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In Vivo Replication Capacity Rather Than In Vitro Macrophage Tropism Predicts Efficiency of Vaginal Transmission of Simian Immunodeficiency Virus or Simian/Human Immunodeficiency Virus in Rhesus Macaques

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We used the rhesus macaque model of heterosexual human immunodeficiency virus (HIV) transmission to test the hypothesis that in vitro measures of macrophage tropism predict the ability of a primate lentivirus to initiate a systemic infection after intravaginal inoculation. A single atraumatic intravaginal inoculation with a T-cell-tropic molecular clone of simian immunodeficiency virus (SIV), SIVmac239, or a dualtropic recombinant molecular clone of SIV, SIVmac239/1A11/239, or uncloned dualtropic SIVmac251 or uncloned dualtropic simian/human immunodeficiency virus (SHIV) 89.6-PD produced systemic infection in all rhesus macaques tested. However, vaginal inoculation with a dualtropic molecular clone of SIV, SIVmac1A11, resulted in transient viremia in one of two rhesus macaques. It has previously been shown that 12 intravaginal inoculations with SIVmac1A11 resulted in infection of one of five rhesus macaques (M. L. Marthas, C. J. Miller, S. Sutjipto, J. Higgins, J. Torten, B. L. Lohman, R. E. Unger, H. Kiyono, J. R. McGhee, P. A. Marx, and N. C. Pedersen, J. Med. Primatol. 21:99–107, 1992). In addition, SHIV HXBc2, which replicates in monkey macrophages, does not infect rhesus macaques following multiple vaginal inoculations, while T-cell-tropic SHIV 89.6 does (Y. Lu, P. B. Brosio, M. Lafaile, J. Li, R. G. Collman, J. Sodroski, and C. J. Miller, J. Virol. 70:3045–3050, 1996). These results demonstrate that in vitro measures of macrophage tropism do not predict if a SIV or SHIV will produce systemic infection after intravaginal inoculation of rhesus macaques. However, we did find that the level to which these viruses replicate in vivo after intravenous inoculation predicts the outcome of intravaginal inoculation with each virus.

Human immunodeficiency virus (HIV) is transmitted primarily by sexual contact, and, by use of the simian immunodeficiency virus (SIV)-rhesus macaque system, an animal model of sexual HIV transmission has been developed (21–24, 27, 28). It has been shown that cell-free SIVmac251 (reviewed in reference 21) and some strains of SIV/HIV chimeric viruses (SHIV) (16) are capable of crossing the vaginal mucosa and initiating a systemic infection in rhesus macaques. It has further been shown that sufficient target cells exist in the vaginal mucosa for SIV to be transmitted after virus infusion into blind vaginal pouches of hysterectomized rhesus macaques (22). These studies utilized uncloned SIVmac251 as the viral inoculum. The SIV-rhesus macaque model has also been used to demonstrate the presence of SIV-infected cells in the vaginal mucosa of acutely and chronically SIV-infected rhesus macaques (28, 41). On the basis of these studies, a hypothesis to explain the dissemination of SIV and HIV after vaginal inoculation has been proposed (27). This hypothesis predicts that macrophages and dendritic cells in the vaginal mucosa are the initial target cells for virus inoculated into the vagina.

Some studies of small numbers of individuals acutely infected with HIV through sexual contact suggest that the virus transmitted during sexual contact represents a variant present at low frequency in the transmitting partner's viral population and that the transmitted virus is macrophage tropic and nonsyncytium inducing (NSI) (51). 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 homogeneous 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 (51). The observation that the transmitted virus represents a minor, macrophage-tropic, NSI variant in the blood of the transmitter is consistent with either of the last two explanations (51). In addition, studies which characterized the immune cell populations in the genital tracts of women and female rhesus macaques have demonstrated that antigen-presenting cells (macrophages and $CD1a^+$ Langerhans cells) are the most abundant $CD4^+$ cells in the cervicovaginal mucosa (25, 38). Thus, it is possible that viruses which can replicate efficiently in macrophages and dendritic cells may be more efficient at crossing the vaginal mucosa and initiating a systemic infection than viruses which cannot replicate in these antigen-presenting cells. Transmission studies utilizing molecular clones of SIV with specific phenotypes may provide insight into the viral variants which are capable of initiating infection after vaginal inoculation. The capacity of a

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virus to infect and productively replicate in discrete populations of cells is defined as tropism (45), and HIV and SIV variants have been classified on the basis of their in vitro ability to replicate in macrophages (macrophage tropic) or T-cell lines (T-cell tropic) or both (dualtropic). We 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 viral clones that were strictly T-cell tropic. We sought to test this hypothesis using well-characterized clones of SIVmac and SHIV and two uncloned viral stocks.

For these studies, three clones of SIVmac were used. The prototype pathogenic molecular clone, SIVmac239, is T-cell tropic in vitro but does not replicate in rhesus macrophages (2, 3, 29, 30). Rhesus macaques inoculated intravenously (i.v.) with cloned SIVmac239 develop persistent viremia, produce antiviral antibodies, and develop a fatal AIDS-like disease (10, 11). This infectious molecular clone has been completely sequenced and has a truncated *nef* gene (30, 35). The second clone used was SIVmac1A11 (20), a nonpathogenic molecular clone of SIV that is macrophage and T-cell tropic in vitro (2). The third SIV clone used was a chimera between SIVmac239 and SIVmac1A11. This recombinant SIV clone, designated SIVmac239/1A11/239, is macrophage and T-cell tropic in vitro (2). SIVmac239/1A11/239 contains the long terminal repeats (LTRs), a small portion of the 3′ end of *gag*, gp41, *nef*, and the second exon of *rev* from SIVmac239, and the rest of the virus is derived from SIVmac1A11. Rhesus macaques inoculated i.v. with this clone develop persistent or intermittent viremia and produce antiviral antibodies, and some of the animals develop a fatal AIDS-like disease (20).

We characterized the in vitro replication kinetics of infectious virus stocks of SIVmac251, SIVmac239, SIVmac1A11, and SIVmac239/1A11/239 in rhesus macaque monocyte-derived macrophages. We also characterized the in vivo replication kinetics of the stocks of SIVmac239, SIVmac1A11, and SIVmac239/1A11/239 after i.v. inoculation of rhesus. We then determined the ability of stocks to initiate systemic infection following vaginal inoculation of rhesus macaques. Further, we characterized the replication of infectious virus stocks of SHIV HXBc2, 89.6, and 89.6-PD in rhesus macaque monocyte-derived macrophages. These results are compared to the published data of others regarding the ability of these SHIV to replicate in rhesus macaques after i.v. inoculation and our previously published studies describing the ability of these viruses to initiate systemic infection after intravaginal inoculation. Our results demonstrate that in vitro measures of the ability of a virus to replicate in rhesus macaque macrophages do not correlate with the ability of the virus to cause systemic infection following vaginal inoculation. However, the ability of a virus to be transmitted vaginally was associated with its in vivo replication capacity. Thus, systemic infection was detected only in rhesus macaques that were intravaginally inoculated with SIV clones (SIVmac239 or SIVmac239/1A11/239), SHIV 89.6, or the uncloned viruses (SIVmac251 and SHIV 89.6-PD), all of which produce plasma viremia after i.v. inoculation.

MATERIALS AND METHODS

Animals. All animals used in this study were colony bred, multiparous, female rhesus macaques (*Macaca mulatta*) from the California Regional Primate Research Center. 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 recommendations of the guide prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Resource Council (5a). Prior to use, the animals were negative for antibodies to HIV type 2 (HIV-2), SIV, type D retrovirus, and simian T-cell lymphotropic virus type 1.

Virus stocks. The SIVmac239 stock used in this study originated from a vial of infectious virus generously supplied by R. Desrosiers (New England Regional Primate Center) and was expanded by a single short-term passage on CEMx174 cells. The second stock was a chimera of SIVmac239 and SIVmac1A11 (designated SIVmac239/1A11/239) and was grown in rhesus peripheral blood mononuclear cells (PBMC). A detailed description of the chimeric virus has been published elsewhere (2). This SIV chimera causes immunodeficiency in some rhesus macaques infected by i.v. inoculation inoculation (20). The SIVmac1A11 stock was grown in rhesus PBMC. A detailed description of this virulence-attenuated virus has been published previously (2). This SIV clone does not cause immunodeficiency in rhesus macaques infected by i.v. inoculation (20). The SIVmac251 stock used in this study was produced by short-term culture of a previously used infectious virus stock in rhesus PBMC. All four of the above SIV virus stocks had a titer of 10^5 tissue culture infective doses ($TCID_{50}$)/ml as determined by endpoint titration on CEMx174 cells and estimation of the 50% endpoint by the method of Reed and Muench (34). This new SIVmac251 stock has not been used in previously published vaginal inoculation studies. A description of the technique used for intravaginal inoculation has been published elsewhere (24). Seminal plasma was not used for the vaginal inoculations, and although no attempt was made to inoculate animals at specific points in the menstrual cycle, animals were not inoculated during menses. i.v. inoculations were performed by standard techniques.

A detailed description of the SHIV stocks has been previously published (16). Briefly, both SHIV clones contain functional HIV-1 *vpu*, *tat*, *rev*, and *env* genes in the context of the SIVmac239 provirus and were grown in rhesus macaque PBMC. The first virus was designated SHIV HXBc2 (14) and was constructed by using the HIV-1 IIIB/LAI variant, which is the prototype of the T-cell-tropic viruses. The second virus, designated SHIV 89.6 (37), was identical to SHIV HXBc2 except for the *Kpm*I (nucleotide 5925)-to-*Bam*HI (nucleotide 8053) fragment, which encodes the ectodomain of the gp120 and gp41 envelope glycoproteins. This *env* fragment in the SHIV 89.6 virus was derived from HIV-1 89.6, a highly cytopathic, macrophage-tropic variant (5). The SHIV HXBc2 stock contained 4,800 $TCID_{50}$/ml and the SHIV 89.6 stock contained 1,800 $TCID_{50}$/ml, as determined by endpoint titration on CEMx174 cells and estimation of the 50% endpoint by the method of Reed and Muench (34). Both SHIV HXBc2 and SHIV 89.6 initiate i.v. infection of rhesus macaques with an inoculum of approximately 1 $TCID_{50}$. The frequency of virus isolation is comparable to that of the animals infected i.v. with SHIV HXBc2 and SHIV 89.6 over the first 2 months of infection (48a). A description of the in vivo passage of SHIV 89.6 has been published elsewhere (36, 37). To produce the SHIV 89.6-PD stock used in this study, a plasma sample was collected from a rhesus macaque 2 weeks after it was inoculated with a SHIV 89.6-P stock. This plasma was generously provided by N. Letvin. The virus stock was amplified by culturing 3 ml of plasma with 2×10^6 CEMx174 cells. The level of SIV p27 antigen in the culture supernatant was higher than 5,000 pg/ml after 10 days, at which time supernatants were collected, filtered, and used to infect fresh concanavalin A-stimulated rhesus macaque PBMC. After 10 days in culture, the supernatant of the PBMC culture was collected and filtered, and aliquots were frozen. This stock is designated SHIV 89.6-PD to indicate that the stock was plasma derived. This designation also distinguishes the SHIV 89.6-PD stock from the SHIV 89.6-P stock (passaged), which was derived from PBMC from the same animal (36). The titer of the SHIV 89.6-PD stock is 25,000 $TCID_{50}$/ml, as determined by endpoint titration on CEMx174 cells and estimation of the 50% endpoint by the method of Reed and Muench (34).

Assessment of in vitro macrophage tropism of SIV and SHIV stocks. In order to characterize the ability of the virus stocks used in the vaginal transmission studies to replicate in rhesus macaque macrophages, a series of in vitro studies was performed, using a modification of previously published techniques (33). Briefly, PBMC were isolated from heparinized whole blood obtained from three naive rhesus macaques. The PBMC were suspended in RPMI 1640 containing 20% fetal bovine serum, 10% human AB serum (Gemini Bio-Products, Inc., Calabasas, Calif.), 2 mM L-glutamine, and 10 U of penicillin-streptomycin per ml. The PBMC (4×10^6/well) were placed in a 24-well plate and incubated overnight. Nonadherent cells were removed by repeated washing with 37°C serum-free RPMI 1640. A second medium, macrophage growth medium (MGM), consisting of RPMI 1640 with 20% fetal bovine serum, 200 U of granulocyte-macrophage colony-stimulating factor (Genzyme, Cambridge, Mass.) per ml, 2 mM L-glutamine, and 10 U of penicillin-streptomycin per ml, was added to each well. The macrophages were cultured for 7 to 10 days, and the MGM was changed twice a week. By the end of this period, each well contained approximately 4×10^5 well-differentiated macrophages. Immunohistochemical analysis of cells harvested from representative wells demonstrated that all cells were $CD68^+$ (EBM-11 monoclonal antibody; Dako, Carpenteria, Calif.), and no $CD3^+$ T cells (anti-CD3 monoclonal antibody; Dako) were detected in these preparations. Aliquots of the same SIV or SHIV stocks used for the animal inoculation studies were added to duplicate wells containing the macrophages. The volume of virus stock added to each well was based on the concentration of the virus stock (see above). For the experiments using the SIV stocks, the multiplicity of infection (MOI) was 0.05; for the experiments using the SHIV stocks, the MOI was 0.001. The virus was incubated with the macrophages

overnight at these MOIs; then 1 ml of MGM was added to the wells, and the incubation was continued for an additional 24 h. Excess virus was then removed from the wells by at least four washes with 37°C serum-free RPMI 1640. Following the last wash, 1.5 ml of MGM was added to each well, and 0.5 ml of the added medium was removed immediately as the day 1 sample. To assess p27 antigen production, beginning at day 4, 0.5 ml of supernatant was removed from each well and replaced. This was repeated every 2 or 3 days until day 15. A commercial p27-SIV antigen assay kit (Coulter Immunology, Hialeah, Fla.) was used to quantify p27 in the culture supernatants.

Virus isolation. Virus was isolated from heparinized whole blood obtained from the SIV-inoculated rhesus macaques. PBMC were isolated by Ficoll gradient separation (Lymphocyte Separation Medium; Organon Teknika, West Chester, Pa.) and cocultured with CEMx174 cells (9) (provided by James A. Hoxie, University of Pennsylvania) as previously described (15). Except where otherwise noted, 5×10^6 PBMC were cocultivated with 2×10^6 to 3×10^6 CEMx174 cells. Aliquots of the culture media were assayed regularly for the presence of SIV major core protein (p27) by antigen capture enzyme-linked immunosorbent assay (ELISA) (15). Cultures were considered positive if they were antigen positive at two consecutive time points. A detailed description of the technique and criteria to determine if an aliquot was antigen positive has been published elsewhere (20). All cultures were maintained for 8 weeks and tested for SIV p27 by ELISA before being scored as virus negative. Blood samples for virus isolation were collected at the times indicated below (see Table 1).

Quantification of cell-free and cell-associated viral load in i.v.-inoculated rhesus macaques. In order to assess the relative in vivo replicative capacities of the SIV molecular clones in the i.v.-inoculated animals, the concentrations of cell-free virus in plasma and cell-associated virus in PBMC were measured in a limiting-dilution assay (47). Fresh plasma was centrifuged to pellet contaminating cells, diluted serially 10-fold in complete medium, and cocultured with CEMx174 cells in 1 ml in a 24-well plate. Likewise, PBMC were washed, diluted serially 10-fold from 10^6 cells per ml, and cocultured with CEMx174 cells. After 4 weeks, cells from all negative wells at 10^6 cells per well were pooled and transferred to a T25 tissue culture flask (Fisher Scientific) and cultured for an additional 4 weeks. In addition, 10^7 PBMC were cocultured with 2×10^6 CEMx174 cells in T75 tissue culture flasks (Fisher Scientific) and maintained for 8 weeks before being terminated as negative. The $TCID_{50}$ of cell-free and cell-associated virus were calculated by the method of Reed and Muench (34). Negative cultures correspond to less than 1.0 $TCID_{50}$ per ml of plasma or less than 0.1 $TCID_{50}$ per 10^6 PBMC, the limits of detection for these assays.

Antigen capture ELISA to quantitate plasma p27 antigen in i.v.-inoculated rhesus macaques. In order to assess the relative in vivo replicative capacities of the SIV molecular clones in the i.v.-inoculated animals, p27 antigen levels in plasma were assessed in the animals inoculated i.v. with SIV clones by using a commercial antigen capture ELISA kit (Coulter Immunology, Hialeah, Fla.) according to the manufacturer's instructions.

bDNA quantitation of SIV RNA in plasma in i.v.-inoculated rhesus macaques. In order to assess the relative in vivo replicative capacities of the SIV molecular clones in the i.v.-inoculated animals, quantitative assays for the measurement of SIV RNA were performed using a branched DNA (bDNA) signal amplification assay specific for SIV (6). This assay is similar to the Quantiplex HIV RNA assay (32) except that target probes were designed to hybridize with the *pol* region of the SIVmac group of strains including SIVmac251 and SIVmac239. SIV *pol* RNA in plasma samples was quantified by comparison with a standard curve produced with serial dilutions of cell-free SIV-infected tissue culture supernatant. The quantitation of this standard curve was done by comparison with purified, quantitated, in vitro-transcribed SIVmac239 *pol* RNA. SIV RNA associated with viral particles was measured after being pelleted from 1 ml of heparinized plasma (23,500 × *g* for 1 h at 4°C). The lower quantitation limit of this assay was 10,000 copies of SIV RNA per ml of plasma.

RT-QC-PCR quantitation of SIV RNA in plasma in i.v.-inoculated rhesus macaques. Reverse transcription quantitative competitive PCR (RT-QC-PCR) was also used to quantify SIV RNA in plasma of i.v.-inoculated animals. The details of this assay have been published previously (42). Briefly, blood was collected into tubes containing acid citrate dextrose as an anticoagulant. The plasma fraction was centrifuged at 17,500 × *g* (model GS-15R centrifuge; Beckman Instruments, Fullerton, Calif.) at 4°C for 1.5 h. RNA was extracted from the resulting viral pellet with Trizol reagent (Gibco-BRL, Gaithersburg, Md.) and was then subjected to chloroform extraction, isopropanol precipitation, and a 70% ethanol wash. The RNA pellets were resuspended in RNase-free water and stored at −70°C until analyzed. To generate wild-type control and competitor RNAs, runoff transcripts were produced (Ribomax Large Scale RNA Production System; Promega, Madison, Wis.) from plasmids (generously provided by S. Staprans, Gladstone Institute, San Francisco, Calif.) containing either a 162-bp fragment of *gag* from SIVmac239 (pSIV*gag*; wild-type control) or an analagous fragment with a 40-bp insert (pSIV*gag*+40; competitor). A 5-μl volume of competitor RNA (10, 50, 100, 500, 1,000, or 5,000 copies) and 5 μl of wild-type RNA (2,000 copies) or sample RNA were added to each reaction mixture. Reverse transcription was carried out, and the resulting cDNA was amplified by using primers and PCR conditions as previously described (42) to generate a 190-bp competitor fragment and a 150-bp sample fragment. PCR products were analyzed on 3% Metaphor (FMC, Rockport, Maine) agarose gels and visualized with ethidium bromide. Densitometry analysis of PCR products was conducted with an ISO 1000 gel analysis and documentation system (Alpha Innotech, San Leandro, Calif.). Quantitation of plasma RNA was accomplished by the method of Staprans et al. (42) and reported as RNA copies per milliliter of plasma.

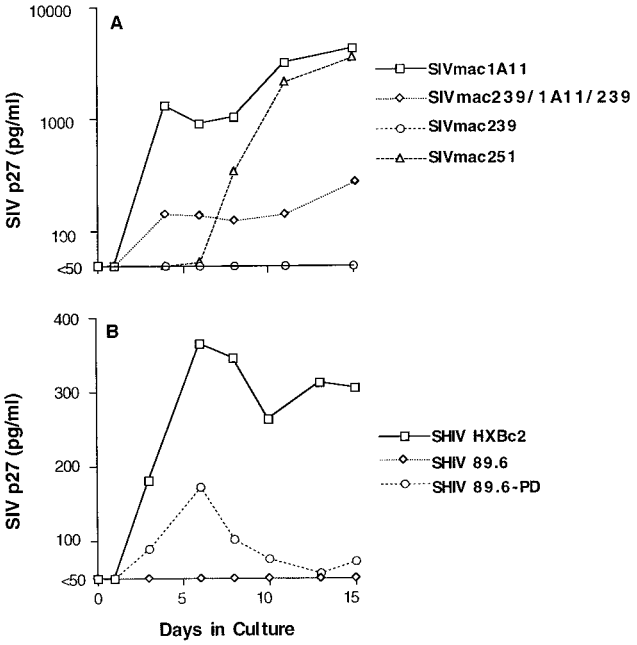

FIG. 1. In vitro replication of SIV and SHIV molecular clones in rhesus macaque monocyte-derived macrophages. The results shown here are representative of three separate experiments using monocyte-derived macrophages isolated from three rhesus macaques. (A) Replication of SIV molecular clones and uncloned SIVmac251. Note that SIVmac1A11, SIVmac239/1A11/239, and SIVmac251 replicate well in rhesus macaque macrophages. (B) Replication of SHIV molecular clones and uncloned SHIV 89.6-PD. Note that only SHIV HXBc2 and SHIV 89.6-PD replicate in rhesus macaque macrophages.

PCR-based detection of SIV *gag*. Nested PCR was carried out on genomic PBMC DNA in a model 9600 Thermal Cycler (Perkin-Elmer Cetus, Emeryville, Calif.) as previously described (26). Briefly, cryopreserved PBMC isolated from whole blood were washed three times in Tris buffer at 4°C and resuspended at 10^7 cells/ml. Ten microliters of the cell suspension was added to 10 μl of PCR lysis buffer (50 mM Tris-HCl [pH 8.3], 0.45% Nonidet P-40, 0.45% Tween 20) with 200 μg of proteinase K per ml. The cells were incubated for 3 h at 55°C and then for 10 min at 96°C. Two rounds of 30 cycles of amplification were performed on aliquots of plasmid DNA containing the complete genome of SIVmac1A11 (18) (positive control) or aliquots of cell lysates under conditions described elsewhere (46). DNA from uninfected CEMx174 cells was amplified as a negative control in all assays to monitor potential reagent contamination. β-Actin DNA sequences were amplified with two rounds of PCR (30 cycles/round) from all PBMC lysates to detect potential inhibitors of *Taq* polymerase in cell lysates (26). Following the second round of amplification, a 10-μl aliquot of the reaction product was removed and run on a 1.5% agarose gel. Amplified products in the gel were visualized by ethidium bromide staining. Blood samples for PCR analysis were collected at the times indicated in Table 1.

Serum antibody responses in inoculated animals. Immunoblots were performed to detect antibody responses to specific SIV proteins in the sera of the vaginally inoculated animals by a previously described technique (44).

RESULTS

In vitro replication of SIV and SHIV molecular clones in rhesus monocyte-derived macrophages. All the virus stocks used in these studies replicate well in mitogen-stimulated rhesus macaque PBMC (data not shown). The macrophage tropism studies were repeated at least three times, and the results were consistent for monocyte-derived macrophages from three rhesus macaques. SIVmac1A11 and SIVmac251 consistently replicated well in rhesus macaque macrophages (Fig. 1A). SIVmac239 did not replicate in rhesus macaque macrophages, and SIVmac239/1A11/239 replicated to intermediate levels in

rhesus macaque macrophages (Fig. 1A). These results are consistent with those of previously published studies (2, 3, 29). Of the SHIV studied, SHIV HXBc2 replicated in rhesus macaque macrophages, SHIV 89.6-PD replicated to only a limited extent (Fig. 1B), and SHIV 89.6 failed to replicate to detectable levels in rhesus macaque macrophages (Fig. 1B). Results of the macrophage replication studies using SHIV 89.6 are similar to previously published results for the same stock; however, our results with the SHIV HXBc2 stock differ from previously published results (37). This difference may be due to the source of the rhesus macaque macrophages (bone marrow versus monocyte derived) or other differences in the culture systems. The replication pattern of the SHIV in rhesus macrophages was unexpected because the HIV clones from which the *env* of the SHIV was derived have exactly the opposite pattern of replication in human macrophages. Thus, HIV HXBc2 does not replicate in human macrophages, while HIV 89.6 replicates well in human macrophages (5). It is possible that the difference between the patterns of replication of the parental HIV in human macrophages and the SHIV in macaque macrophages reflects differential expression of virus coreceptors (chemokine receptors) on the cells from the two species. It is perhaps equally likely that the inability to detect p27 in the supernatants of some of the macrophage cultures is due to postentry blocks to virus replication or assembly.

In vivo replication of SIV molecular clones following i.v. inoculation of rhesus macaques as assessed by virus load in plasma and PBMC. In order to assess the in vivo replication capacity of each of the stocks derived from SIV molecular clones and used for vaginal inoculation, two naive rhesus macaques were inoculated i.v. with 0.5 ml of each virus stock (5×10^4 $TCID_{50}$). The level of plasma p27 antigenemia, the number of SIV RNA copies in plasma as determined by using bDNA and RT-QC-PCR assays, the $TCID_{50}$ of cell-free SIV in plasma (plasma viremia), and infectious virus in PBMC were determined at 1, 2, 3, and 4 weeks postinoculation (p.i.). Animals inoculated with each of the three molecular clones of SIV exhibited distinct patterns of plasma antigenemia, infectious virus titer, and viral RNA as measured by both RT-QC-PCR and bDNA assay (Fig. 2). By all measures, the monkeys infected with SIVmac239 had the highest levels of virus replication (Fig. 2). While levels of SIV p27 and viral RNA in plasma and the infectious titer in PBMC peaked at week 2 p.i., the peak level of infectious virus in plasma occurred at 2 weeks in 1 animal (22317) and at 3 weeks in the other animal (24190). The SIVmac239-inoculated animals maintained a greater virus load throughout the study period than the monkeys infected with SIVmac1A11 or SIVmac239/1A11/239. The monkeys inoculated with SIVmac239/1A11/239 had intermediate levels of virus replication (Fig. 2). SIV p27 and infectious virus in plasma were detected in only one of the two animals (19519) inoculated with SIVmac239/1A11/239. Viral RNA was detected in the plasma of both animals, although the peak levels were at least 1 log lower than in the SIVmac239-infected animals. The peak levels of infectious virus in PBMC were more similar in the SIVmac239- and SIVmac239/1A11/239-inoculated animals. Interestingly, the peak antigenemia and viremia (infectious virus, RNA, and cell-associated virus) occurred at 3 weeks p.i., 1 week later than in the SIVmac239-inoculated animals. The SIVmac239/1A11/239-inoculated animals maintained an intermediate virus load throughout the study period compared to that of the monkeys infected with SIVmac1A11 or SIVmac239. The monkeys inoculated with SIVmac1A11 had the lowest levels of virus replication (Fig. 2). SIV p27 and infectious virus in plasma were not detected in animals inoculated with SIVmac1A11. Viral RNA was detected in the plasma of both animals, although the peak levels were at least 3 log lower than in the SIVmac239-infected animals (Fig. 2). The peak levels of infectious virus in PBMC were lower than in the SIVmac239- and SIVmac239/1A11/239-inoculated animals, but significant levels (approximately 10^3 $TCID_{50}/10^6$ PBMC) were found in the SIVmac1A11-inoculated animals. Interestingly, the peak viral RNA levels occurred at 1 week p.i., while the peak cell-associated virus occurred at 2 or 3 weeks p.i. The SIVmac1A11-inoculated animals maintained the lowest virus load throughout the study period, compared to the monkeys infected with SIVmac239/1A11/239 or SIVmac239.

The analysis of four parameters of virus replication by five assays in animals inoculated i.v. with three SIV molecular clones demonstrated three distinct levels of virus replication. These results demonstrate that SIVmac239 replicates well and SIVmac1A11 replicates poorly in i.v.-inoculated rhesus macaques. Furthermore, the chimeric virus SIVmac239/1A11/239 has a capacity to replicate in vivo that is intermediate to that of the two parental viruses. The most obvious distinction was that infectious virus and viral antigen were detected in the plasma of both animals inoculated with SIVmac239 and one of two animals inoculated i.v. with SIVmac239/1A11/239. In sharp contrast, plasma viremia or antigenemia was never detected in the animals inoculated i.v. with SIVmac1A11. These results are consistent with previously published, but less detailed, comparisons of the in vivo replication capacities of these viruses (12, 20). Thus, it seems that SIVmac1A11 does not replicate sufficiently well in rhesus macaques to produce detectable plasma viremia or antigenemia. The analysis also demonstrated that two assays (bDNA and RT-QC-PCR) for measuring SIV RNA in plasma of rhesus macaques yielded very similar but not identical results.

Vaginal transmission of SIVmac239. At 14 days p.i., SIV was isolated from the PBMC of both of the female rhesus macaques inoculated intravaginally with SIVmac239 (Table 1). PCR analysis of proviral DNA in PBMC confirmed the results of the virus isolation studies: both animals had detectable proviral DNA in PBMC. The PBMC from the animals remained PCR positive throughout the 24-week observation period. In addition, proviral DNA was detected in lymph node mononuclear cells (LNMC) collected at 2, 8, and 16 weeks p.i. Both animals seroconverted to SIV antigens by 16 weeks p.i. (data not shown). Thus, both rhesus macaques intravaginally inoculated once with SIVmac239 developed a persistent viremia.

Vaginal transmission of SIVmac239/1A11/239. All four female rhesus macaques inoculated intravaginally with SIVmac239/1A11/239 were virus isolation positive at 14 days p.i. (Table 1). At 24 weeks p.i., virus could not be isolated from the PBMC of two of these animals (25815 and 25578), while the other two animals (25273 and 25418) remained virus isolation positive. PCR analysis of proviral DNA in PBMC confirmed the results of the virus isolation studies: all four animals had detectable proviral DNA in PBMC collected at 14 days p.i., and one animal (25273) was PCR positive at 7 days p.i. The PBMC of all animals remained PCR positive throughout the 24-week observation period. Thus, at 24 weeks p.i., proviral DNA was detected in the PBMC of the animals (28515 and 25578) that were virus isolation negative. With the exception of the 2-week LNMC sample from one animal (25273), proviral DNA was detected in LNMC collected at 2, 8, and 16 weeks p.i. from all four animals. All four of the animals inoculated intravaginally with SIVmac239/1A11/239 were seropositive for SIV antigens by 16 weeks p.i. (data not shown). Thus, rhesus macaques inoculated intravaginally once with SIVmac239/1A11/239 developed a persistent viremia, and they remained PCR positive for at least 24 weeks p.i.

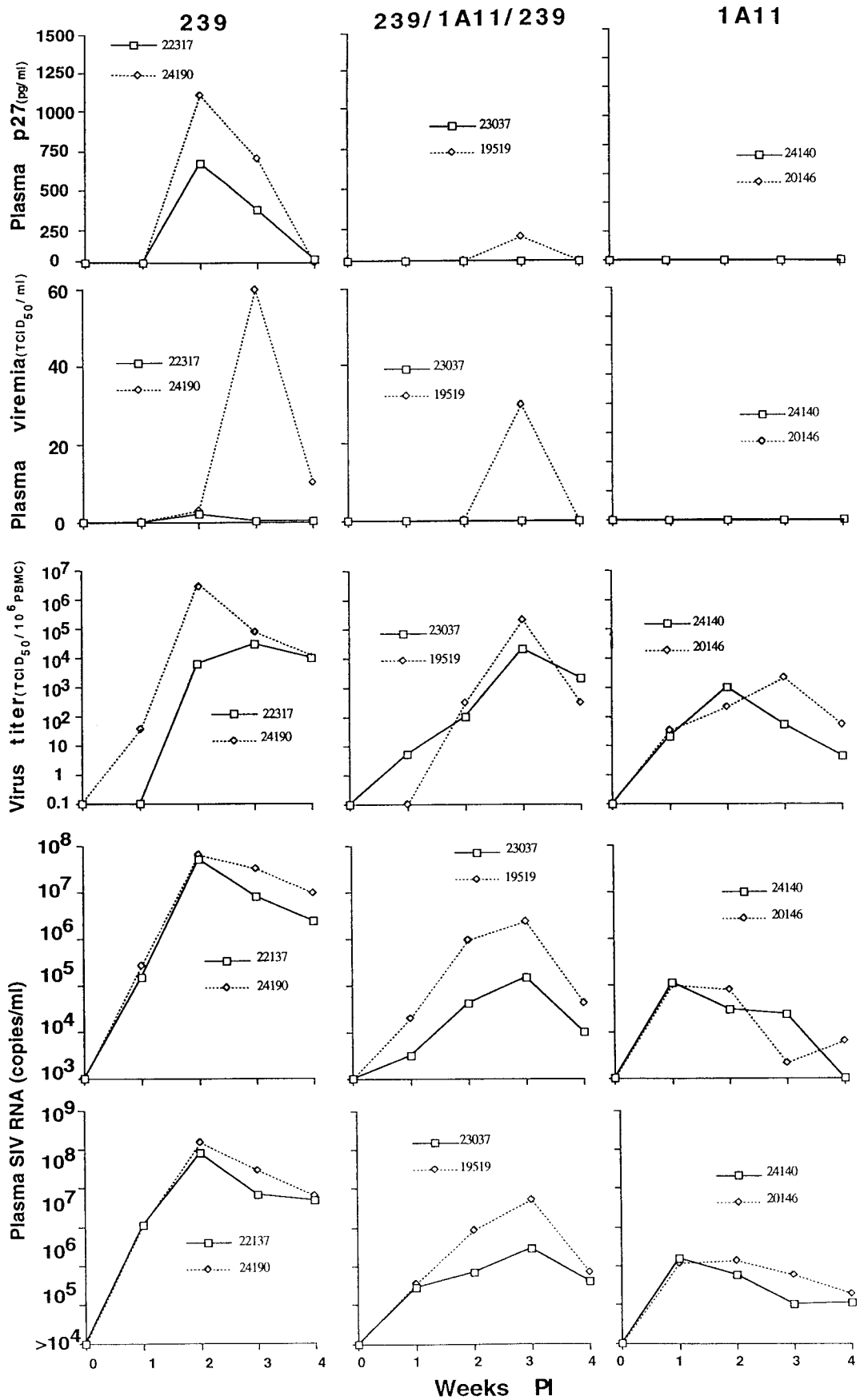

FIG. 2. In vivo replication capacities of SIV molecular clones in rhesus macaques following i.v. inoculation. Three groups of animals (two animals per group) were inoculated i.v. with one of the three SIV molecular clones. The results of the analysis shown for each group of animals are arranged in columns, and the virus used to inoculate the animals in each group is noted at the top of each column. The rows are the data from a single assay: plasma p27 level (A), titer of infectious virus in plasma (B), cell-associated virus titer (C), viral RNA in plasma as measured by RT-QC-PCR (D), and viral RNA in plasma as measured by bDNA (E). Note that by all measures SIVmac239 has the highest replicative capacity, SIVmac1A11 has the lowest replicative capacity, and SIVmac239/1A11/239 has an intermediate replicative capacity. The most distinctive difference among the three SIV clones is that, unlike SIVmac239 and SIVmac239/1A11/239, i.v. inoculation with SIVmac1A11 did not result in sufficient virus replication to produce plasma antigenemia or infectious virus in plasma.

Vaginal transmission of SIVmac1A11. Two rhesus macaques were inoculated intravaginally with 1 ml of SIVmac1A11 (10^5 $TCID_{50}$). Virus could not be isolated from PBMC collected at any time point from one animal (Table 1). This animal did not develop detectable serum antibodies to SIV antigens. Virus was isolated from the PBMC of a second animal (21237) at 6 and 8 weeks p.i. (Table 1). Subsequent attempts to isolate virus from this animal were unsuccessful. Thus, this animal had a transient viremia but did not seroconvert to SIV antigens by 16 weeks p.i. (data not shown). It was previously shown that 12 intravaginal inoculations with SIVmac1A11 resulted in transient viremia in only one of five rhesus macaques (19) (see Table 3).

Vaginal transmission of uncloned SIVmac251. In order to compare the ability of uncloned SIVmac251 to produce systemic infection after vaginal inoculation with the results of the transmission studies using the SIV clones, we intravaginally inoculated eight mature female rhesus macaques with 1 ml of uncloned SIVmac251 (10^5 $TCID_{50}$/ml). All eight animals became viremic (Table 2) and seroconverted to SIV antigens by 8 weeks p.i. (data not shown). Our previous experience with vaginal transmission using several different SIVmac251 stocks is summarized in Table 3.

In vivo replication of SHIV 89.6-PD following i.v. inoculation of rhesus macaques as assessed by plasma antigenemia. Two animals (20458 and 19854) inoculated i.v. with 1 ml of the undiluted SHIV 89.6-PD stock (25,000 $TCID_{50}$) became persistently virus isolation positive (Table 4). Viral antigen was detected at 1 week p.i. (Fig. 3). In both animals, the peak plasma antigenemia occurred at 7 days p.i., but antigen was still detectable at 14 days p.i. By 21 days p.i., SIV p27 was detectable in plasma of one animal (20458) but undetectable in the other animal. At 2 weeks p.i., 1 week after the antigenemia peak, the two i.v.-inoculated monkeys had a precipitous drop in $CD4^+$ T-cell counts (data not shown). Neither animal seroconverted to SIV or HIV antigens (data not shown), and both were euthanized with clinical signs of AIDS by 28 weeks p.i.

Vaginal transmission of SHIV molecular clones and uncloned SHIV 89.6-PD. A summary of published vaginal transmission studies (16) using the two SHIV molecular clone stocks is provided in Table 3. SHIV HXBc2 does not infect rhesus macaques even after four vaginal inoculations, whereas a less concentrated stock of SHIV 89.6 can be transmitted to rhesus macaques after three intravaginal inoculations (16). Both of these SHIV infect rhesus macaques after i.v. inoculation but do not cause disease (13, 16, 37). Five of six animals inoculated intravaginally once with 1 ml of the undiluted SHIV 89.6-PD stock (25,000 $TCID_{50}$) became persistently virus isolation positive (Table 4). The five infected animals developed a peak level of p27 antigen in plasma at 2 weeks p.i. (Fig. 3). One animal (25772) remained virus isolation negative after the first intravaginal inoculation and was reinoculated with the same virus dose 145 days after the first inoculation. This animal remained virus isolation negative for more than 170 days after the second inoculation. One of two animals inoculated once intravaginally with 1 ml of the 10^{-1} dilution of the SHIV 89.6-PD stock (2,500 $TCID_{50}$) became persistently virus isolation positive (Table 4). Three of the animals infected by intravaginal inoculation (25902, 25788, and 26814) did not seroconvert to SIV and HIV antigens (data not shown), and they were killed with clinical signs of AIDS by 24 weeks p.i. The other three infected animals made anti-SIV antibody responses which were detectable at 4 weeks p.i. (data not shown), and they have survived for more than 44 weeks p.i. Anti-SIV or anti-HIV antibodies in serum were not detected in the multiply exposed uninfected animal (25772).

DISCUSSION

For the purposes of this study, vaginal transmission of SIV or SHIV is defined as the ability to detect virus in PBMC, by virus isolation or PCR, after vaginal inoculation. This defini-

TABLE 1. Virus isolation from PBMC of rhesus macaques inoculated intravaginally with SIV molecular clones[a]

Animal	Virus	Isolation of virus at the indicated wk p.i.[b]						
		2	4	6	8	12	16	24
25817	SIVmac239	+	+	+	+	+	+	+
25835	SIVmac239	+	+	+	+	+	+	+
25418	SIVmac239/1A11/239	+	+	+	+	+	+	+
25578	SIVmac239/1A11/239	+	+	+	+	+	+	+
25273	SIVmac239/1A11/239	+	+	+	+	+	+	+
25815	SIVmac239/1A11/239	+	+	+	+	+	+	+
21237	SIVmac1A11	−	−	+	+	−	−	−
25742	SIVmac1A11	−	−	−	−	−	−	−

[a] All virus inoculations consisted of 1 ml of virus stock, and all inoculations summarized in this table were done without addition of seminal plasma.
[b] +, virus isolated; −, no virus isolated.

TABLE 2. Virus isolation from PBMC of rhesus macaques inoculated intravaginally with uncloned SIVmac251[a]

Animal	Isolation of virus at the indicated wk p.i.[b]				
	1	2	4	8	12
20573	−	+	+	+	+
20960	−	+	+	+	+
24668	+	+	+	+	+
24786	−	+	+	+	+
25790	+	+	+	+	+
26875	+	+	+	+	+
21743	ND	+	+	+[c]	
24762	ND	+	+	+[c]	

[a] All virus inoculations consisted of 1 ml of virus stock (10^5 $TCID_{50}$), and all inoculations summarized in this table were done without addition of seminal plasma.
[b] +, virus isolated; −, no virus isolated; ND, not determined.
[c] This animal was necropsied at this time for reasons unrelated to this study.

TABLE 3. Summary of relevant previously published vaginal transmission studies from this laboratory

Virus	$TCID_{50}$ of inoculum[a]	No. of inoculations	No. of animals infected[b]/no. of animals inoculated	Reference
SIVmac251	10^5	1	8/8[c]	23
	10^4	1	8/8[c]	23
	10^5	1	4/4[c]	23
SIVmac1A11	10^5	12	1/5	15
SIVmac239	10^5	2	2/2	25
SHIVHXBc2	4,800	4	0/2	12
	4,800	3	0/2	12
SHIV 89.6	1,800	4	2/2	12
	1,800	3	2/2	12

[a] All virus inoculations consisted of 1 ml of virus stock, and all inoculations summarized in this table were done without addition of seminal plasma.
[b] An animal was considered infected if virus could be isolated from its PBMC on at least one occasion.
[c] Transient viremia was noted in some of the animals inoculated intravaginally with this virus stock. The animals inoculated with the lower doses of virus were more likely to become transiently viremic than animals inoculated with the higher doses of virus. Please refer to reference 23 for details.

tion does not rule out the possibility that a virus can cross the mucosa and not disseminate systemically. The in vitro phenotype of a virus stock with regard to macrophage tropism did not predict the ability of the virus to be transmitted following vaginal inoculation. Of the three SIV clones used in this study, two (SIVmac239/1A11/239 and SIVmac1A11) replicated well in rhesus macaque macrophages in vitro, while one clone (SIVmac239) did not. The uncloned SIVmac251 stock replicates well in rhesus macaque macrophages and adherent-cell-depleted PBMC. SIVmac239, a T-cell-tropic molecular clone, and SIVmac1A11/239, a dualtropic molecular clone, readily initiated systemic infection in rhesus macaques after one intravaginal inoculation. Dualtropic SIVmac1A11 was not efficiently transmitted by vaginal inoculation. In this study, one of two animals became transiently viremic after one vaginal inoculation with SIVmac1A11, but it has previously been shown that only one of five rhesus macaques intravaginally inoculated a total of 12 times each with SIVmac1A11 became viremic (19) (Table 3). Of the three SHIV stocks tested, two (SHIV HXBc2 and 89.6-PD) replicated in rhesus macaque macrophages, while one (SHIV 89.6) did not. It has previously been shown that four intravaginal inoculations of SHIV HXBc2 do not produce viremia in rhesus macaques, while three intravaginal inoculations of SHIV 89.6 do infect rhesus monkeys (16). We have also shown that one intravaginal inoculation with SHIV 89.6-PD (25,000 $TCID_{50}$) results in infection of five of six rhesus macaques and one of two animals inoculated intravaginally with SHIV 89.6-PD (2,500 $TCID_{50}$) became infected. These results demonstrate that two viruses (SIVmac1A11 and SHIV HXBc2) which replicate well in rhesus macaque macrophages do not efficiently initiate infection after vaginal inoculation, while two viruses (SIVmac239 and SHIV 89.6) which do not replicate well in rhesus macaque macrophages ("T-cell tropic") and three viruses (SIVmac239/1A11/239, SIVmac251, and SHIV 89.6-PD) which replicate in rhesus macaque macrophages ("dualtropic") do infect rhesus macaques after intravaginal inoculation. Thus, the ability of a virus to infect a rhesus macaque after intravaginal inoculation is not predicted by the capacity of the virus to replicate in rhesus macaque macrophages in vitro.

The results of this study should not be used to suggest that macrophages in the vaginal mucosa are not target cells for virus during vaginal transmission of SIV. Although SIVmac239 is unable to replicate in specific types of differentiated macrophages in vitro, SIVmac239 mRNA can be detected in bone marrow macrophages 3 days after i.v. inoculation of rhesus macaques (17). Because the readout for the in vitro macrophage assay used in our study was p27 production, it is possible that the failure to produce antigen is due to a postentry block in virus replication. In fact, it has been previously demonstrated that SIVmac239 can enter macrophages and integrate into the host genome as a provirus and that viral genes are transcribed and proteins are produced but that virion assembly does not occur (43). Similar studies using SHIV 89.6 and SIVmac239/1A11/239 have not been conducted, but on the basis of the results of the studies using SIVmac239, it is clear that in vitro experiments do not predict the range of cells that a virus can infect in vivo. This leaves intact the possibility that mucosal antigen-presenting cells, including macrophages, are targets for early infection after intravaginal inoculation with SIV. It should also be noted that SIVmac1A11 mRNA can be detected in macrophages of the bone marrow (17) after i.v. inoculation of rhesus macaques. Thus, even the ability to repli-

TABLE 4. Virus isolation from PBMC of rhesus macaques inoculated once with SHIV 89.6-PD[a]

Animal	Route of inoculation	Virus dose ($TCID_{50}$)	Isolation of virus at the indicated wk p.i.[b]													
			1	2	3	4	6	8	12	16	20	24	28	32	36	40	44
19854	i.v.	25,000	+	+	+	+	+	+	+	+	+[c]						
20458	i.v.	25,000	+	+	+	+	+	+	+	+	+	+	+[c]				
25757	IVAG[d]	25,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25788	IVAG	25,000	+	+	+	+	+	+	+	+	+	+[c]					
26904	IVAG	25,000	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
25772	IVAG	25,000	−	−	−	−	−	−	−	−	−[e]	−	−	−	−	−	−
26428	IVAG	25,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25902	IVAG	25,000	+	+	+	+	+	+	+	+	+[c]						
26261	IVAG	2,500	−	−	−	−	−	−	−	−	−	−					
26814	IVAG	2,500	+	+	+	+	+	+	+	+	+[c]						

[a] All virus inoculations consisted of 1 ml of virus stock, and all inoculations summarized in this table were done without addition of seminal plasma.
[b] +, virus isolated; −, no virus isolated.
[c] Results of virus isolation from PBMC at the time of necropsy. Animals were necropsied due to severe clinical signs of AIDS.
[d] IVAG, intravaginal.
[e] This animal was reinoculated intravaginally with 1 ml of undiluted SHIV 89.6-PD at this time.

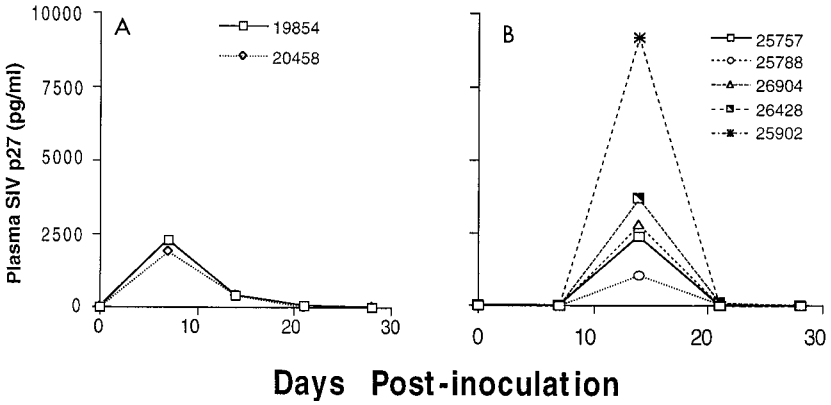

FIG. 3. Viral p27 antigen in plasma of rhesus macaques inoculated with SHIV 89.6-PD. Plasma collected at the time points shown was tested for the presence of SIV p27 with a commercial antigen capture kit (Coulter Immunology). (A) Results for two animals i.v. inoculated with 25,000 $TCID_{50}$ of SHIV 89.6-PD. Note that for both animals, the peak antigenemia occurs at 7 days p.i. (B) Results for five animals intravaginally inoculated with 25,000 $TCID_{50}$ of SHIV 89.6-PD. Animal 25722 was negative in plasma p27 and virus isolation assays and is not included in the graph. Note that for the five infected animals, the peak plasma antigenemia occurs at 14 days p.i. Thus, the detection of viral antigen in the plasma of intravaginally inoculated animals was delayed by 7 days compared to that of the i.v. inoculated animals.

cate in macrophages in vivo is not sufficient to confer on a virus the ability to initiate a systemic infection after vaginal inoculation.

In contrast, the in vivo phenotype of a virus stock as related to replication capacity was predictive of the ability of a virus to produce systemic infection after intravaginal inoculation of rhesus macaques. After i.v. inoculation of rhesus macaques, plasma antigenemia, titers of infectious virus in plasma (viremia), levels of viral RNA in plasma, and cell-associated virus load are measures of the capacity of the virus to replicate in vivo. In rhesus macaques acutely infected with SIV by i.v. inoculation, viruses which produce plasma antigenemia and viremia, high viral RNA levels in plasma, and high cell-associated viral loads have a relatively high in vivo replication capacity compared to that of viruses which do not produce plasma antigenemia and viremia and produce relatively low levels of viral RNA in plasma and low cell-associated viral loads. Of the SIV stocks used in this study, SIVmac239 consistently produced plasma antigenemia and viremia and high levels of viral RNA in plasma and cell-associated virus in i.v.-inoculated animals. Thus, SIVmac239 has a relatively high in vivo replicative capacity, and SIVmac239 is efficiently transmitted by vaginal inoculation. SIVmac239/1A11/239 produced plasma antigenemia and viremia in one of two i.v.-inoculated macaques and moderate levels of viral RNA in plasma and cell-associated virus in both i.v.-inoculated animals. SIVmac239/1A11/239 has a moderate in vivo replicative capacity, and it is efficiently transmitted by vaginal inoculation. SIVmac1A11 did not produce plasma viremia and produced only low levels of viral RNA in plasma and cell-associated virus in i.v.-inoculated macaques. SIVmac1A11 has a relatively low in vivo replicative capacity and is not transmitted efficiently by vaginal inoculation. SIVmac251 produces plasma antigenemia (12) and high levels of viral RNA in plasma and cell-associated virus after i.v. inoculation of rhesus macaques (data not shown). SIVmac251 has a high in vivo replicative capacity and is transmitted efficiently by vaginal inoculation. Rhesus macaques inoculated i.v. with SHIV 89.6 have higher levels of antigenemia and increased numbers of in situ hybridization-positive cells in lymph nodes than macaques inoculated i.v. with SHIV HXBc2 (37). Thus, SHIV 89.6 has an increased ability to replicate in rhesus macaques compared to that of SHIV HXBc2, and SHIV 89.6 initiates systemic infection in rhesus macaques after multiple vaginal inoculations, whereas SHIV HXBc2 does not. SHIV 89.6-PD produces high levels of plasma antigenemia following i.v. inoculation of rhesus macaques. SHIV 89.6-PD has a high in vivo replication capacity and can be efficiently transmitted to rhesus macaques by a single vaginal inoculation. In summary, of the five virus clones and two uncloned virus stocks tested, the five viruses (SIVmac239, SIVmac251, SIVmac239/1A11/239, SHIV 89.6, and SHIV 89.6-PD) which have moderate to high in vivo replication capacities can be transmitted to rhesus macaques by vaginal inoculation, whereas the two viruses (SIVmac1A11 and SHIV HXBc2) that have relatively low in vivo replication capacities are not efficiently transmitted to rhesus macaques by vaginal inoculation. Thus, the in vivo replicative capacities of the SHIV and SIV clones or isolates used in these studies predicted the ability of each virus to produce systemic infection after intravaginal inoculation. These results are consistent with a recently published study of heterosexual HIV transmission cases which demonstrated that men who transmitted HIV to their female partners had higher levels of viral RNA in plasma than men who did not transmit the virus to their partners (8).

These results demonstrate that SIV and SHIV variants can be transmitted across the vaginal mucosa regardless of whether they are able to replicate in rhesus macrophages in vitro. Because viruses which replicate well in vivo are consistently transmitted by vaginal inoculation, it is likely that the major virus variant in the plasma of an HIV-1-infected individual at the time of a sexual contact will be transmitted to a sexual partner. Numerous studies have demonstrated that individuals acutely infected with HIV-1 have virus variants which can grow in primary macrophages in vitro but not in T-cell lines and that these variants do not cause syncitia in MT-2 cells, defined as a macrophage-tropic, NSI phenotype. It has been widely presumed that viral variants with a macrophage-tropic, NSI phenotype are selectively transmitted by sexual contact. However, 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-phase 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 (7). In that 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 dualtropic. Furthermore, studies of 10 pairs of individuals con-

sisting of the index case and the seroconverting sexual partner showed that, when the viral phenotypes in the two individuals forming a transmission pair were compared, the phenotypes of the HIV-1 were the same in both individuals in 9 of the 10 transmission pairs. Moreover, both of the individuals in five of the pairs were infected with a syncytium-inducing (SI) variant. Thus, the study found that there was no selection for macrophage-tropic NSI viruses during sexual transmission (7). As the authors of that paper (7) 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 (4, 31, 39, 49, 51). Thus, in approximately half of the published cases of suspected sexual HIV transmission, the seroconverting partner became infected with a T-cell-tropic SI HIV-1 variant.

The preceding discussion has focused on the categorization of viral variants based on their in vivo or in vitro phenotype, but viral variants can also be defined as genotypic variants on the basis of their nucleotide sequence. Studies of acute-phase HIV-1 seroconverters have demonstrated that a limited number of viral genotypes, as characterized by the nucleotide sequence in specific regions of the envelope gene, are found in humans recently infected with HIV-1. Most of these studies have concluded that, on the basis of the envelope gene sequence, there is selection for a limited number of genotypes in acute HIV infection (48, 50, 51). However, this restriction on genotype does not extend to all regions of HIV-1 *env* or *gag* (1, 50, 51). In one study, it was shown that two individuals infected by heterosexual contact had provirus variants with a gp120 sequence that was found in only a minor fraction of the proviral population in the blood of the transmitter (51). A more recent study by the same group characterized proviral 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 sequences of the envelope V1-V2 region in the recipient were found in only a minor variant of the donor (52). It is also not clear that this restriction on the viral genotype in acute infection is related to the route of HIV transmission. A study involving a relatively large number of acute-phase seroconverters found that when the nucleotide sequences of viruses in each individual were compared, there was little sequence heterogeneity in two normally hypervariable regions of *env* (50). However, this result was not limited to individuals that had been infected by sexual contact but was also found in individuals infected parenterally (50). A similar result has been reported for HIV-1-infected neonates (48). Thus, selection of particular genotypic variants is observed regardless of the route of HIV-1 transmission. This suggests that only a limited number of the viral genetic variants in a donor have the replicative fitness to initiate an infection in a naive recipient, regardless of the route of transmission.

We have shown that not all of the SIV or SHIV genotypes that produce systemic infection following i.v. inoculation are capable of producing systemic infection by intravaginal inoculation. SHIV HXBc2 and SHIV 89.6 are chimeras between SIVmac239 and HIV-1 molecular clones (14, 16, 37). Both SHIV reliably produce infection in i.v. inoculated macaques (37). As summarized in Table 3, it has previously been reported (16) that four intravaginal inoculations of SHIV HXBc2 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 (16). One experiment in this study demonstrated that after intravaginal inoculation of animals with a mixed inoculum containing both viruses, only the SHIV 89.6 genome could be detected in the PBMC of the animals. The only difference in the genotypes of these two viruses is that *env* is derived from different parental HIV-1 clones. Thus, this is clear evidence that in the SHIV-rhesus macaque system, the *env* gene influences the ability of a virus to produce systemic infection after intravaginal inoculation. Use of three molecular clones of SIV to assess transmission of viral variants during vaginal inoculation yielded similar results. i.v. inoculation of SIVmac239, SIVmac1A11, and SIVmac239/1A11/239 reliably results in infection of rhesus macaques (20). However, while animals inoculated intravaginally with SIVmac239 and SIVmac239/1A11/239 reliably become infected after a single intravaginal inoculation, animals inoculated intravaginally with SIVmac1A11 only rarely become infected. The only difference in the genotypes of SIVmac1A11 and SIVmac239/1A11/239 is that the latter contains the LTRs, a small portion of the 3′ end of *gag*, gp41, *nef*, and the second exon of *rev* from SIVmac239 (20). Thus, the nucleotide sequences of *nef*, the gp41 gene, and/or the LTR can influence the ability of a virus to produce systemic infection after intravaginal inoculation. These results clearly demonstrate that the genotypic determinants that permit SIV or SHIV to produce systemic infection in rhesus macaques differ, depending on the route of virus inoculation. The findings in the SHIV and SIV studies summarized here are a clear demonstration that there is selection, or exclusion, of specific lentivirus genotypes during vaginal transmission.

When all the published data is reviewed, it seems clear that there is no restriction on the sexual transmission of HIV-1 variants which can replicate in vitro in T-cell lines but not in macrophages. The results of the in vivo SIV and SHIV studies described here support that conclusion. It is likely that viral determinants of vaginal transmission are multifactorial and include in vivo cell tropism and in vivo replicative capacity. A recent report suggests that the HIV-1 strains that are associated with the heterosexually transmitted epidemics in southeast Asia and Africa replicate relatively efficiently in Langerhans cells in vitro (40). To date, we have been unable to establish an in vitro infection assay using rhesus macaque Langerhans cells, and we do not know if any of the SIV clones used in this study can replicate in Langerhans cells in vitro. It has been shown that rhesus macaques chronically infected with SIVmac251 do have infected cells in the vaginal epithelium and that these cells are likely Langerhans cells (28). Understanding of the types of viruses which cross the vaginal mucosa and initiate systemic infection is important for development of vaccines to prevent sexual transmission of HIV. The results presented here suggest that vaccine development efforts should not be limited to preventing transmission of macrophage-tropic HIV variants but rather should be focused on preventing transmission of HIV variants with a high in vivo replicative capacity.

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REFERENCES

1. **Albert, J., J. Walhberg, T. Leitner, D. Escanilla, and M. Uhlen.** 1994. Analysis of a rape case by direct sequencing of the human immunodeficiency virus type 1 *pol* and *gag* genes. J. Virol. **68:**5918–5924.
2. **Banapour, B., M. Marthas, R. Munn, and P. Luciw.** 1991. *In vitro* macrophage tropism of pathogenic and nonpathogenic molecular clones of simian

immunodeficiency virus (SIVmac). Virology **183:**12–19.
3. **Banapour, B., M. Marthas, R. Ramos, B. Lohman, R. Unger, M. Gardner, N. Pedersen, and P. Luciw.** 1991. Identification of viral determinants of macrophage tropism for simian immunodeficiency virus (SIVmac). J. Virol. **65:** 5798–5805.
4. **Clark, S. J., M. S. Saag, W. D. Decker, S. Campbell-Hill, J. L. Roberson, P. J. Velderkamp, J. C. Kappes, B. H. Hahn, and G. M. Shaw.** 1991. High titers of cytopathic virus in the plasma of patients with symptomatic primary HIV-1 infection. N. Engl. J. Med. **324:**954–960.
5. **Collman, R., J. W. Balliet, S. A. Gregory, H. Friedman, D. L. Kolson, N. Nathanson, and A. Srinivasan.** 1992. An infectious molecular clone of an unusual macrophage-tropic and highly cytopathic strain of human immunodeficiency virus type 1. J. Virol. **66:**7517–7521.

5a. **Committee on Care and Use of Laboratory Animals.** 1996. Guide for the care and use of laboratory animals. Institute of Laboratory Resources, National Resource Council, National Academy Press, Washington, D.C.

6. **Dailey, P. J., M. Zamroud, R. Kelso, J. Kolberg, and M. Urdea.** 1995. Quantitation of simian immunodeficiency virus (SIV) RNA in plasma of acute and chronically infected rhesus macaques using a branched DNA (bDNA) signal amplification assay, abstr. 99, p. 180. *In* Abstracts of the 13th Annual Symposium on Nonhuman Primate Models of AIDS.
7. **Fiore, J. R., A. Bjorndal, K. A. Peipke, M. Di Stefano, G. Angarano, P. Giuseppe, H. Gaines, E. M. Fenyo, and J. Albert.** 1994. The biologic phenotype of HIV-1 is usually retained during and after sexual transmission. Virology **204:**297–303.
8. **Fiore, J. R., Y.-J. Zhang, A. Bjondal, M. DiStefano, G. Angarano, G. Pastore, and E. M. Fenyo.** 1997. Biological correlates of HIV-1 heterosexual transmission. AIDS **11:**1089–1094.
9. **Hoxie, J. A., B. S. Haggarty, S. E. Bonser, J. L. Rackowski, H. Shan, and P. J. Kanki.** 1988. Biological characterization of a simian immunodeficiency virus-like retrovirus (HTLV-IV): evidence for CD4-associated molecules required for infection. J. Virol. **62:**2557–2568.
10. **Kestler, H., T. Kodama, D. Ringler, M. Marthas, N. Pedersen, A. Lackner, D. Regier, P. Sehgal, M. Daniel, N. King, and R. Desrosiers.** 1990. Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. Science **248:**1109–1112.
11. **Kestler, H., D. Ringler, K. Mori, D. Panicali, P. Sehgal, M. Daniel, and R. Desrosiers.** 1991. Importance of the *nef* gene for maintenance of high virus loads and for development of AIDS. Cell **65:**651–662.
12. **Lackner, A. A., P. Vogel, R. A. Ramos, J. D. Kluge, and M. Marthas.** 1994. Early events in tissues during infection with pathogenic (SIVmac239) and nonpathogenic (SIVmac1A11) molecular clones of simian immunodeficiency virus. Am. J. Pathol. **145:**428–439.
13. **Li, J., M. Halloran, C. Lord, A. Watson, J. Ranchalis, M. Fung, N. Letvin, and J. Sodroski.** 1995. Persistent infection of macaques with simian-human immunodeficiency viruses. J. Virol. **69:**7061–7071.
14. **Li, J., C. I. Lord, W. Haseltine, N. L. Letvin, and J. Sodroski.** 1992. Infection of cynomologous monkeys with a chimeric HIV-1/SIVmac virus expressing the HIV-1 envelope glycoproteins. J. Acquired Immune Defic. Syndr. **5:** 639–646.
15. **Lohman, B., J. Higgins, M. Marthas, P. Marx, and N. Pedersen.** 1991. Development of simian immunodeficiency virus isolation, titration, and neutralization assays which use whole blood from rhesus monkeys and an antigen capture enzyme-linked immunosorbent assay. J. Clin. Microbiol. **29:** 2187–2192.
16. **Lu, Y., P. B. Brosio, M. Lafaile, J. Li, R. G. Collman, J. Sodroski, and C. J. Miller.** 1996. Vaginal transmission of chimeric simian/human immunodeficiency viruses in rhesus macaques. J. Virol. **70:**3045–3050.
17. **Mandel, C. P., N. C. Jain, C. J. Miller, and S. Dandekar.** 1995. Bone marrow macrophages are an early cellular target of pathogenic and nonpathogenic isolates of simian immunodeficiency virus (SIVmac) in rhesus macaques. Lab. Invest. **72:**323–333.
18. **Marthas, M. L., B. Banapour, S. Sutjipto, M. E. Siegel, P. A. Marx, M. B. Gardner, N. C. Pedersen, and P. A. Luciw.** 1989. Rhesus macaques inoculated with molecularly cloned simian immunodeficiency virus. J. Med. Primatol. **18:**311–319.
19. **Marthas, M. L., C. J. Miller, S. Sutjipto, J. Higgins, J. Torten, B. L. Lohman, R. E. Unger, H. Kiyono, J. R. McGhee, P. A. Marx, and N. C. Pedersen.** 1992. Efficacy of live-attenuated and whole-inactivated simian immunodeficiency virus vaccines against vaginal challenge with virulent SIV. J. Med. Primatol. **21:**99–107.
20. **Marthas, M. L., R. A. Ramos, B. L. Lohman, K. K. A. Van Rompay, R. E. Unger, C. J. Miller, B. Banapour, N. C. Pedersen, and P. A. Luciw.** 1993. Viral determinants of simian immunodeficiency (SIV) virulence in rhesus macaques assessed by using attenuated and pathogenic molecular clones of SIVmac. J. Virol. **67:**6047–6055.
21. **Miller, C. J.** 1994. Mucosal transmission of SIV. Curr. Top. Microbiol. Immunol. **188:**107–122.
22. **Miller, C. J., N. J. Alexander, P. Vogel, J. Anderson, and P. A. Marx.** 1992. Mechanism of genital transmission of SIV: a hypothesis based on transmission studies and the location of SIV in the genital tract of chronically infected female rhesus macaques. J. Med. Primatol. **21:**64–68.
23. **Miller, C. J., N. J. Alexander, S. Sutjipto, A. A. Lackner, A. G. Hendrickx, A. Gettie, L. J. Lowenstine, M. Jennings, and P. A. Marx.** 1989. Genital mucosal transmission of simian immunodeficiency virus: animal model for heterosexual transmission of human immunodeficiency virus. J. Virol. **63:**4277–4284.
24. **Miller, C. J., M. Marthas, J. Torten, N. J. Alexander, J. P. Moore, G. F. Doncel, and A. G. Hendrickx.** 1994. Intravaginal inoculation of rhesus macaques with cell-free simian immunodeficiency virus results in persistent or transient viremia. J. Virol. **68:**6391–6400.
25. **Miller, C. J., M. McChesney, and P. F. Moore.** 1992. Langerhans cells, macrophages and lymphocyte subsets in the cervix and vagina of rhesus macaques. Lab. Invest. **67:**628–634.
26. **Miller, C. J., M. B. McChesney, X. S. Lü, P. J. Dailey, C. Chutkowski, D. Lu, P. Brosio, B. Roberts, and Y. Lu.** 1997. Rhesus macaques previously infected with simian/human immunodeficiency virus are protected from vaginal challenge with pathogenic SIVmac239. J. Virol. **71:**1911–1921.
27. **Miller, C. J., J. R. McGhee, and M. B. Gardner.** 1992. Mucosal immunity, HIV transmission and AIDS. Lab. Invest. **68:**129–145.
28. **Miller, C. J., P. Vogel, N. J. Alexander, S. Sutjipto, A. G. Hendrickx, and P. A. Marx.** 1992. Localization of SIV in the genital tract of chronically infected female rhesus macaques. Am. J. Pathol. **141:**655–660.
29. **Mori, K., D. J. Ringler, T. Kodama, and R. C. Desrosiers.** 1992. Complex determinants of macrophage tropism in *env* of simian immunodeficiency virus. J. Virol. **66:**2067–2075.
30. **Naidu, Y. M., H. W. Kestler, Y. Li, C. V. Butler, D. P. Silva, D. K. Schmidt, C. D. Troup, P. K. Sehgal, P. Sonigo, and M. D. Daniel.** 1988. Characterization of infectious molecular clones of simian immunodeficiency virus (SIVmac) and human immunodeficiency virus type 2: persistent infection of rhesus monkeys with molecularly cloned SIVmac. J. Virol. **62:**4691–4696.
31. **Nielsen, C., C. Pedersen, J. D. Lundgren, and J. Gerstoft.** 1993. Biological properties of HIV isolates in primary infection: consequences for subsequent course of infection. AIDS **7:**1035–1040.
32. **Pachl, C., J. A. Todd, D. G. Kern, P. J. Sheridan, S.-J. Fong, M. Stempien, B. Hoo, D. Besemer, T. Yeghiazarian, B. Irvine, J. Kolberg, R. Kokka, P. Neuwald, and M. S. Urdea.** 1995. Rapid and precise quantification of HIV-1 RNA in plasma using a branched DNA signal amplification assay. J. Acquired Immune Defic. Syndr. Hum. Retrovirol. **8:**446–454.
33. **Perno, C.-F., R. Yarchoan, D. A. Cooney, N. R. Hartman, D. S. A. Webb, Z. Hao, H. Mitsuya, D. G. Johns, and S. Broder.** 1989. Replication of human immunodeficiency virus in monocytes. Granulocyte/macrophage colony-stimulating factor (GM-CSF) potentiates viral production yet enhances the antiviral effect mediated by 3′-azido-2′,3′-dioxythymidine (AZT) and other dideoxynucleoside congeners of thymidine. J. Exp. Med. **169:**933–951.
34. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27:**493–497.
35. **Regier, D. A., and R. C. Desrosiers.** 1990. The complete nucleotide sequence of a pathogenic molecular clone of simian immunodeficiency virus. AIDS Res. Hum. Retroviruses **6:**1221–1231.
36. **Reimann, K. A., J. T. Li, R. Veazey, M. Halloran, I. W. Park, G. B. Karlsson, J. Sodroski, and N. L. Letvin.** 1996. 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. **70:**6922–6928.
37. **Reimann, K. A., J. T. Li, G. Voss, C. Lekutis, K. Tenner-Racz, P. Racz, W. Lin, D. C. Montefiori, D. E. Lee-Parritz, R. G. Collman, J. Sodroski, and N. L. Letvin.** 1996. An *env* gene derived from a primary human immunodeficiency virus type 1 isolate confers high in vivo replicative capacity to a chimeric simian/human immunodeficiency virus in rhesus monkeys. J. Virol. **70:**3198–3206.
38. **Roncalli, M., M. Sideri, P. Gie, and E. Servida.** 1988. Immunophenotypic analysis of the transformation zone of human cervix. Lab. Invest. **58:**141–149.
39. **Roos, M. T., J. M. Lange, R. de Goede, R. A. Coutinho, P. T. Schellekens, F. Miedema, and M. Tersmette.** 1992. Viral phenotype and immune response in primary human immunodeficiency virus type 1 infection. J. Infect. Dis. **165:** 427–432.
40. **Soto-Ramirez, L. E., B. Renjifo, M. F. McLane, R. Marlink, C. O'Hara, R. Sutthent, C. Wasi, P. Vithayasi, V. Vithayasi, C. Apichartpiyakul, P. Auewarakul, V. Pena Cruz, D.-S. Chui, R. Osathanondh, K. Mayer, T.-H. Lee, and M. Essex.** 1996. HIV-1 Langerhans cell tropism associated with heterosexual transmission of HIV. Science **271:**1291–1293.
41. **Spira, A. I., P. A. Marx, B. K. Patterson, J. Mahoney, R. A. Koup, S. M. Wolinsky, and D. D. Ho.** 1996. Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. J. Exp. Med. **183:**215–225.
42. **Staprans, S. I., B. C. Corliss, J. L. Guthrie, and M. B. Feinberg.** 1996. Quantitative methods to monitor viral load in simian immunodeficiency virus infections, p. 167–184. *In* K. W. Adolph (ed.), Viral genome methods. CRC Press, New York, N.Y.
43. **Stephens, E. B., H. M. McClure, and O. Narayan.** 1995. The proteins of lymphocyte- and macrophage-tropic strains of simian immunodeficiency virus are processed differently in macrophages. Virology **206:**535–544.
44. **Sutjipto, S., N. C. Pedersen, C. J. Miller, M. B. Gardner, C. V. Hanson, A. Gettie, M. Jennings, J. Higgins, and P. A. Marx.** 1990. Inactivated simian

immunodeficiency virus vaccine failed to protect rhesus macaques from intravenous or genital mucosal infection but delayed disease in intravenously exposed animals. J. Virol. **64:**2290–2297.

45. **Tyler, K. L., and B. N. Fields.** 1996. Pathogenesis of viral infections, p. 173–218. *In* B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), Fields virology, 3rd ed. Lippincott-Raven, Philadelphia, Pa.
46. **Unger, R. E., M. L. Marthas, A. A. Lackner, E. Pratt-Lowe, B. L. Lohman, K. Van Rompay, and P. A. Luciw.** 1992. Detection of simian immunodeficiency virus DNA in macrophages from infected rhesus macaques. J. Med. Primatol. **21:**74–81.
47. **Van Rompay, K. K. A., M. L. Marthas, R. A. Ramos, C. P. Mandell, E. K. McGowan, S. M. Joye, and N. C. Pedersen.** 1992. Simian immunodeficiency virus (SIV) infection of infant rhesus macaques as a model to test antiretroviral drug prophylaxis and therapy: oral 3′-azido-3′-deoxythymidine prevents SIV infection. Antimicrob. Agents Chemother. **36:**2381–2386.
48. **Wolinsky, S. M., C. M. Wike, B. T. Korber, C. Hutto, W. P. Parks, L. L. Rosenblum, K. J. Kunstman, M. R. Furtado, and J. L. Munoz.** 1992. Selective transmission of human immunodeficiency virus type-1 variants from mothers to infants. Science **255:**1134–1137.

48a. **Wyand, M., Y. Lu, J. Li, and J. Sodroski.** Unpublished observations.

49. **Yoshiyama, H., N. Kobayashi, T. Matsui, H. Nakashima, T. Kajii, K. Yamato, S. Kotani, I. Miyoshi, and N. Yamamoto.** 1987. Transmission and genetic shift of human immunodeficiency virus (HIV) in vivo. Mol. Biol. Med. **4:**385–396.
50. **Zhang, L. Q., P. MacKenzie, A. Cleland, E. C. Holmes, A. J. Leigh Brown, and P. Simmonds.** 1993. Selection for specific sequences in the external envelope protein of human immunodeficiency virus type 1 upon primary infection. J. Virol. **67:**3345–3356.
51. **Zhu, T., H. Mo, N. Wang, D. S. Nam, Y. Cao, R. A. Koup, and D. D. Ho.** 1993. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science **261:**1179–1181.
52. **Zhu, T., N. Wang, A. Carr, D. S. Nam, R. Moor-Jankowski, D. A. Cooper, and D. D. Ho.** 1996. Genetic characterization of human immunodeficiency virus type 1 in blood and genital secretions: evidence for virus compartmentalization and selection during sexual transmission. J. Virol. **70:**3098–3107.

ERRATA

In Vivo Replication Capacity Rather Than In Vitro Macrophage Tropism Predicts Efficiency of Vaginal Transmission of Simian Immunodeficiency Virus or Simian/Human Immunodeficiency Virus in Rhesus Macaques

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Volume 72, no. 4, p. 3252, Fig. 2B: The *y* axis numbers "0, 20, 40, and 60" should read "0, 2.0×10^4, 4.0×10^4, and 6.0×10^4;" in Fig. 2C, the *y* axis numbers "0.1, 1, 10, 10^2, 10^3, 10^4, 10^5, 10^6, and 10^7" should read "0, 0.1, 1, 10, 10^2, 10^3, 10^4, 10^5, and 10^6."

Page 3254, Table 3: In the reference column, "23, 23, 23, 15, 25, 12, 12, 12, 12" should read as "24, 24, 24, 19, 26, 16, 16, 16, 16." In footnote *c*, last sentence, "23" should read as "24."

On Viruses, Sex, and Motherhood

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Volume 71, no. 2, p. 859: The author's name should appear as shown above.