

Vaginal Transmission of Chimeric Simian/Human Immunodeficiency Viruses in Rhesus Macaques

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Chimeric simian/human immunodeficiency viruses (SHIVs) that express the *env* genes derived from distinct HIV type 1 (HIV-1) isolates were tested for the ability to infect rhesus macaques following intravaginal inoculation. SHIVs containing either the HIV-1 HXBc2 or the HIV-1 89.6 envelope glycoproteins were capable of replicating in intravenously inoculated rhesus macaques. However, intravaginal inoculation of animals with these two SHIVs resulted in infection only with the SHIV containing the HIV-1 89.6 glycoprotein. Thus, properties conferred by the envelope glycoproteins in the chimeric virus affect the ability of particular SHIVs to initiate a systemic infection following vaginal inoculation. These results provide indirect support for the hypothesis that the selection of specific viral variants occurs in the genital tracts of individuals exposed to HIV by sexual contact.

Recent studies have shown that, immediately following sexual transmission, the virus population in human immunodeficiency virus (HIV) infection is homogeneous with respect to cell tropism and their nucleic acid sequences (16, 24, 25). In a few cases in which the transmitting and newly infected partners have been identified, it appears that the virus transmitted represents a macrophage-tropic and non-syncytium-inducing variant present at a low frequency in the donor viral population (25). However, viral variability can develop rapidly after infection (16, 25). Three hypotheses have been proposed to explain the discrepancy between the heterogeneous virus population in the transmitting partner and the homogeneous virus recovered from a recently infected partner. The homogenous virus observed in a newly infected person could reflect (i) exposure to a low titer of virus from the transmitter, (ii) selective amplification of one variant after entering the new host, or (iii) selective transmission of viral variants across the genital mucosa (25). The observation that the transmitted virus represents a minor, macrophage-tropic, non-syncytium-inducing variant in the blood of the transmitter is consistent with either of the last two explanations (25).

The similarity between human and rhesus monkey reproductive anatomies and physiologies (4) and the close genetic and biologic homology of HIV and simian immunodeficiency virus (SIV) have led to the development of several nonhuman primate models of mucosal transmission of lentiviruses (reviewed in reference 11). We have shown that a simple application of SIV onto the intact genital mucosa of mature and immature rhesus macaques results in virus transmission and that the disease induced by this route of inoculation is indistinguishable from that seen in intravenously inoculated animals (12, 13).

This model is being used to understand the interaction between the virus and host during the sexual transmission of HIV. Chimeric viruses consisting of the HIV *env* gene in a SIVmac239 backbone have been constructed, and it has been demonstrated that intravenous inoculation of these viruses into rhesus macaques results in productive infection. SIV/HIV chimeric viruses (SHIVs) which contain the *env* gene from distinct HIV isolates could be used to determine if a specific *env* gene can confer a specific phenotype to a molecularly cloned virus which allows virus transmission across the vaginal mucosa. To test the hypothesis that the *env* gene sequence of HIV type 1 (HIV-1) contributes to the ability of the virus to cross a mucosal surface, we used two SHIV viruses containing *env* genes from HXBc2, a lymphotropic HIV-1 IIIB isolate, or HIV-1 89.6, a primary isolate that is lymphotropic and monocytotropic. In rhesus macaques, only the SHIV virus containing the *env* gene from HIV-1 89.6 was capable of initiating an infection across the vaginal mucosa.

MATERIALS AND METHODS

Animals. All animals used in this study were captive bred, mature, cycling, female rhesus macaques (*Macaca mulatta*) or juvenile, male rhesus macaques (for the intravenous inoculations) from the California Regional Primate Research Center. The ages of the animals at the time of the virus inoculations are provided in Table 1. The animals were housed in accordance with American Association for Accreditation of Laboratory Animal Care standards. When necessary, the animals were immobilized with 10 mg of ketamine HCl (Parke-Davis, Morris Plains, N.J.) per kg of body weight injected intramuscularly. The investigators adhered to the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Resource Council (1a). Prior to use, the animals were negative for antibodies to HIV-2, SIV, type D retrovirus, and simian T-cell leukemia virus type 1.

Virus stocks and animal inoculations. Two stocks of SHIVs were used for the animal inoculations. Both SHIVs contained functional HIV-1 *vpu*, *tat*, *rev*, and *env* genes in the context of the SIVmac239 provirus and were grown in rhesus macaque peripheral blood mononuclear cells (PBMC). The first virus was designated SHIV(HXBc2) (7) and was constructed with the HIV-1 IIIB/LAI variant, which is the prototype of the T-tropic viruses. The second virus, designated SHIV(89.6) (17), was identical to SHIV(HXBc2) except for the *Kpm1* (nucleo-

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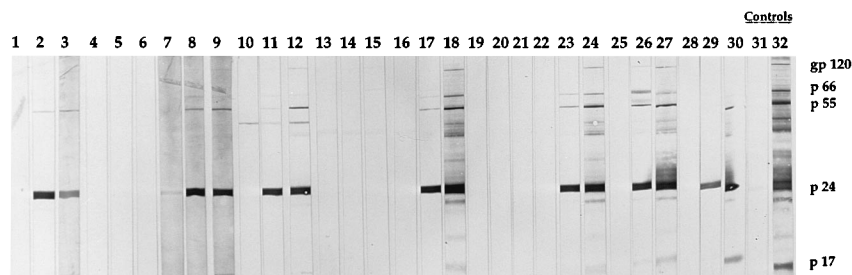


FIG. 1. Reaction of sera from SHIV-inoculated animals to SIV antigens in a Western blot assay. All animals from which SHIV was isolated seroconverted to SIV *gag* and *pol* antigens (p66, p55, p27, and p17). Note that the sera in lanes 12, 18, 24, and 27 react weakly with SIV gp120. Seroconversion occurred by 6 weeks p.i. The animals from which SHIV was not isolated remained seronegative. Into the following lanes (in groups of three), sera from the specified macaque taken at weeks 0, 6, and 16 p.i., respectively, were loaded: lanes 1 to 3, monkey 17376; lanes 4 to 6, monkey 25505; lanes 7 to 9, monkey 20429; lanes 10 to 12, monkey 21170; lanes 13 to 15, monkey 22190 (first set of inoculations); lanes 16 to 18, monkey 22190 (second set of inoculations); lanes 19 to 21, monkey 25726 (first set of inoculations); lanes 22 to 24, monkey 25726 (second set of inoculations); lanes 25 to 27, monkey 26457; lanes 28 to 30, monkey 27923. Lane 31 contained negative control serum from a naive rhesus macaque; lane 32 contained positive control serum from a known seropositive rhesus macaque.

III.). All of the DNA *env* sequences of the SHIV clones were compared with the published nucleotide sequences of the HIV-1 89.6 and HIV-1 HXB molecular clones with a Macintosh computer (Apple Inc., Cupertino, Calif.) and the MacVector program (Eastman Kodak Corp., Rochester, N.Y.). On the basis of the match score assigned by the DNA Database Matrix with a hash value of 1, a SHIV clone *env* sequence was assigned to the HIV-1 89.6 or HXB clone. If the match score between the SHIV clone *env* sequence and the HIV-1 89.6 reference *env* sequence was greater than the match score between the SHIV clone *env* sequence and the HIV-1 HXB reference *env* sequence, the SHIV *env* clone was assigned to HIV-1 89.6. If the match score between a SHIV clone *env* sequence and the HIV-1 HXB reference *env* sequence was greater than the match score between the SHIV clone *env* sequence and the HIV-1 89.6 *env* sequence, the SHIV *env* clone was assigned to HIV-1 HXB. If the match scores between a SHIV clone *env* sequence and the HIV-1 89.6 and HIV-1 HXB *env* sequences were equal, the SHIV *env* clone was not assigned to either HIV clone.

RESULTS

Two rhesus macaques (17376 and 25505) were intravaginally inoculated with 1 ml of SHIV(HXBc2) (4,800 TCID₅₀) twice a week for 2 weeks (a total of four inoculations) and monitored for virus infection. No evidence of infection could be detected over a period of 24 weeks. The animals were negative for SHIV by virus isolation (Table 1), PCR (Table 2), and serological

analysis (data not shown). The animals were reinoculated with SHIV(89.6) (1,800 TCID₅₀/ml) by the same inoculation protocol as before (twice a week for 2 weeks; a total of four inoculations). The animals were monitored for infection as before, except the observation period extended to 52 weeks. One animal (25505) remained uninfected and seronegative; however, the second animal (17376) was virus isolation positive at 2, 4, 6, 8, and 10 weeks p.i. By 6 weeks after the first SHIV(89.6) inoculation, monkey 17376 developed serum antibodies to SIV *gag* and *pol* antigens and HIV *gag* and *env* antigens (Fig. 1 and 2).

Two additional rhesus macaques were inoculated with SHIV(89.6), but these animals were exposed to three inoculations of 1 ml of the virus stock over 2 weeks. Both animals became infected (Tables 1 and 2) and seroconverted to SIV *gag/pol* and HIV *gag/env* antigens by 6 weeks p.i. (Fig. 1 and 2). The animals were strongly viremic until 12 weeks p.i., at which time they became virus isolation negative according to the results of all but one additional blood sample.

These results suggested that the properties conferred on SHIV(89.6) by the HIV-1 89.6 *env* gene allow infection follow-

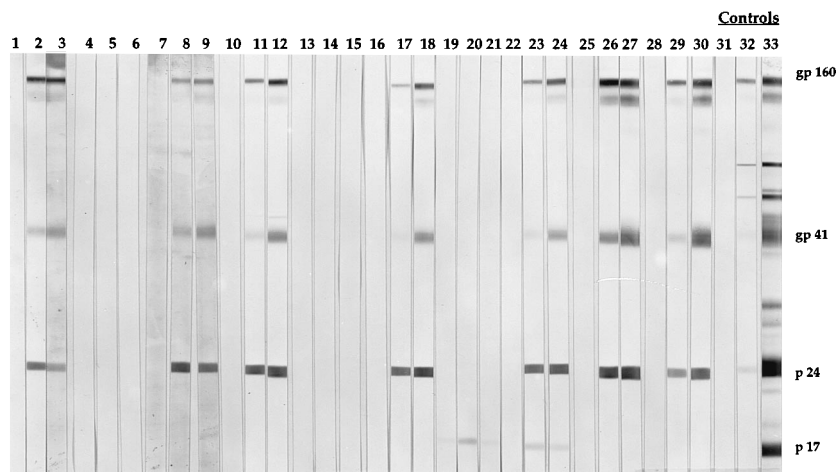


FIG. 2. Reaction of sera from SHIV-inoculated animals to HIV antigens in a Western blot assay. All animals from which SHIV was isolated seroconverted to SIV *env* antigens (gp160, gp120, p41, and p32). Note that significant cross-reaction to HIV *gag* antigens (p24 and p17) occurred in many of the serum samples. Seroconversion occurred by 6 weeks p.i. The animals from which SHIV was not isolated remained seronegative. Into the following lanes (in groups of three), sera from the specified macaque taken at weeks 0, 6, and 16 p.i., respectively, were loaded: lanes 1 to 3, monkey 17376; lanes 4 to 6, monkey 25505; lanes 7 to 9, monkey 20429; lanes 10 to 12, monkey 21170; lanes 13 to 15, monkey 22190 (first set of inoculations); lanes 16 to 18, monkey 22190 (second set of inoculations); lanes 19 to 21, monkey 25726 (first set of inoculations); lanes 22 to 24, monkey 25726 (second set of inoculations); lanes 25 to 27, monkey 26457; lanes 28 to 30, monkey 27923. Lane 31 contained negative control human serum, lane 32 contained weak positive control human serum, and lane 33 contained strong positive control human serum.

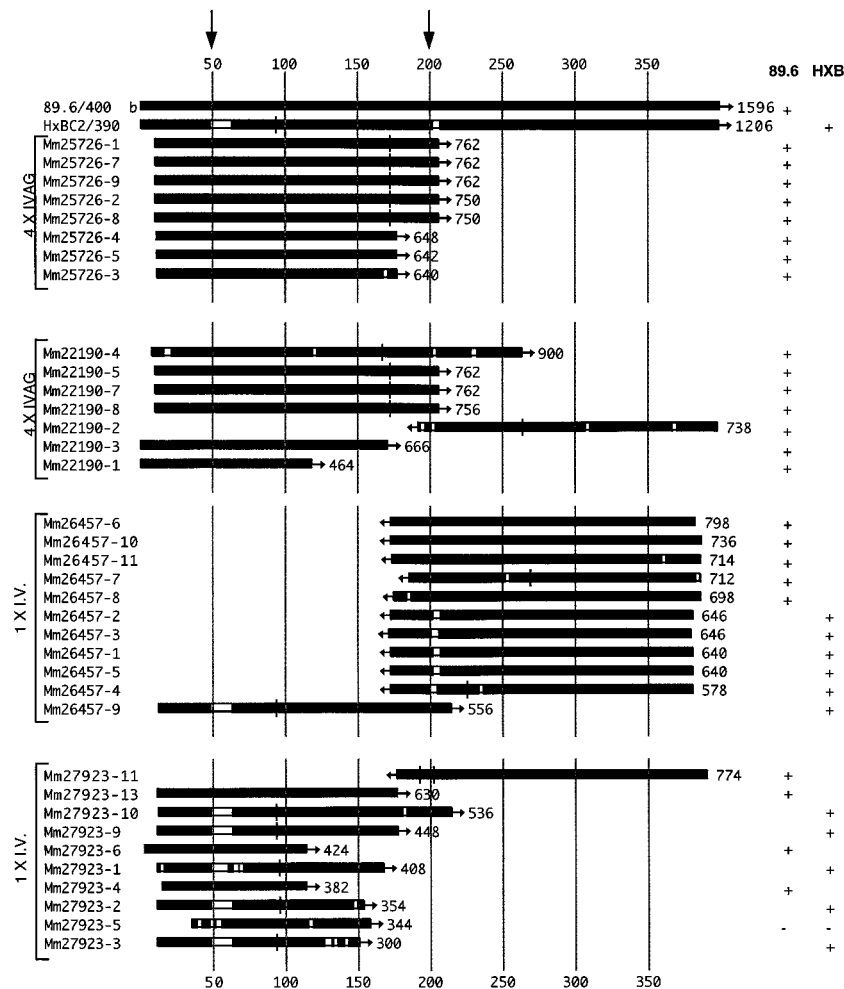


FIG. 3. Sequence comparisons of the HIV-1 89.6 and HIV-1 HXBc2 *env* genes with viral DNA amplified from rhesus monkeys inoculated with the SHIV (89.6)-SHIV (HXBc2) viral mixture. The left-hand column contains the SHIV *env* gene clone number (which consists of the animal number and the clone number) and the route of inoculation for each of the four animals. Horizontal bars graphically demonstrate each SHIV *env* gene sequence alignment with the compared sequence, a 400-bp fragment from the HIV-1 89.6 *env* gene. Open portions of the bars represent regions where gaps were introduced in the sequences to improve the alignments. The short, solid, vertical lines through the bars indicate where insertions were found in the sequences relative to the compared sequence. The assignment on the basis of criteria described in Materials and Methods of each amplified SHIV *env* gene fragment to a specific HIV-1 *env* gene sequence (89.6 or HXB) is noted in the columns at the far right-hand side of the figure. A + in the 89.6 column indicates that the SHIV *env* sequence was assigned to the HIV-1 89.6 *env* sequence, and a + in the HXB column indicates that the SHIV *env* sequence was assigned to the HIV-1 HXB *env* sequence. A - in both columns indicates that the SHIV *env* sequence was not assigned to either HIV-1 clone sequence. I.V., intravenous inoculation; IVAG, intravaginal inoculation.

ing vaginal inoculation while the properties conferred on SHIV (HXBc2) by the HIV-1 HXBc2 *env* gene do not permit systemic infection following vaginal inoculation. To investigate this possibility and to rule out the effects of host factors (the stage of menstrual cycle at inoculation, age, reproductive history, etc.), two female rhesus macaques (22190 and 25725) were inoculated intravaginally with a mixture of the two SHIVs twice in a single week (for a total of two inoculations each). The mixture consisted of 1 ml of SHIV(HXBc2) (4,800 TCID₅₀) and 1 ml of SHIV(89.6) (1,800 TCID₅₀). Both animals remained virus isolation negative, PCR negative, and seronegative over a 6-month observation period (Tables 1 and 2). Six months after the first series of inoculations, the same two animals (22190 and 25725) were inoculated intravaginally with the same SHIV mixture twice a week for 2 weeks (for a total of four inoculations each). Virus was isolated from the PBMC of both animals up to 12 weeks p.i. (Tables 1 and 2), and both animals seroconverted to SIV and HIV-1 antigens by 6 weeks p.i. (Fig. 1 and 2). To determine if one or both of the SHIVs

were present in the animals, *env* fragments from proviruses in PBMC were amplified by PCR and cloned. Eight clones were isolated from the 2-week-p.i. PBMC sample of one animal, and seven clones were isolated from the 2-week-p.i. PBMC of the other animal. The sequences of the *env* genes from the clones from both of these animals were consistent with that of SHIV(89.6) (Fig. 3). One animal (22190) had two clones (Mm 22190, clones 2 and 4) with *env* sequences that had all the characteristics of HIV-1 89.6 except that these two clones had multiple random-base-pair deletions. Thus, only SHIV(89.6) was detected in the 2-week-p.i. PBMC samples of the animals. This result supports the conclusion that SHIV(89.6), and not SHIV(HXBc2), is capable of initiating an infection following vaginal inoculation.

To examine the relative abilities of the SHIV variants to initiate infection of rhesus macaques by another route, two juvenile male rhesus macaques were inoculated intravenously with 1 ml of the SHIV mixture [0.5 ml of SHIV(HXBc2) (2,400 TCID₅₀) and 0.5 ml of SHIV(89.6) (900 TCID₅₀)]. Virus was

consistently isolated from the PBMC of both animals up to 10 weeks p.i., and the animals were intermittently negative for virus isolation thereafter. Both animals seroconverted to SIV and HIV-1 antigens by 6 weeks p.i. (Fig. 1 and 2). To determine if one or both of the SHIVs were present in the animals, *env* fragments from proviruses in PBMC were amplified by PCR and cloned. Eleven clones were isolated from the 2-week-p.i. PBMC sample of one animal, and 10 clones were isolated from the 2-week-p.i. PBMC of the other animal (Fig. 3). The *env* gene sequences from approximately half of the clones from each of these animals were consistent with that of SHIV(89.6), and the other half of the clones exhibited *env* sequences consistent with that of SHIV(HXBc2) (Fig. 3). One animal (27923) had a single clone (Mm 27923, clone 5) with an *env* sequence that had characteristics distinct from those of both SHIV(89.6) and SHIV(HXBc2). This result demonstrates that both SHIV(89.6) and SHIV(HXBc2) are capable of replicating simultaneously in an animal inoculated with a mixture of the viruses. Although a rigorous quantitative assessment of the relative frequencies of the two SHIVs was not undertaken, it is worth noting that in both of the intravenously inoculated animals, *env* sequences of the two SHIVs were detected at similar frequencies. Thus, the presence of SHIV(89.6) in a rhesus macaque does not prevent replication of SHIV(HXBc2). The results of all of these studies support the concept that SHIV(89.6) is capable of initiating infection by intravaginal inoculation while SHIV(HXBc2) is not.

DISCUSSION

It has previously been shown that SHIVs can productively infect a variety of macaque species following intravenous inoculation (7, 20, 23a). Here we demonstrate that at least some SHIV clones can productively infect rhesus macaques after intravaginal inoculation. With this finding, the SHIV-macaque model can now be used to test the abilities of HIV-1 envelope-based vaccines to prevent infection following intravenous and intravaginal challenge. The SHIV-macaque model may also be useful in studying HIV-1-specific determinants of lentivirus infection and transmission. We have demonstrated that when rhesus macaques are intravaginally inoculated with two SHIV viruses that differ only in the sequences of their HIV *env* genes, there is infection only with the SHIV(89.6) variant. By contrast, both SHIV(HXBc2) and SHIV(89.6) are capable of replicating in intravenously inoculated rhesus macaques. The backbones of the two SHIV clones used for this study were identical and consisted of the SIVmac239 genome and the HIV-1 *tat*, *rev*, and *vpu* genes. Because only the *env* gene sequences of SHIV(HXBc2) and SHIV(89.6) differ, our results demonstrate that the *env* gene confers properties on these SHIV viruses that directly affect the abilities of the viruses to initiate infection by the vaginal route.

In order to initiate a systemic infection following vaginal inoculation, a specific SHIV variant must be able to cross the barrier of the vaginal mucosa and replicate in the tissues of the reproductive tract and draining lymph nodes. It is possible that SHIV(89.6) replicates more efficiently in these tissues than SHIV(HXBc2); however, at 2 weeks p.i., both viruses were present at similar frequencies in the intravenously inoculated rhesus macaques used in this study. In rhesus PBMC, SHIV(89.6) replicates more slowly and at a lower level than SHIV(HXBc2) (17). These *in vitro* results are in contrast to the *in vivo* results of the same investigators. When compared with rhesus macaques inoculated intravenously with SHIV(HXBc2), rhesus macaques inoculated intravenously with SHIV 89.6 have high levels of antigenemia and increased numbers of *in*

situ hybridization-positive cells in lymph nodes (17). Thus, in primary infection, SHIV(89.6) appears to have an increased ability to replicate in rhesus macaques compared with SHIV HXBc2. This high *in vivo* replication capacity may account for the ability of SHIV(89.6) to initiate systemic infection after intravaginal inoculation.

It is also possible that the ability of SHIV(89.6) to initiate infection via the vaginal route is due to the ability of this virus to cross the vaginal mucosa. Thus, the results reported here provide indirect evidence that the *env* gene of SHIV(89.6) confers on the virus the ability to cross the vaginal mucosa. It has been previously reported that SHIV(HXBc2) can be transmitted to rhesus macaques by rectal transmission (22). Thus, the *env* gene of HXBc2 allows rectal transmission of the same SHIV construct that could not be transmitted by vaginal inoculation. This implies that the properties of the HIV envelope glycoproteins required for vaginal transmission differ from those required for rectal transmission. Although the target cells for HIV in the genital and rectal mucosae have not been determined, the anatomies of these sites differ and these differences may provide an explanation for the fact that SHIV(HXBc2) could be transmitted rectally but not vaginally. The *in vitro* cell tropism of the HIV-1 isolates from which the SHIV *env* sequences were derived has been studied. The HIV-1 HXBc2 isolate replicates well in T cells but not in macrophages (3), while the HIV-1 89.6 isolate replicates well in both macrophages and T cells (1). Recent unpublished studies have shown that the HIV 89.6 isolate replicates in Langerhans cells, while the HIV HXBc2 isolate does not replicate well in the Langerhans cells (2a). There are numerous CD4⁺ Langerhans cells in the cervicovaginal epithelia of both rhesus macaques and humans (2, 6, 15, 18). The *in vitro* tropism of the HXBc2 and 89.6 SHIV constructs in rhesus macaque primary cells has not yet been determined. However, the *in vivo* results with the SHIV constructs and the *in vitro* results with the parent HIV-1 viruses are consistent with the hypothesis that Langerhans cells in the vaginal mucosa are the target cells for vaginal HIV transmission.

It has been reported that a limited number of *env* sequences are found in viral clones obtained from individuals acutely infected with HIV-1 (16, 24, 25). This finding has been interpreted to suggest that viruses with particular envelope glycoprotein structures are preferentially transmitted from one individual to another. In the experiments described in this paper, we have tested the hypothesis that certain HIV isolates contain specific *env* sequences which confer upon these viruses the ability to be transmitted by mucosal routes. The results provide indirect support for the idea that the selection of specific viral variants occurs on the vaginal surfaces of individuals exposed to HIV. The phenotypes of the selected variants need to be characterized further. This result does not imply that only one virus genotype is transmitted when an individual is exposed to a complex mixture of viral genotypes but that certain genotypes in the mixture have an advantage in successfully initiating infection following vaginal exposure. It remains to be determined how rare or common these variants are in the genital secretions and semen of HIV-1-infected individuals.

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