individual were compared, there was little sequence heterogeneity in two normally hyper-variable regions of the *env* gene.1 However, this result was not limited to individuals that had been infected by sexual contact but was also found in individuals infected parenterally.1 This finding does 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, 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. Interestingly, in these studies the sequence homogeneity of the envelope did not extend to the *gag* gene.1,2 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 but not in T cell lines and does not cause syncytia in MT-2 cells. It has been widely presumed that these viral variants with a macrophage-tropic/non-syncytium-inducing (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 a 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. In addition, 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.10 In this study, all of 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. Studies on 10 pairs of individuals consisting of the index case and seroconverting sexual partner showed that, when the viral phenoypes in the two individuals forming a transmission pair were compared, the phenotype of the HIV-1 was the same in both individuals in 9 of the 10 transmission pairs. Further, both of the individuals in five of the pairs were infected with a syncytium-inducing (SI) variant. Thus, this study found that there was no selection for macrophage-tropic/NSI viruses during sexual transmission.10 As the authors of this study10 point out, there are a number of case reports and smaller studies in which index cases with T cell-tropic/SI variants infected a sexual partner.2,11–14 In approximately half of these transmission events, the seroconverting partner became infected with a T cell-tropic/SI variant.

The significance of macrophage-tropic versus T cell-tropic HIV-1 phenotypes has been called into question. In a carefully controlled *in vitro* study, six of eight macrophage-tropic HIV-1 isolates or clones were able to infect one or more T cell lines productively, producing high titers of viral p24 antigen and infectious progeny virus.14 This demonstrates that most “macrophage-tropic” HIV isolates are actually dual tropic. It has long been recognized that essentially all “macrophage-tropic” HIV-isolates replicate in primary T cells. The differences in the results of the studies reported above may have resulted from differences in culture techniques, but there also have been differences in the way the same results have been interpreted by different groups. Fundamentally, it is difficult, perhaps impossible, to extend any *in vitro* finding to the *in vivo* situation. When all the published data are 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 *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 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 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 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 levels of plasma viral RNA. On the basis of these results, it is likely that viruses that are adapted to replicate to high levels *in vivo* are capable of being transmitted by vaginal inoculation. This principle may also apply to the transmission of HIV in humans.

Acknowledgments

Drs. Joseph Sodroski, Norman Letvin, and Yichen Lu graciously provided the SHIV viruses used in this study. This work was supported by NIH Grants AI35545 and RR00169.

REFERENCES

1. Zhang LQ, MacKenzie P, Cleland A, Holmes EC, Leigh Brown AJ, Simmonds P. Selection for specific sequences in the external envelope protein of human immunodeficiency virus type 1 upon primary infection. J Virol. 1993; 67:3345. [PubMed: 8497055]
2. Zhu T, Mo H, Wang N, et al. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science. 1993; 261:1179. [PubMed: 8356453]
3. Wolinsky SM, Wike CM, Korber BT, et al. Selective transmission of human immunodeficiency virus type-1 variants from mothers to infants. Science. 1992; 255:1134. [See comments]. [PubMed: 1546316]
4. Albert J, Walhberg J, Leitner T, Escanilla D, Uhlen M. Analysis of a rape case by direct sequencing of the human immunodeficiency virus type 1 *pol* and *gag* genes. J Virol. 1994; 68:5918. [PubMed: 7520096]

4a. Miller, CJ.; Marthas, M.; Greenier, J.; Lu, D.; Dailey, PJ.; Lu, Y. In-vivo replication capacity rather than in-vitro macrophage tropism predicts efficiency of vaginal transmission of simian immunodeficiency virus (SIV) or simian/human immunodeficiency virus (SHIV) in rhesus macaques. 1997. Submitted

5. Reimann KA, Li JT, Voss G, et al. An *env* gene derived from a primary HIV-1 isolate confers high *in vivo* replicative capacity to a chimeric simian/human immunodeficiency virus in rhesus monkeys. J Virol. 1996; 70:1996.
6. Lu Y, Brosio B, Lafaile M, et al. Vaginal transmission of chimeric simian/human immunodeficiency viruses in rhesus macaques. J Virol. 1996; 70:3045. [PubMed: 8627782]
7. Reimann KA, Li JT, Veazey R, et al. A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate *env* causes an AIDS-like disease after in vivo passage in rhesus monkeys. J Virol. 1996; 70:6922. [PubMed: 8794335]
8. Marthas ML, Ramos RA, Lohman BL, et al. Viral determinants of simian immunodeficiency (SIV) virulence in rhesus macaques assessed by using attenuated and pathogenic molecular clones of SIVmac. J Virol. 1993; 67:6047. [PubMed: 8371353]
9. Zhu T, Wang N, Cart A, Nam DS, Moor-Jankowski R, Cooper DA, Ho DD. Genetic characterization of human immunodeficiency virus type-1 in blood and secretions: evidence for virus compartmentalization and selection during sexual transmission. J Virol. 1996; 70:3098. [PubMed: 8627789]
10. Fiore JR, Bjomdal A, Peipke KA, et al. The biologic phenotype of HIV-1 is usually retained during and after sexual transmission. Virology. 1994; 204:297. [PubMed: 8091660]
11. Nielsen C, Pedersen C, Lundgren JD, Gerstoft J. Biological properties of HIV isolates in primary infection: Consequences for subsequent course of infection. AIDS. 1993; 7:1035. [PubMed: 8104421]
12. Roos MT, Lange JM, de Goed RE, et al. Viral phenotype and immune response in primary human immunodeficiency virus type 1 infection. J Infect Dis. 1992; 165:427. [PubMed: 1347054]
13. Yoshiyama H, Kobayashi N, Matsui T, et al. Transmission and genetic shift of human immunodeficiency virus (HIV) in vivo. Mol Biol Med. 1987; 4:385. [PubMed: 3501821]

14. Chowdhury IH, Potash MJ, Volsky DJ. Redifinition of tropism of common macrophage-tropic human immunodeficiency virus type 1. AIDS Res Hum Retroviruses. 1995; 11:1467. [PubMed: 8679290]

Table 1

Relationship of in Vitro Phenotype of SIV and SHIV to Vaginal Transmission in Rhesus

Virus isolate/clone	Replication in T cell lines	Replication in macrophages	Replication kinetics	Vaginal transmission
SIVmac251	+	+	Rapid	Yes
SHIV89.6PD	+	+	Intermediate	Yes
SI VmaclAll/239	+	+	Delayed	Yes
SHIV89.6	+	–	Delayed	Yes
SIVmac239	+	–	Delayed	Yes
SIVmaclAll	+	+	Rapid	No
SHIVHXBc2	+	+	Intermediate	No

Table 2

Relationship of *in Vitro* Phenotype[a] of SIV and SHIV to Vaginal Transmission in Rhesus Macaques

Virus isolate/clone	Plasma antigenemia after intravenous inoculation	PBMC load after intravenous inoculation	Vaginal transmission
SIVmac251	+	High	Yes
SIVmac239	+	High	Yes
SIVmaclAll/239	+	Intermediate	Yes
SHIV89.6	+	Intermediate	Yes
SHIV89.6PD	+	High	Yes
SIVmaclAll	–	Low	No
SHIVHXBc2	–	Intermediate	No

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