Infection of Macaques after Vaginal Exposure to Cell-Associated Simian Immunodeficiency Virus

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(See the editorial commentary by Anderson, on pages 333–336.)

Background. The contribution of infected semen cells to sexual transmission of human immunodeficiency virus (HIV) is still debated. We addressed this issue in the model of experimental infection of macaques with simian immunodeficiency virus (SIV).

Methods. Frozen stocks of cells obtained from the spleen of macaques at the peak of SIVmac251 viremia were prepared. After being thawed and washed, cells were deposited at different concentrations in the vaginas of adult macaques treated with medroxyprogesterone acetate (Depo-Provera). To unravel mechanisms of infection, stock cells labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) were inoculated intravaginally. Follow-up testing of samples from the mucosa and different lymphoid tissues obtained 21 and 45 h later was performed by flow cytometry, immunohistochemical analysis, and in situ hybridization.

Results. Systemic and persistent infection was achieved after vaginal exposure of macaques to SIV-infected cells. The dose needed to infect 50% of females was $6.69 \times 10^3 \pm 0.08 \times 10^5$ viral DNA copies. At days 1 and 2 after exposure to cell-associated SIV labeled with CFSE, SIV-positive cells were detected in proximal and distal lymphoid tissues.

Conclusions. Infection with SIV after exposure of vaginal and cervical mucosa to cell-associated virus represents a new mechanism of sexual transmission of HIV and SIV that may have significant impacts in the development of preventive approaches like microbicides.

The development of strategies for the control of the human immunodeficiency virus (HIV) epidemic requires an improved understanding of the mechanism underlying sexual transmission of the virus [1]. Studies of AIDS performed in vitro with reconstituted mucosal barriers or in nonhuman primate models highlight the diversity of mechanisms by which HIV and simian immunodeficiency virus (SIV) virions may cross the cervico-vaginal or rectal mucosa and disseminate into the host to establish persistent infection [2]. Many studies have emphasized the role played by infectious viral particles in contaminating fluids. However, genital fluids, especially semen, may also contain significant amounts of infected cells that may contribute to transmission of HIV infection. Semen of untreated patients contains high levels of cell-free HIV particles and significant amounts of HIV-infected cells [3]. HIV DNA is present in the leukocyte cell fractions of semen in 4%–65% of HIV-infected patients, at levels of $10^{-80,000}$ viral DNA copies/mL [3–5], which may represent high numbers of infected cells assuming that infected cells contain few [1–3] copies of integrated DNA. In addition, leukocytes in semen may also actively replicate the virus because intracellular HIV RNA (27,000–70,000 copies/mL) could be detected in significant amounts [4]. Provirus load may significantly increase during inflammation of the genital tract [5] in individuals with ure-
Stocks K833 and X801 contained 10^7 spleen cells per ampoule. The cell stocks were then frozen in 10% dimethyl sulfoxide (DMSO).

Twenty-five Cynomolgus macaques (K833 and X801) at day 12 after infection with SIVmac251 using Ficoll, from the spleen of 2 adult female cynomolgus macaques (*Macaca fascicularis*), each weighing between 4 and 5 kg and imported from Mauritius, were used for animal care (Directive 86/609/EEC). Animals were anesthetized and handled in accordance with European guidelines for animal care (Directive 86/609/EEC), and protocols were approved by the Ethical Committee “Ile-De-France SUD,” as requested by our Institution (CEA).

**Animals and cell-associated virus stocks.** Fourteen adult female cynomolgus macaques (*Macaca fascicularis*), each weighing between 4 and 5 kg and imported from Mauritius, were maintained and handled in accordance with European guidelines for animal care (Directive 86/609/EEC). Animals were anesthetized with 10 mg/kg of ketamin (Imalgene). Cells were isolated, using Ficoll, from the spleen of 2 adult female cynomolgus macaques (K833 and X801) at day 12 after infection with SIVmac251 strain (provided by A.-M. Aubertin, Strasbourg, France). Spleen cell stocks were then frozen in 10% dimethyl sulfoxide (DMSO).

Stocks K833 and X801 contained 10^7 spleen cells per ampoule. Cynomolgus macaque females were treated with 30 mg/kg of medroxyprogesterone acetate (Depo-Provera) and then inoculated 30 days later by the vaginal route. Stock cells were thawed 1 h before challenge and washed twice before being suspended in 1 mL Roswell Park Memorial Institute 1640 medium (RPMI 1640) for administration to macaques. Before inoculation, the lower female reproductive tract was inspected for signs of pre-existing inflammation. A lubricated nasogastric tube was used (Centrevat) for the inoculation of cells in the vaginal vault. Animals were then kept in prone position for 5 min. Twelve animals were followed up over 6 months after inoculation with infected cells; 2 macaques were killed at days 1 and 2 after inoculation.

**Carboxyfluorescein succinimidyl ester labeling of stock cells.** Spleen cells from stock K833 were labeled with 5 μmol/L of carboxyfluorescein diacetate succinimidyl ester (CFSE), using the CellTrace TM CFSE Proliferation Kit (C34554 Molecular probes; Invitrogen) as described elsewhere [3].

**Plasma viral load.** Viral RNA was prepared from 200 μL of cell-free plasma or supernatants, using the HighPure viral RNA kit (Roche Diagnostics) and used for quantification by reverse-transcription polymerase chain reaction (RT-PCR) as we previously described [10, 11].

**Phenotype of T cells and monocyte/macrophages.** Cell-associated virus stocks were immunostained with anti-CD3 Alexa 700 (clone SP34; BD Biosciences), anti-CD4 PerCP (clone L200; BD Biosciences), anti-CD8 fluorescein isothiocyanate (FITC) (clone DK25; Dako), anti-CD95 allophycocyanin (APC) (clone DX2; BD Pharmingen), anti-CD28 PE (clone CD28.2; BD Pharmingen), anti-CD20 PC5 (clone B9E9; Beckman Coulter), or anti-CD14 PE (clone M5E2; BD Pharmingen) and anti-CD11b FITC (clone Bear1; Immunotech). All samples were incubated with antibodies for 30 min at room temperature. Red blood cells were then lysed with 600 μL of fluorescence-activated cell sorting (FACS) lysing solution (BD Biosciences). Stained cells were washed in phosphate-buffered saline (PBS) and fixed (Cell-Fix; BD Biosciences). Data were acquired on a LSRII instrument (BD Biosciences) using Diva software (BD Biosciences). Proportions of CD3^+CD4^+ and CD3^+CD8^+ cells were determined for the lymphocyte gate, defined in terms of light-scattering properties, using Diva software. Absolute counts were calculated from the absolute blood count of lymphocytes, obtained by automated cell counting (Coulter MDII; Coultronics). Naive, central memory, and effector memory T cells were defined as CD28^−CD95^−, CD28^+CD95^−, and CD28^+CD95^+, respectively, as we previously described [4]. Lymph nodes from females challenged with CFSE-labeled cells were mechanically disrupted and passed through a strainer with 40-μm pores (BD Biosciences). Cells were then washed in PBS. We characterized CFSE-labeled populations from blood, fresh tissues, and cervico-vaginal washes, using anti-CD3 Alexa700 (clone SP34-3; BD Biosciences), anti-CD4 PerCP (clone L200; BD Biosciences) and CD8 APC (clone SK1; BD Biosciences), with the appropriate mouse IgG1 isotype control (all BD Biosciences).

**Cell sorting.** Lymphocyte subsets and CD14^+ monocytes were sorted from splenocytes on a FACS Aria (BD Biosciences). The following antibodies were used: CD3 Alexa Fluor 700 (clone SP34-2; BD Biosciences), CD4 PerCP (clone L200; BD Biosciences), CD8 FITC (clone DK25; Dako), CD28 PE (clone 28.2; BD Biosciences), CD95 APC (clone DX2; BD Biosciences) and CD14 PE (clone M5E2; BD Biosciences). CD4^+CD28^−CD95^− cells were considered naive T cells; CD4^+CD28^+CD95^+ cells, central memory cells; and CD4^+CD28^−CD95^+ cells, effector memory cells, as we previously described [4]. Stained cells were washed twice in PBS and were analyzed by simultaneous 4-way sorting on FACS ARIA.
The purity of isolated cells was analyzed by flow cytometry. FlowJo software (TreeStar) was used for data analysis.

**Nucleic acid extraction.** We collected 10,000 cells in each cell subpopulation directly after sorting in lysis buffer RA1 from the NucleoSpin RNAXS kit (Machery-Nagel). Purified cell lysates from each subpopulation were divided into 2 parts (5000 lysate cells per part), for either RNA extraction using NucleoSpin RNAXS kit or DNA extraction using NucleoSpin Tissue XS kit (Machery-Nagel). All extractions were performed according to the manufacturer’s instructions. RNA or DNA was eluted in 40 μL of nuclease-free water and frozen immediately at −80°C until analysis.

**Quantification of cellular viral messenger RNA.** Cellular RNA was analyzed in duplicate by real-time PCR Taqman. SIVmac251 virus, titrated by the branched-chain DNA assay and diluted in 5000 cells from uninfected macaques, was used to generate a standard curve (serial 10-fold dilutions). Standards and cellular RNA samples were extracted and tested in parallel under the same conditions. One-tube RT-PCR was performed in an iCycler real-time thermocycler (Bio-Rad) using the SuperScript III Platinum One-Step QRT-PCR System kit (Invitrogen) with SIV-gag primers and probe, as described elsewhere [5]. The probe comprised a 743 bp SIVmac251 gag complementary DNA sequence, ligated into pCR4-TOPO (Invitrogen) plasmid and purified with the HiSpeed Maxiprep Kit (Invitrogen), was used as standard dilute in 5000 cells from uninfected macaques (serial 10-fold dilutions). One-tube RT-PCR was performed in an iCycler real-time thermocycler (Bio-Rad) using the SuperScript III Platinum One-Step QRT-PCR System kit (Invitrogen) with SIV-gag primers and probe, as described elsewhere [4, 5].

**Quantification of total viral DNA.** DNA extracted from cells was analyzed in duplicate by real-time PCR Taqman assay using the Platinum qPCR SuperMix-UDG kit (Invitrogen) with SIV-gag primers and probe, as described elsewhere [5]. The SIVmac251 gag complementary DNA sequence, ligated into pCR4-TOPO (Invitrogen) plasmid and purified with the HiSpeed Maxiprep Kit (Invitrogen), was used as standard dilute in 5000 cells from uninfected macaques (serial 10-fold dilutions). We obtained a quantification threshold of ≥300 copies per 10^6 cells and a detection threshold of ≥100 copies per 10^6 cells. The reaction, data acquisition, and analysis were performed with the iCycler real-time thermocycler (Bio-Rad).

**Normalization of viral DNA and viral messenger RNA.** Nucleic acids were extracted from 5000 stock cells. Thus, we verified cell numbers in each unknown sample. RNA or DNA from 5000 cells from uninfected macaques was serially diluted 1 in 10 (up to 10^-5). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was then amplified simultaneously using a primer set and probe, as described elsewhere [5]. GAPDH-RNA or GAPDH-DNA levels in unknown samples were inferred by comparing threshold cycle (Ct) value against a calibration curve. Unknown samples displayed levels of amplifiable complementary DNA or DNA equivalent to the levels obtained from 5000 cells. Absolute numbers of viral DNA or cellular viral RNA were normalized to 5000 cells. Results were expressed as SIV DNA or SIV RNA copy numbers per 10^6 cells.

**Identification of CFSE-labeled cells in fixed tissues.** Formalin-fixed tissues were embedded in paraffin, and 5 μm sections were obtained. All slides were stained using the Dako Autostainer (Dako). PBS with 0.05% Tween 20 (Dako) was used for all washings, and Primary Antibody Diluting Buffer (Dako) was used for all antibody dilutions. Primary antibodies included polyclonal antifluorescein rabbit antibody (Invitrogen), anti-CD3 (clone SP34, BD Biosciences), and anti-HLA-DR (clone CR3/43, Dako). Slides were incubated for 30 min with their respective primary antibody; then stained cells were visualized with anti-rabbit biotinylated goat immunoglobulin secondary antibody (Dako) using final substrate DAB (diamin-3,3’benzidine) (Ultratech, Immunotech) and haematoxylin as counterstain. For each tissue, 1 control section was stained with only HES (haematoxylin, eosin, safranin).

**In situ hybridization.** Cells expressing SIV RNA in the vagina and lymph nodes were identified by in situ hybridization in fixed tissue sections, as described elsewhere [4]. The gag probe comprised a 743 bp SIVmac251 gag complementary DNA fragment (Genbank accession number M19499, nucleotides 1386–2129) subcloned into pCR4-TOPO. The 35S-UTP-labeled riboprobes were synthesized by in vitro transcription of the linearized plasmid template, using T7 or SP6 RNA polymerase to generate the antisense or corresponding sense probe. The orientation of the inserted fragment was checked by sequencing. Radioactive in situ hybridization was performed as described elsewhere [5]. Slides were dipped into NTB emulsion and exposed in the dark for 10 days at 4°C. Slides were then developed, counterstained with Mayer’s hemalum solution, and covered with cover slips in Eukitt (CML). Slides were observed and photographed with an Axiosplan II and Axiosphot photomicroscope (Zeiss). The specificity of the hybridization signal was systematically checked by hybridizing sense probes on parallel sections.

**RESULTS**

**Transmission of infection to female macaques after single cervico-vaginal exposure to SIV-infected cells.** Many reports suggest that viruses produced in culture assays, including those using primary cells, may not necessarily reflect replicating populations in vivo because of specific in vivo constraints that shape the viral genome [12–15]. We therefore used 2 cell-associated stocks prepared with spleen cells obtained directly from 2 cynomolgus macaque (K833 and X801) infected with an SIVmac251 isolate. We previously confirmed this virus to be pathogenic in this species [10, 11]. We collected cells at peak of plasma viremia (day 12 after infection). The prepared spleen cell stock K833 consisted of 2% CD14+ monocyte/macrophages, 41% of CD20 cells, 47% of CD3+CD4+ CD8+ T cells, and 27% CD3+CD4+CD8+ T cells. Using differentiation markers we had previously reported [10], we found that most of CD4+ T cells were represented by central memory T cells (90% of CD28−CD95high), with smaller proportions of naive (7%;
infected population (2.17 × 10^5 viral DNA copies per 10^6 cells) and is also the most active in viral replication (7.92 × 10^5 viral messenger RNA copies per 10^6 cells). We observed much lower copies of viral DNA and messenger RNA in naïve T cells and effector memory cells. The monocyte/macrophage cell population displayed the lowest level of infection (5.37 × 10^3 viral DNA copies per 10^6 cells) but with a high level of viral replication (1.64 × 10^6 viral messenger RNA copies per 10^6 cells). Stock of cells obtained from macaque X801 have similar representation of total T lymphocytes (CD3^+; 44%) and monocyte/macrophages (CD14^+; 2%) but different proportion of B cells (CD20^+, 29%) and CD4^+ T cells; 15%; comprising 65% of central memory cells, 10% of effector memory cells, and 22% naïve T cells and 71% CD8^+ T cells). Lower numbers but similar relative representation of infected cells were observed when compared with those of stock K833 (Figure 1).

Nine adult females were treated with medroxyprogesterone acetate (30 mg) to thin the vaginal epithelial layer and facilitate transmission [16]. We inoculated these animals 30 days later with different concentrations of cells that we thawed, washed twice, and resuspended in culture medium (Table 1). The 3 females inoculated with 10^7 cells (B118, X946, and F234) of the K833 stock became persistently infected (median plasma viral load at peak of viremia and at set point were 6.34 × 10^8 ± 3.79 × 10^8 and 2.69 × 10^8 ± 4.2 × 10^7 copies/mL, respectively). We observed seroconversion (Table 1) and a progressive decrease of circulating CD4^+ T cells (data not shown) in all animals. We did not detect infection in females inoculated with lower numbers of infected cells (Table 1). Finally, to demonstrate that infection occurs through SIV associated with

Table 1. Viral Loads and Antibody Levels in Female Macaques Vaginally Exposed to Simian Immunodeficiency Virus (SIV) Stocks K833 and X801

<table>
<thead>
<tr>
<th>Type of exposure, animal</th>
<th>SIV stock</th>
<th>SIV DNA copies per 10^6 cells</th>
<th>Plasma viral load, vRNA copies/mL</th>
<th>Titer of anti-SIV antibodies in serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 14</td>
</tr>
<tr>
<td>Infected cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B118</td>
<td>K833</td>
<td>4.2 × 10^6</td>
<td>&lt;60</td>
<td>3 × 10^6</td>
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<td>K833</td>
<td>4.2 × 10^6</td>
<td>&lt;60</td>
<td>5 × 10^6</td>
</tr>
<tr>
<td>F234</td>
<td>K833</td>
<td>4.2 × 10^6</td>
<td>&lt;60</td>
<td>1 × 10^6</td>
</tr>
<tr>
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<td>K833</td>
<td>4.2 × 10^5</td>
<td>&lt;60</td>
<td>&lt;60</td>
</tr>
<tr>
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<td>K833</td>
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<td>&lt;60</td>
<td>&lt;60</td>
</tr>
<tr>
<td>V166</td>
<td>K833</td>
<td>4.2 × 10^5</td>
<td>&lt;60</td>
<td>&lt;60</td>
</tr>
<tr>
<td>V401</td>
<td>K833</td>
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<td>&lt;60</td>
<td>&lt;60</td>
</tr>
<tr>
<td>AF3986</td>
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<td>&lt;60</td>
<td>5 × 10^6</td>
</tr>
<tr>
<td>AF344</td>
<td>X801</td>
<td>4.0 × 10^5</td>
<td>&lt;60</td>
<td>&lt;60</td>
</tr>
<tr>
<td>Supernatant*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N802D</td>
<td>K833</td>
<td>3.5 × 10^5</td>
<td>&lt;60</td>
<td>&lt;60</td>
</tr>
</tbody>
</table>

NOTE. ND, not data; vRNA, viral RNA.
* Supernatant of the last washing of SIV-infected cells (see Materials and Methods).
* Copies per milliliter of supernatant.

Figure 1. Infection of spleen cell stocks used for vaginal inoculation of macaques. The number of viral DNA copies per million cells is represented on the x-axis, and the number of viral messenger RNA copies per million cells is represented on the Y axis. Naïve CD4^+ T cells (CD3^+CD4^+CD8^+CD95low; diamonds), central memory CD4^+ T cells (CD3^+CD4^+CD8^+CD95high; squares) and memory effector CD4^+ T cells (CD3^+CD4^+CD8^+CD95high; circles) were defined as described elsewhere [14]. CD14^+ monocytes/macrophages are represented by triangles. Cells from stocks K833 and X801 are represented by black and white symbols, respectively.

Cells from stocks K833 and X801 are represented by black and white symbols, respectively.
occulated cells and not through free viral particles contaminating the inoculum, we collected the supernatant of the last wash of the $10^7$ cells of K833 stock before inoculation of cells to macaques and used this supernatant (2 mL) to inoculate an additional naive animal by the vaginal route. This animal did not become infected, although we detected residual amounts of viral RNA ($3.51 \times 10^4$ copies/mL) in supernatant. This residual ma-

terial may not correspond to infectious viral particles because infection of the highly susceptible MT4 cell line could not be transmitted in vitro (data not shown). Moreover, if transmission of infection of the 3 females inoculated with K833 stock cells had occurred through free infectious particles, there was low chance ($P = .006$ using statistical analysis of sparse infection data method) [17] that animal N802D inoculated with the corresponding supernatant did not become infected.

**Number of SIV-infected cells needed to transmit infection.** Transmission of infection with cell-associated SIV was reproducible using the second viral stock obtained from a macaque (X801) at the peak of initial viremia. This second stock con-

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**Table 2. Supernatant Residual Infectious Titer**

This table is available in its entirety in the online version of the *Journal of Infectious Diseases.*

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**Figure 2.** Dissemination of carboxyfluorescein succinimidyl ester (CFSE)–labeled cells into the host after vaginal exposure. CFSE-positive cells were detected by flow cytometry at 21 h and 45 h after vaginal exposure to $10^7$ CFSE-labeled cells from stock K833. **A** and **B**, CFSE-positive cells in lymph nodes and peripheral blood mononuclear cells (PBMCs) and cervicovaginal wash fluid (CVW). **C**, CFSE-positive cells were gated on lymphocytes and monocytes at 21 h (gray) and 45 h (black) after exposure (acquisition of $4 \times 10^4$ to $1.4 \times 10^5$ events in CVW and $6.1 \times 10^4$ to $3.1 \times 10^5$ events in lymph nodes and PBMCs); negative controls are shown in white. **D**, Cytometry analysis of CFSE-labeled cells in macaque lymph nodes (LNs) and PBMCs 45 h after exposure; Cells of the right panel are gated on CD3$^+$ cells identified in left panel. **E**, Percentage of CFSE$^+$ cells accounted for CD3$^+$ cells at 21 h (gray) and 45 h (black) after inoculation in lymph nodes and PBMCs (no data for 21 h after exposure). Detection limit was assessed by diluting ex vivo CFSE labeled cells in normal lymph node cells.
Figure 3. Carboxyfluorescein succinimidyl ester (CFSE)–labeled cells and infected cells in tissues after vaginal exposure to simian immunodeficiency virus (SIV)–infected cells. A–C, CFSE-labeled cells revealed by immunohistochemical analysis (arrows) in macaque tissues (magnification, ×100 and ×1000) using rabbit antifluorescein antibody and anti-rabbit biotinylated goat secondary antibody (Dako) and DAB as final substrate. D–F, in situ hybridization using SIV complementary DNA probes in the same macaque tissues (magnification, ×100 and ×630). At 21 h after inoculation of 10⁷ CFSE-labeled SIV-infected cells from stock K833, most CFSE⁺ and SIV⁺ cells are present in the vagina (A and D) and proximal lymph nodes (LNs) (panels B and E). At 45 h after inoculation, CFSE⁺ cells have probably migrated from the vagina and are found in proximal and distal lymph nodes (C); SIV⁺ cells are also found in axillary lymph nodes at the same time point (F). Inset A, CFSE-labeled cell with lymphocyte morphology in the superficial submucosa of the vagina 21 h after infection. Inset B, CFSE-labeled cell with lymphocyte morphology at the entry of internal iliac lymph node 21 h after infection. Inset C, CFSE-labeled cell with lymphocyte morphology in the subcapsular sinus of the axillary lymph node 45 h after infection. Inset D, 2 SIV⁺ cells in the vaginal lamina propria 21 h after infection. Inset E, SIV⁺ cell in the internal iliac lymph node 21 h after infection. F, SIV⁺ cell in the axillary lymph node 45 h after infection.

Donor-infected cells may cross the mucosal barrier and rapidly disseminate to lymphoid tissues. With the aim of elucidating the mechanisms of transmission of SIVmac251 after cervico-vaginal exposure to infected cells, 2 macaques treated with medroxyprogesterone acetate were inoculated intravaginally with 10⁷ cells of the K833 stock that had previously been maintained lower numbers of infected cells (4.0 × 10⁴ viral DNA copies per 10⁶ cells) (Table 1). One of the 2 females (AF344 and AF386) subjected to vaginal exposure of 10⁷ spleen cells of X801 stock became persistently infected (Table 1). Assuming a similar transmission efficiency in these 2 experiments, and using the method of Spouge et al [17], the calculated dose needed to infect 50% of the females (animal infectious dose of 50% [AID₅₀]) is 6.69 × 10⁵ ± 2.08 × 10⁵ viral DNA copies. This is not significantly different from our experimental data (Table 1). In addition, this value did not differ significantly if we repeat calculation with macaques infected only with stock K833: 8.74 × 10⁵ ± 2.47 × 10⁵ (Table 2).

Table 3. Detection Sensitivity of CFSE⁺ Cells by Flow Cytometry

This table is available in its entirety in the online version of the Journal of Infectious Diseases.
labeled with CFSE. Animals were killed at 21 or 45 h after inoculation. Surprisingly, we observed that infected cells may rapidly disseminate in macaque tissues. Indeed, we identified CFSE+ cells using flow cytometry (Figures 2) as early as 21 h in the cervico-vagina tissue, internal iliac lymph node, or sacral lymph nodes (0.2% of total lymphocytes). We also found CFSE+ cells in distant lymphoid tissues, including inguinal and axillary lymph nodes (Figures 2 and 3). In axillary lymph nodes, 27.7% ± 2.0% of CFSE+ cells were CD3+ T cells, which may derive from the inoculated stock rather than from host cells that have taken up the tracer, as may be expected for phagocytic cells like macrophages (Table 3). At 45 h after exposure, CFSE+ cells could also be identified in peripheral blood mononuclear cells. More than 60% of these cells were CD3+ cells, of which 28% were CD3+CD4+ cells and 56% were CD3+CD8+ cells. We used immunohistochemical analysis to confirm the presence of CFSE+ cells in tissue sections where such cells appeared to consist mostly of lymphocytes, with some rare monocytes (Figure 3). Infected cells identified by in situ hybridization were found in the lamina propria of vaginal mucosa at 21 h after exposure, as well as in T-cell areas of distal lymph nodes at 21 and 45 h after exposure (Figure 3).

**DISCUSSION**

Here, we demonstrated in one of the most relevant animal models of HIV infection and AIDS, that exposure of intact mucosa of the vagina and cervix of macaques to cells infected with SIV results in transmission of infection. The stock of infected cells we used to model transmission appeared to mimic populations of HIV-infected cells present in the semen. In our previous studies, we found that both CD3+ T cells and macrophages actively produce viral messenger RNA in prostate and seminal glands of SIVmac251-infected macaques [9]. In human semen, T cells and macrophages have also been reported to represent the majority of infected cells [8]. Thus, the presence of these 2 infected populations in our viral stock is highly relevant for the model we are developing.

We estimated that we would need 6.69 × 10^5 ± 2.08 × 10^5 viral DNA copies to transmit infection to 50% of females macaques. This finding is compatible to current knowledge on natural HIV infection in humans and previous published studies of animal models [23]. Although highly variable, a significant number of leucocytes (from 3.0 × 10^4 up to 5.6 × 10^7 cells/mL; median, 1.0 × 10^5 cells/mL) are present in the semen of treatment naive infected patients (copy number of HIV DNA varying between 10,000 and 80,000 per mL) [18]. Considering the volume of a single ejaculate (2–3 mL), we may therefore assume that in our studies, an inoculum of 10^7 cells containing ~10,000–100,000 infected cells is compatible to what is reported in humans and may indicate that in the natural situation, sexual transmission of HIV through cell-associated virus is highly probable, although it may occur at low frequency. In addition, the dose we have evaluated for macaques is the one infecting 50% of the exposed animals, and by this estimation method, lowering 10 times this dose in macaques may result in transmission efficacy similar to that during male to female transmission in humans (0.3% to 0.003%).

Here, we propose new mechanisms for sexual transmission of HIV and SIV, including rapid spread of donor-infected cells in the host. Our observations suggest that infected cells present in semen of infected partners may efficiently cross the mucosal barrier and rapidly disseminate into the host. Additional studies are needed to fully elucidate the mechanisms involved. However, our results strongly suggest that T lymphocytes cross the mucosal barrier and very rapidly disseminate in host tissues, including distant lymphoid tissues, where they can transmit infection to resident cells. Similarly, donor lymphocytes and monocyte/macrophages migrating across the cervico-vaginal mucosa and to distal lymphoid tissues have been reported in mouse models of reproductive physiology and infection [19–22].

Previous attempts to transmit SIV infection after vaginal exposure to infected cells in macaques have either failed or required repeated inoculation with infected cells or treatment of the mucosa with compounds that result in significant erosion [23–25]. Our results clearly demonstrated for the first time that efficient transmission of SIVmac251 strain could be achieved through a single exposure of the intact mucosa. In our model, we have used medroxyprogesterone acetate to facilitate infection, which may mimic the luteal phase of the menstrual cycle, but certainly this does not represent the general situation of individuals exposed to HIV. In addition, medroxyprogesterone acetate treatment certainly exaggerates the effect of progesterin on the mucosa thickness. It was reported in animal models that estrogen, in contrast, reduces the transmission efficacy of SIV. Future work will be needed to address the influence of the menstrual cycle in transmission of infection after exposure of the cervix or vagina to cell-associated SIV.

Although the model we are developing may need additional improvement, our study highlights the need to consider the role played by HIV-infected cells in the semen in preventive approaches for HIV sexual transmission such as using microbicides.

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References


19. Ibata B, Parr EL, King NJ, Parr MB. Migration of foreign lymphocytes from the mouse vagina into the cervicovaginal mucosa and to the iliac lymph nodes. Biol Reprod 1997;56:537–543.


