JOURNAL OF VIROLOGY, Dec. 1995, p. 7982–7990 Vol. 69, No. 12
0022-538X/95/$04.00+0
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Genotypic Selection of Simian Immunodeficiency Virus in Macaque Infants Infected Transplacentally†

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Received 23 June 1995/Accepted 18 September 1995

To understand viral and host factors that contribute to transplacental transmission of human immunodeficiency virus, we developed an animal model using pregnant female macaques infected with simian immunodeficiency virus (SIV). Pregnant females were inoculated intravenously during midgestation with either a well-characterized primary isolate of SIV (SIV/DeltaB670) or a combination of SIV/DeltaB670 and the macrophage-tropic molecular clone SIV/17E-Fr. The viral genetic diversity in five infected female macaques and their in utero-infected infants was analyzed. All of the mothers harbored a genetically diverse virus population at parturition, whereas a single genotype from the maternal quasispecies was identified in the infants at birth. Only one of two variants was found in the infants: SIV/17E-Fr (two cases) or a genotype contained within the SIV/DeltaB670 quasispecies (three cases). The macrophage-tropic properties of both transmitted genotypes were suggested by productive replication in primary rhesus macrophage cultures in vitro and the clonal presence in central nervous system tissue of infected monkeys with encephalitis. These observations provide compelling evidence for both genotypic and phenotypic selection in transplacental transmission of SIV and suggest a critical role for macrophages in fetal infection in utero.

Perinatal transmission of human immunodeficiency virus (HIV) from an infected mother to a developing fetus accounts for most cases of pediatric AIDS. The prognosis for infected children is very poor, with a median survival time of 38 months (16). Transfer of the virus from the infected mother to the fetus is believed to occur transplacentally during pregnancy (prenatally), at the time of delivery through vaginal secretions (perinatally), or by breast feeding (postnatally). Understanding the mechanism(s) by which each type of transfer is effected is vital to the development of strategies directed at prevention.

The simian immunodeficiency virus (SIV)-macaque model has been instrumental in advancing our knowledge of the pathogenesis of HIV infection, the development of strategies effective in prevention and/or treatment of HIV infection, and more recently, perinatal transmission of HIV (2, 5, 6, 9, 11). SIV inoculation via the amniotic fluid or oral exposure immediately after birth has provided a model for the transmission of HIV late in gestation or at the time of delivery through skin or mucous membrane exposure (2, 6, 9). Transplacentally infected infants delivered by cesarean section from female pigtail macaques (*Macaca nemestrina*) inoculated during midgestation have also been identified at the Washington Primate Center (11). The transmitted viruses in that study, however, were not genetically analyzed.

Although our early attempts to develop a model for transplacental transmission of SIV in rhesus macaques (*Macaca mulatta*) using a limited number of animals were unsuccessful (5), we subsequently identified infected infants in a study utilizing larger numbers. This report describes the genetic analysis of paired isolates from five SIV-infected female macaque monkeys and their in utero-infected infants. Sequence analysis was performed on mother and infant envelope sequences amplified by PCR directly from lymphoid organs, peripheral blood mononuclear cells (PBMC), or virus from high-speed plasma pellets to identify the specific genotypes present in each animal. In three infants, the same variant found within the quasispecies of a primary virulent isolate used to infect the mothers was identified. This variant replicated well in rhesus macrophages in vitro and was identified in brain homogenates from animals with encephalitis. A well-characterized macrophage-tropic infectious molecular clone was identified in two additional infants. These studies demonstrate that like maternal-fetal transmission of HIV in humans, transplacental transmission of SIV involves a single variant found in the maternal quasispecies. The controlled conditions employed in the SIV-macaque model have further demonstrated that specific strains are preferred for this event.

MATERIALS AND METHODS

Animal manipulations. Female monkeys were subjected to timed mating with exogenous progesterone administration and withdrawal. Females received 10 daily injections of progesterone (0.5 mg/0.25 ml of corn oil, intramuscularly); 14 days after the last injection, they were placed with fertile males. The fertility of the males was judged on the basis of breeding history and semen analyses. Pairing sessions ranged from 5 to 7 days. The midpoint of the breeding session was taken as the estimated time of conception. Standard ultrasound examination of the females was utilized for early pregnancy determination. Subsequent fetal growth and development were monitored weekly thereafter with a real-time mechanical sector scanner (Siemens Sonoline ST-250) with a 7.5/6.0-MHz sector transducer. All routine manipulations were performed while monkeys were anesthetized with ketamine HCl (Parke-Davis, Morris Plains, N.J.).

Females were under 24-h surveillance, and vaginal deliveries were permitted in uncomplicated births. Vaginally delivered newborns were immediately removed from their mothers prior to nursing and hand reared. In cases of fetal distress (as

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† This work is dedicated to the memory of Elizabeth Glaser, whose profound courage made this study possible.

TABLE 1. Summary of macaque mother-infant pairs analyzed for viral genetic diversity

Mother-infant pair	Species	Virus inoculum (mother)	SIV-positive tissue(s) in infant	Time of birth (days p.i.)[a]	Fetal outcome
J110-L535	*M. mulatta*	SIV/DeltaB670hu	Spleen, lymph node, thymus	32	Stillbirth (term delivery)
M464-M893	*M. fascicularis*	SIV/DeltaB670hu	Spleen, lymph node, thymus	79	Death at 1 day of age (term delivery)
M820-R638	*M. nemestrina*	SIV/DeltaB670rh, SIV/17E-Fr	Spleen, lymph node	6	Abortion (61 days premature)
N861-R352	*M. fascicularis*	SIV/DeltaB670rh, SIV/17E-Fr	Spleen, lymph node, lung, thymus	48	Death in utero (19 days premature)
M692-R644	*M. nemestrina*	SIV/DeltaB670rh, SIV/17E-Fr	Thymus	8	Abortion (71 days premature)

[a] p.i., postinfection.

observed by ultrasonography), cesarean deliveries were performed under isoflurane inhalation anesthesia.

Three virus stocks were used to infect the females in these studies. SIV/DeltaB670hu was obtained by coculturing lymph node tissue from SIV-infected monkey B670 with primary human phytohemagglutinin-stimulated PBMC (PHA blasts). SIV/Delta/B670rh was obtained by a single passage of the SIV/DeltaB670hu stock onto rhesus PHA blasts. SIV/17E-Fr is an infectious molecular clone that is macrophage tropic (1). The minimal animal infectious dose (AID_{50}) of SIV/DeltaB670hu and SIV/DeltaB670rh was determined by inoculating a series of monkeys with 10-fold dilutions of the cryopreserved virus preparation and assaying for infection. The AID_{50} was calculated by using software developed by John Spouge (19).

Pregnant females M464 and J110 were inoculated with 1,000 AID_{50}s of SIV/DeltaB670hu during the second and third trimesters of pregnancy, respectively. Females M820, N861, and M692 were inoculated during the second trimester with a mixture of 50 AID_{50}s of SIV/DeltaB670rh and 10,000 AID_{50}s (estimated) of SIV/17E-Fr. Inoculations were performed via cannulation of the saphanous vein with a 22-gauge butterfly catheter. The butterfly tubing was flushed with sterile saline before and after inoculation to ensure accurate delivery of the infectious material.

Complete necropsies were performed on all cesarean section-derived dead fetuses and infants that died following delivery. Representative lymph node, brain, thymus, lung, and spleen tissue samples were snap-frozen in liquid nitrogen and stored at −70°C.

PCR. PBMC were isolated from heparanized blood on Ficoll-Hypaque density gradients. Where indicated, mononuclear cells were isolated from tissue biopsies by gentle teasing. Cells were washed with RPMI culture medium prior to lysis, and the DNA was purified from detergent-disrupted cells by solvent extraction and spooling on glass rods. SIV infection was identified by nested PCR using conserved sequences in viral *env* genes. PCR amplification was performed with 1 μg of DNA. The viral sequences were amplified in an automated thermal cycler (Perkin-Elmer Corp., Norwalk, Conn.) programmed for 30 cycles of denaturation at 94°C for 1 min and annealing and extension at 55°C for 2 min. Each reaction mixture contained 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.75 mM $MgCl_2$, 0.01% Triton X-100, 0.2 mM deoxynucleoside triphosphates, 20 nM 5′ and 3′ oligonucleotide primers, and 2.5 U of *Taq* polymerase (Promega, Madison, Wis.). One microliter of the first reaction mixture was used in a second round of PCR containing internal primers and amplified with the same parameters. Primers used for amplification of the V1 hypervariable region of gp120 from SIV/DeltaB670 and SIV/17E-Fr in the first- and second-round amplification reactions are shown below, with nucleotide references from the SIV_{mac239} sequence. The first-round primers were (nucleotides 6709 to 6728) 5′-AGG-AAT-GCG-ACA-ATT-CCC-CT-3′ and (nucleotides 7406 to 7385) 5′-TCC-ATC-ATC-CTT-GTG-CAT-GAA-G-3′, and the second-round primers were (nucleotides 6845 to 6868) 5′-CAG-TCA-CAG-AAC-AGG-CAA-TAG-A-3′ and (nucleotides 7327 to 7305) 5′-TAA-GCA-AAG-CAT-AAC-CTG-GCG-GT-3′.

To minimize contamination of the reagents used in the PCR, several measures were taken. Isolation of DNA from PBMC and tissues was performed in a separate laboratory to prevent contamination by plasmid DNA. Separate reagents were used and stored specifically for these manipulations. PCR amplification mixtures were prepared in a PCR clean room, and aerosol-resistant plugged pipette tips were used for all pipetting. Addition of first-round PCR products to the second-round amplification mixtures was done in a laminar-flow hood to protect further from contamination. To detect possible contamination, several experimental controls were utilized. Uninfected rhesus PBMC were processed in parallel with infected samples and were used in amplification reactions to monitor contamination through these processes. Several (four to eight) PCR amplifications containing only reagents (no template DNA) were also performed in each experiment to control for possible cross-contamination or contamination of buffers and other reagents.

Reverse transcription-PCR. To evaluate viral variants being expressed, virus in 1 ml of plasma was pelleted by centrifugation at 12,400 × *g* for 45 min. Virus from a cryopreserved virus stock was also pelleted in the same manner. The viral pellet was solubilized in 1 ml of RNAzol B reagent (Biotecx, Houston, Tex.), and the RNA was pelleted and resuspended in RNase-free water. The RNA was reverse transcribed to cDNA with the Gene Amp RNA PCR kit (Perkin-Elmer Corp.). Reverse transcription was done with random hexamers as primers, murine leukemia virus reverse transcriptase, and all of the other reagents and protocols provided with the kit. The first round of PCR amplification was done with reagents provided in the Gene Amp kit and the first-round primers described above. The amplification cycle conditions and performance of the second-round amplification reactions were as described above.

When plasma virus pellets were unavailable, viral variants that were being actively transcribed in PBMC were analyzed by solubilizing approximately 5×10^5 PBMC in 1 ml of RNAzol B reagent. The RNA was pelleted and resuspended in RNase-free water. To remove any traces of DNA that may have precipitated with the cellular RNA, the sample was treated with a cocktail containing 10 mM $MgCl_2$, 1 mM dithiothreitol, 10 U of placental RNase inhibitor (RNasin; Promega), and 4 U of DNase I (U.S. Biochemical, Cleveland, Ohio) for 15 min at 37°C. The DNase reaction was stopped by adding 25 μl of a stop mixture containing 50 mM EDTA, 1.5 M sodium acetate, and 1% sodium dodecyl sulfate; the RNA was then extracted with an equal volume of phenol-chloroform and precipitated with 2.5 volumes of ethanol. The RNA was reverse transcribed to cDNA by using the Gene Amp kit and the random hexamer primers, and nested PCR amplification of the envelope V1 region was done as described above.

Cloning and sequencing. PCR products from two to four independent amplifications with 1 μg of undiluted DNA (from DNA of PBMC or cDNA from plasma virus or cellular RNA) were pooled and cloned into the TA cloning vector (Invitrogen, San Diego, Calif.). Following transformation, colonies containing appropriate-size inserts were selected and plasmid DNA was purified by alkaline lysis. Nine to 20 clones from each sample were sequenced by dideoxy sequencing with Sequenase (U.S. Biochemical). From previous experiments in our laboratory, we had determined the rate of nucleotide misincorporation by *Taq* polymerase to be similar to that reported by other laboratories (12).

In the case of one infant, R644, the PCR-amplified envelope region was not cloned and the PCR products were directly sequenced with the fmol DNA sequencing kit (Promega). In all other cases, the envelope region was PCR amplified, cloned, and sequenced as already described.

The predominant viral variant(s) in a given sample was confirmed by serial dilution of the DNA prior to amplification by PCR. The PCR product obtained from the highest dilution of DNA was directly sequenced by using 3 μl of the PCR product and the fmol DNA sequencing kit (Promega).

Viral envelope sequences were analyzed with a SUN sparkstation and the IG suite of programs (Intelligenetics, Mountain View, Calif.). Preliminary sequence alignments were performed with the IG program GENALIGN and the DOTS alignment program provided by the laboratory of J. Mullins at Stanford University (18). The DOTS program performs divergence analysis and prints out nucleotide and derived amino acid sequence alignments. Divergence at the nucleotide level is calculated, and each point mutation has a score of 1. Insertions and deletions are disregarded in these calculations.

RESULTS

In a large study to define the parameters of transplacental transmission of SIV in three species of macaques, we obtained evidence for the infection of five infants born of mothers inoculated intravenously during gestation (Table 1). In all cases, the prognosis of the infected infants was poor. Two fetuses were aborted during the primary viremic episode of the mother, one died in utero 19 days before gestational maturity, one was stillborn at term, and one died 1 day following a term delivery. SIV was detectable by PCR in lymphoid tissues obtained at necropsy from all of the infants.

To identify specific viral factors involved in transplacental infection, viral variants present in the mother at delivery were

A

SIV/DeltaB670hu

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B670-Cl 1   —B16  W G L T G N - - V T T - T - - T T - A A P - T A T A N V V N E T S S C V I N N T C A G L E P E P
B670-Cl 2   —B13  . . . . . . I - P . - . - V P . - . T . S K E . . . . . . . . . . . . K . . N . T . . . . . .
             ┌B22 . . . . . . V - P . - . - A . . - S T T T P K E T . . . . . . . . . . K . . N . T . . . . . .
             │B17 . . . . . . V - P . - . - A . . - S T T T P K E T . . . . . . . . . . K . . N . T . . . . . .
             │B15 . . . . . . V - P . - . - A . . - S T T T P K E T . . . . . . . . . . K . . N . T . . . . . .
             │B9  . . . . . . V - P . - . - A . . - S T T T P K E T . . . . . . . . . . K . . N . T . . . . . .
             │B12 . . . . . . V - P . - . - A . . - S T T T P K E T . . . . . . . . . . K . . N . T . . . . . .
             │B7  . . . . . . A - P . - . - A . . - S T T T P K E T . . . . . . . . . . K . . N . T . . . . . .
B670-Cl 3    │B10 . . . . . . V - P . - . - A . . - S T T T P K E T . . . . . . . . . . K . . N . T . . . . . .
             │B8  . . . . . . V - P . - . - A . . - S T T T P K E T . . . . . . . . . . K . . N . T . . . . . .
             │B4  . . . . . . V - P . - . - A . . - S T T T P K E T . . . . . . . . . . K . . N . T . . . . . .
             │B21 . . . . . . V - P . - . - A . . - S T T T P K E T . . . . . . . . . . K . . N . T . . . . . .
             └B1  . . . . . . V - P . - . - A . . - S T T T P K E T D . . . . . . . . . K . . N . T . . . . . .
B670-Cl 4    ┌B24 . . . . R . V E A . . - K T T . . V - S T T . P K . D . . . . . . . . . K . . N . T . . . Q . .
             └B6  . . . . R . V E A . . - K T T . . V - S T T . P K . D . . . . . . . . . K . . N . T . . . Q . .
B670-Cl 5   —B18  . . . . R . V E A . . - . T T . . A T T T T . P K . D . . . . . . . . . . . . N . T . . . Q . .
             ┌B19 . . . . . . A T T . . A . T A A P S T R T P K L . . . . . . . . . . . K . . N . T . . . Q . .
             │B14 . . . . . . A T T . . A . T A A P S T R T P K V . . . . . . . . . . . K . . N . T . . . Q . .
B670-Cl 6    │B5  . . . . . . A T T . . A . T A A P S T R T P K V . . . . . . . . . . . K . . N . T . . . Q . .
             └B20 . . . . . . A T T . . A . T A A P S T R T P K V . . . . I . . . . . . . K . . N . T . . . Q . .
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B

SIV/DeltaB670rh

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             ┌C7  W G L T G N V - P T - T T V P T A - T - P S K E T A N V V N E T S S C V K N N N C T G L E P E P
B670-Cl 2    │C20 . . . . . . . - . . - . . . . . . - . . . . . . . . . . . . . . . . . . . . . . . . . . Q . .
             └L44 . . . . . . . - . . - . . . . . . - . . . . . . . . . . . . . . . . . . . . . . . . . . Q . .
             ┌C8  . . . . . . . - . . - . . A T . S - . - T T P K E T . . G . . . . . . . . . . . . . . . Q . .
             │L6  . . . . . . . - . . - . . A T . S - . - T T P K E T . . . . . . . . . . . . . . . . . . . . .
             │L9  . . . . . . . - . . - . . A T . S - . - T T P K E T . . . . . . . . . . . . . . . . . . . . .
B670-Cl 3    │L31 . . . . . . . - . . - . . A T . S - . - T T P K E T . . . . . . . . . . . . . . . . . . . . .
             │C16 . . . . . . . - . . - . . A T . S - . - T T P K E T . . . . . . . . . . . . . . . . . . . . .
             └L46 . . . . . . . - . . - . . A T . S - . - T T P K E T . . . . . . . . . . . . . . . . . . . . .
B670-Cl 7   —L15  . . . . . . . - . . - . . A T . S - . - I P . G E . D . . . . . . . . . . . . . . . . . . . .
B670-Cl 8    ┌C17 . . . . . . A - T . K . . A T V P - . - T A P P E V K . . . . S . P . . . . . T . A . . . . . .
             └L8  . . . . . . A - T . K . . A T V P - . - T A P P E V K . . . . S . P . . . . . T . A . . . . . .
B670-Cl 9   —C3   . . . . . . . T T . T A P T T A T - . - T P . . E . D . . . . . . . . . . . . . . . . . . Q . .
B670-Cl 10  —L40  . . . . R . . E T . T . . T T . T - S - T T T P K V E I . . . . . . . . . . . . . . . . . . . .
B670-Cl 4   —L22  . . . . R . . E A . T K . T T . V - S - T T T P K . D . . . . . . . . . . . . . . . . . . . .
B670-Cl 5   —L43  . . . . R . . E A . T . . T T . V T . - T T T P K . D . . . . . . . . . I . . . . . . . . Q . .
B670-Cl 11  —L35  . . . . . . A T T K T A . T V S T P . - T P . . K T D . . . . . . . . . . . . . . . . . . Q . .
B670-Cl 6   —C4   . . . . . . A T T . T A . T A A P S . R T P . V . . . . . . . . . . . . . . . . . . . . Q . .
             ┌C11 . . . . . . A T T . T . . T T . . S . T T P . G R . D . . . . . . . . . . . . . . . . . . Q . .
             │L45 . . . . . . A A T . T . . T T . . S . T T P . G R . D . . . . . . . . . . . . . . . . . . Q . .
             │L30 . . . . . . A A T . T . . T T . . S . T T P . G R . D . . . . . . . . . . . S . . . . . . Q . .
             │L10 . . . . . . A A T . T . . T T . . S . T T P . G R . D . . . . . . . . . . . . . . . . . . Q . .
             │C25 . . . . . . A A T . T . . T T . . S . T T P . G R . D . . . . . . . . . . . . . . . . . . Q . .
B670-Cl 12   │C1  . . . . . . A A T . T . . T T . . S . T T P . G R . D . . . . . . . . . . . . . . . . . . Q . .
             │L37 . . . . . . A A T . T . . T T . . S . T T P . G R . D . . . . . . . . . . . . . . . . . . Q . .
             │L39 . . . . . . A A T . T . . T T . . S . T T P . G R . D . . . . . . . . . . . . . . . . . . Q . .
             │L42 . . . . . . A A T . T . . T T . . S . T T P . G R . D . . . . . . . . . . . . . . . . . . Q . .
             │L27 . . . . . . A A T . T . . T T . . S . T T P . G R . D . . . . . . . . . . . . . . . . . . Q . .
             └L7  . . . . . . A A T . T . . T T . . S . T T P . G R . D . . . . . . . . . . . . . . . . . . Q . .
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C

SIV/17E-Fr

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17E-Fr      —Fr1  . . . . K S I - T . - . A S T . - S . - T A S A R V D M . . . . . . . I A Q D . . I . . . Q . Q
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FIG. 1. Genetic variants identified in SIV/DeltaB670 standard inocula. Virus was purified from cryopreserved stocks, and envelope V1 regions were amplified by reverse transcription-PCR, cloned, and then sequenced. The derived amino acid sequences are shown. Dots indicate amino acids identical to those in the reference sequence. Dashes indicate gaps that were introduced to maintain alignments. The 12 different variants identified in these virus stocks are indicated at the left. (A) The V1 region from 20 envelope clones obtained from the SIV/DeltaB670hu stock prepared by culture of lymph node tissue from monkey B670 on primary human PBMC. (B) The V1 region from 30 envelope clones obtained from the SIV/DeltaB670rh stock prepared by culture of the DeltaB670hu stock on primary rhesus PBMC. (C) The V1 region from infectious molecular clone 17E-Fr (1).

identified by sequence analysis and compared with those present in the fetus. For these assays, the first hypervariable region (V1) of the SIV envelope gene was amplified directly from tissues by nested PCR, cloned, and sequenced. This region was used to identify specific variants found in a given sample because it has been shown to be the most variable region of SIV gp160 (3, 12).

Genetic characterization of viral inocula. The genetic composition of the stock preparations used for the inoculations, derived by sequence analysis of PCR-amplified V1 regions of cDNA clones from high-speed pellets of the cryopreserved cell culture supernatants, is shown in Fig. 1. As expected, comparison of the two SIV/DeltaB670 preparations showed a high degree of similarity, with all but one of the viral sequences found in the SIV/DeltaB670hu preparation also identified in the rhesus monkey-grown stock. Interestingly, this one ex-

ception (B670-Cl 1) was one of the original SIV/DeltaB670 sequences identified in a genomic library obtained from chronically infected human H9 cells. Shifts in the proportion of these sequences were apparent between the two preparations, with the identification of additional sequences in the SIV/DeltaB670rh preparation not found among the 20 bacterial clones sequenced from the amplified human-grown stock. Variants identified only in SIV/DeltaB670rh were apparently also present in SIV/DeltaB670hu, however, because they have frequently been identified in monkeys inoculated with this preparation (e.g., B670-Cl 12; this study). In monkeys inoculated with doses of SIV/DeltaB670 similar to those used in this study, we observed various distributions of genotypes. We have been able to identify repeatedly at least 9 of 12 SIV/DeltaB670 variants in the virus inoculum, as well as additional variants not identified in the virus stock, in infected monkeys (unpublished observations), a finding which confirms the infectivity of these genotypes.

Repeated use of these stock preparations in multiple experiments revealed useful information regarding some of the variants identified by sequence analysis. In vitro propagation of either preparation of SIV/DeltaB670 on CEMX174, a human T-lymphoid cell line commonly used in in vitro assays, produced the B670-Cl 3 sequence, a finding which suggests a T-cell-tropic phenotype for this variant. On the other hand, PCR amplification of brain tissue obtained from two of three SIV/DeltaB670-infected monkeys with encephalitis revealed only B670-Cl 12; coculture of these brain homogenates on primary rhesus macrophages produced virus in the culture supernatant with the B670-Cl 12 genotype (data not shown). Taken together, these data demonstrate a macrophage-tropic phenotype for this variant.

SIV/17E-Fr is an infectious molecular clone of SIV whose tropism has been well characterized (1). SIV/17E-Fr, derived from SIV_{mac239} by serial brain passage in macaques, has been shown to replicate well in vitro in both primary macrophages and central nervous system-derived endothelial cells (8, 17). In adult monkeys infected with SIV/17E-Fr alone, the viral phenotype has remained stable, with virus isolated only from purified monocytes-macrophages up to 8 months postinfection (4).

The 12 variants identified in the two SIV/DeltaB670 stock preparations vary from each other by 4 to 21%, whereas SIV/17E-Fr varies from each of the 12 SIV/DeltaB670 sequences by 24 to 30% at the nucleotide level over this region.

Genetic analysis of SIV-infected macaque females. SIV V1 envelope sequences were amplified from PBMC DNAs of all infected mothers within 1 day of giving birth and at 7 days postinoculation from monkey N861 to determine the extent of genetic diversity in the maternal circulation during pregnancy. Viral RNA, obtained either from high-speed plasma pellets, or from PBMC when plasma pellets were unavailable (monkeys J110 and M464), was also evaluated within 1 day of parturition or 20 days prior to parturition (monkey N861) to determine the specific provirus(es) expressed at or near delivery. Sequences obtained from maternal samples were then compared with those identified in fetal tissues.

The deduced amino acid sequences of viral variants found in maternal blood taken at parturition are shown in Fig. 2. As expected, all females had a swarm of genetically distinct variants in the peripheral blood and the specific proviruses expressed at these time points were, in general, less complex than the provirus found in PBMC.

Genetic analysis of SIV-infected infants. In contrast, sequence analysis of SIV proviruses found in lymphoid tissues of all of the infants revealed predominantly either one of two variants: B670-Cl 12 or SIV/17E-Fr (Fig. 3). Sequence analysis of 28 clones from the spleen, lymph node, and thymus tissues of infant L535, more than 40 clones from the spleen, lymph node, and thymus tissues of infant M893, and 12 clones from the spleen and lymph node tissues of infant R638 revealed only the B670-12 variant. As shown in Fig. 3, the V1 sequences identified in all of these infants were identical, with respect to both one another and sequences identified in the brains of monkeys with encephalitis.

Sequence analysis of more than 30 clones from the spleen, lymph node, and lung tissues of infant R352 and direct sequencing of the PCR product from the thymus of infant R644 revealed only the macrophage-tropic variant SIV/17E-Fr. In one infant (R352), two additional sequences were identified (among 13 clones sequenced) from thymic tissue; one was novel, and the other was identical to that found in the mother (data not shown). The significance of this finding is unknown.

A summary of the genetic analysis is shown in Table 2. The provirus identified in each infant is indicated by an asterisk. In three mothers (J110, M820, and M692), the variant transmitted to the infant was most closely related to a minor virus strain found in the mother's peripheral blood, while in two other mothers (monkeys M464 and N861), the transmitted variant was most closely related to a major strain. In only two cases (monkeys M820 and N861) was the variant transmitted to the infant being transcribed in the mother near parturition.

The presence of only two variants in the infant does not appear to be due to the dose used for the maternal inoculations. B670-Cl 12 sequences were identified in two infants born of mothers inoculated with SIV/DeltaB670hu, for whom this variant was present as a minor form in both the inoculum and maternal PBMC at parturition (monkeys J110 and M820). In addition, in mothers inoculated with both SIV/DeltaB670 and SIV/17E-Fr and in whose babies SIV/17E-Fr alone was found, SIV/17E-Fr was not identified among 15 clones analyzed from maternal PBMC (monkey N861) taken at 7 days postinoculation or among 17 clones from maternal PBMC (monkey M692) taken at parturition.

Both B670-Cl and SIV/17E-Fr V1 sequences were readily identified in rhesus macrophage cultures of tissue homogenates (data not shown). In addition, in two females which developed encephalitis after delivery (monkeys N861 and M692), the same variant found in the fetus was also selectively identified in the brain. Taken together, these findings suggest that viruses with the ability to replicate well in macrophages and to cross the blood-brain barrier have a selective advantage in crossing the maternal-fetal barrier and successfully replicating in fetal tissues.

DISCUSSION

Sequence analysis of five paired maternal and fetal macaque isolates has demonstrated the selective infection of fetal tissues with specific SIV genotypes. All five macaque females harbored a heterogeneous virus population at parturition, while the virus identified in the infants' lymphoid tissues (with the exception of two minor variants identified in the thymus of one infant) was homogeneous. In all cases, the consensus sequence found in infant tissues could be identified in the maternal viral quasispecies in the peripheral circulation and/or in the original inoculum.

Infection of neonates with a single genetic variant found in the swarm of genetically distinct forms in the mothers is in

A Monkey J110

Provirus

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          ┌MJ 1  W G L T G N A T T T T A T T A A P S T R T P K V T A N V V N E T S S C V K N N N C T G L E Q E P
          │MJ 3  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │MJ 4  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
B670-Cl 6 │MJ 6  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │NJ 1  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │MJ 2  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          └NJ 3  . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          ┌NJ 6  . . . . . . . A . . . T . . T T A . . T . . . G R . D . . . . . . . . . . . . . . . . . . . . .
B670-Cl 12│NJ 5  . . . . . . . A . . . T . . T - A . . T . . . G R . D . . . . . . . . . . . . . . . . . . . . .
          └MJ 7  . . . . . . . A . . . T . . T - A . . T . . . G R . D . . . . . . . . . . . . . . . . . . . . .
B670-Cl 3 ─NJ 2  . . . . . . V P . . . - A . T - - . . T . . . E - T S . . . . . . . . I . . . . . . . . . P . .
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Viral RNA (cell associated)

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          ┌RJ 17 W G L T G N A T T T T A T T A A P S T R T P K V T A N V V N E T S S C V K N N N C T G L E P E P
          │RJ 11 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │RJ 9  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │QJ 8  . . . . . . V . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Q . .
          │QJ 14 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . S . . . . Q . .
          │QJ 2  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . K . . . . . . . . . . . . . . . Q . .
          │QJ 13 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Q . .
          │QJ 7  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Q . .
          │QJ 5  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Q . .
B670-Cl 6 │RJ 10 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Q . .
          │QJ 3  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Q . .
          │QJ 12 . . . . . . . . . . . . . I . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Q . .
          │QJ 10 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │RJ 6  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │RJ 12 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │RJ 4  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . . . . . . .
          │RJ 1  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . .
          │RJ 7  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │QJ 15 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Q . .
          └RJ 3  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
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B Monkey M464

Provirus

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          ┌KM 6  W G L T G N A A T T T T T T T T A S T T T P K G R A D V V N E T S S C V K N N N C T G L E Q E P
          │KM 13 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │KM 8  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │KM 3  . . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
B670-Cl 12│KM 14 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │KM 11 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │KM 7  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │KM 5  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G .
          └KM 17 . . . . . . . . ~ . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
B670-Cl 11─KM 16 . . . . . . . - . . K . A . . V S T P . . . . E K T . . . . . . . . . . . . S . . . . . . . . .
B670-Cl 21─KM 1  . . . . . . . - A . . . . . . A S T - . . . . . . R . . . . . . . . . . . . . . . . . . . . . .
          ┌KM 9  . . . . . . V - - P . . . A . . S T - . . . . E - T N . . D . . . . . . . . . . . . . . . P . .
B670-Cl 3 │KM 10 . . . . . . V - - P . . . A . . S T - . . V . E - T N . . . K . . . . . . . . . . . . . . P . .
          └KM 4  . . . . . . V - - P . . . A . . S T - . . . . E - T N . . . . . . . . . . . . . . . . . . P . .
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Viral RNA (cell associated)

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          ┌AM 20 W G L T G N V P T T T A T T S T T T P K E T N V V N E T S S C V K N N N C T G L E P E P
          │AM 16 . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 15 . . . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 1  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 2  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 3  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 4  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 5  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
B670-Cl 3 │AM 6  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 7  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 12 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 13 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 18 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 19 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 14 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 17 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
          │AM 8  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . L
          └AM 10 . . . . . . . . . . . . . . . . . . . . . K . K I . . . . . . . . . . . . . . . . . . .
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FIG. 2. Genetic variants identified in SIV-infected macaque females. The derived amino acid sequences are shown. Dots indicate amino acids identical to those in the reference sequence, dashes indicate gaps that were introduced to maintain alignments, and the asterisk indicates a stop codon. The major variants are identified at the left with the designations assigned from the SIV/DeltaB670 virus stocks (Fig. 1). SIV/DeltaB670 variant designations above number 12 (B670-Cl 13 through B670-Cl 21) indicate variants not identified in the sequencing of 30 clones from either virus stock. (A) Genetic variants identified in the DNA and RNA from PBMC of monkey J110 at parturition. (B) Genetic variants identified in the DNA and RNA from PBMC of monkey M464 at parturition. (C) Genetic variants identified in the DNA from PBMC and plasma-associated virus from monkey M820 at parturition. (D) Genetic variants identified in monkey N861 from the DNA of PBMC 7 days postinoculation, at parturition, and from plasma-associated virus 20 days prior to parturition (28 days postinoculation). (E) Genetic variants identified in the DNA of PBMC and from plasma-associated virus from monkey M692 at parturition.

C Monkey M820

Provirus

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            CM 14 W G L T G N A T T T - T A T T A A P S T R T P K V T R N V V N E T S S C V K N N N R T G L E Q E P
B670-Cl 6   CM 20 . . . . . . . . . . - . . . . . . . . . . . . . . . A . . . . . . . . . . . . . . C . . . . . . .
            CM 5  . . . . . . . . . . - . . . . . . . . . . . . E . . A . . . . . . . . . . . . . . C . . . . . . .
B670-Cl 12  CM 11 . . . . . . . A . . - . T . . T T T . . T . . . G R A D . . . . . . . . . . . . . C . . . . . . .
B670-Cl 9   CM 18 . . . . . . V . . . - . . P . T - - A . T . . . E E A D . . . . . . . . . . . . . C . . . . . . .
            CM 10 . . . . R . V E A . - . K . . T - - T V S . T T P K A D . . . . . . . . . . . . . C . . . . . . .
B670-Cl 4   CM 21 . . . . R . V E A . - . K . . T - - T V S . T T P K A D . I . . . . . . . . . . . C . . . . . . .
            CM 13 . . . . . . V - P . - . T A . T - - - S T . T P K E T . . . . . . . . . . . . . . C . . . . P . .
            CM 19 . . . . . . V - P . - . T A . T - - - S T . T P K E T . . . . . . . . . . . . . . C . . . . P . .
            CM 23 . . . . . . V - P . - . T A . T - - - S T . T P K E T . . . . . . . . . . . . . . C . . . . P . .
B670-Cl 3   CM 2  . . . . . . V - P . - . T A . T - - - S T . T P K E T . . . . . . . . . . . . . . C . . . . P . .
            CM 9  . . . . . . V - P . - . T A . T - - - S T . T P K E T . . . . . . . . . . . . . . C . . . . P . .
            CM 16 . . . . . . V - P . - . T A . T - - - S T . T P K E T . . . . . . . . . . . . . . C . . . . P . .
B670-Cl 8   CM 6  . . . . . . . - . . K . T A . V - - - P T . A P P E V K . . . . S . P . . . . . T C A . . . P . .
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Viral RNA (virion associated)

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B670-Cl 3   O10  W G L T G N V - P T T T - A T T S T T T P K E - - T N V V N E T S S C V K N N N C T G L E P E P
            O6   . . . . . . . - . . . . - . . . . . . . . . . - - . . . . . . . . . . . . . . . . . . . . . . .
B670-Cl 4   O3   . . . . R . . E R . . K T K . . V S . . T P K - - R D . . . . . . . . . . . . . . . . . D Q . .
            O8   . . . . R . . E A . . K T T . . V S . . T P K - - A D . . . . . G . . . . . . . . . . . . Q . .
            O7   . . . . . . A A A . . . T T . . A S . . T P K G R A D . . . . . . . . . . . . . . . . . . Q . .
B670-Cl 12  O12  . . . . . . A A T . . . T T . . A S . . T P K G R A D . . . . . . . . . . . . . . . . . . Q . .
            O14  . . . . . . A A T . . . T T . . A S . . T P K G R A D . . . . . . . . . . . . . . . . . . Q . .
            O11  . . . . . . A T T . . A T T A A P S . R T P . V T A . . . . . S . . . . . . . . . . . . . Q . .
B670-Cl 6   O15  . . . . . . A T T . . A T T A A P S . R T P . V T A . . . . . S . . . . . . . . . . . . . Q . .
            O16  . . . . . . A T T . . A T T A A P S . R T P . V T A . . . . . S . . . . . . . . . . . . . Q . .
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D Monkey N861

Provirus (7 days pi)

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            ON 3  W G L T G N V P T T T V P T A T P S K E - - - - T A N V V N E T S S C A K N N N C T G L E Q E P
B670-Cl 2   ON 20 . . . . . . . . . . . . . . . . . . . . - - - - . . . . . . . . . . . V . . . . . . . . . . . .
            ON 11 . . . . . . . . . . . . . . . . . . . . - - - - . . . . . . . . . . . V . . . . . . . . . . . .
            ON 14 . . . . . . . . . . . A T . . . . . . . - - - - . . . . . . . . . . . V . . . . . . . . . . . .
            ON 21 . . . . . A T . . . . A T . . A . . T R T P K V . . . . . . . . . . . V . . . . . . . . . . . .
            ON 1  . . . . . A T . . . . A T . . A . . T R T P K V . . . . . . . . . . . V . . . . . . . . . . . .
            ON 4  . . . . . A T . . . . A T . . A . . T R T P K V . . . . . . . . . . . V . . . . . . . . . . . .
B670-Cl 6   ON 7  . . . . . A T . . . . A T . . A . . T R T P K V . . . . . . . . . . . V . . . . . . . . . . . .
            ON 8  . . . . . A T . . . . A T . . A . . T R T P K V . . . . . . . . . . . V . . . . . . . . . . . .
            ON 15 . . . . . A T . . . . A T . . A . . T R T P K V . . . . . . . . . . . V . . . . . . . . . . . .
            ON 2  . . . . . A T . . . . A T . . A . . T R T P K V . . D . . . . . . . . V . . . . . . . . . . . .
            ON 5  . . . . . A A . . . . T T . T . A . T T T P K G R . D . . . . . . . . V . . . . . . . . . . . .
B670-Cl 12  ON 18 . . . . . A A . . . . T T . T . A . T T T P K G R . D . . . . . . . . V . . . . . . . . . . . .
            ON 10 . . . . . A A . . . . T T . T . A . T T T P K G R . D . . . . . . . . V . . . S . . . . . . . .
            ON 22 . . . . . A A . . . . T T . T . A . T T T P K G R . D . . . . . . . . V . . . . . A . . . . . .
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Viral RNA (virion associated; 28 days pi)

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            J 10  W G L T G N V P T T T A T T S T T S P K E T N V V N E T S S C V K N N N C T G L E P E P
            J 20  . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . .
            J 19  . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . .
            J 16  . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . .
B670-Cl 3   J 11  . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . .
            J 9   . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . .
            J 6   . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . .
            J 4   . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . .
            J 1   . . . . . . . . . . . . . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . .
            J 8   . . . . K S I T . . A S . . . . . A S A R V D M . . . . . . . I A Q D . . I . . . Q . Q
17E-Fr      J 12  . . . . K S I T . . A S . . . . . A S A R V D M . . . . . . . I A Q D . . I . . . Q . Q
            J 14  . . . . K S I T . . A S . . . . . A S A R V D M . . . . . . . I A Q D . . I . . . Q . Q
            J 2   . . . . K S I T . . A S . . . . . A S A R V D M . . . . . . . I A Q D . . I . . . Q . Q
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Provirus (at parturition)

```
            AN 35 W G L T K S I T T T A - - S T T - S T T A S A G V D M V N E T S S C I A Q D N C I G L E Q E Q
            AN 5  . . . . . . . . . . . - - . . . - . . . . . . R . . . . . . . . . . . . . . . . . . . . . . .
            AN 2  . . . . . . . . . . . - - . . . - . . . . . . R . . . . . . . . . . . . . . . . . . . . . . .
            AN 24 . . . . . . . . . . . - - . . . - . . . . . . R . . . . . . . . . . . . . . . . . . . . . . .
            AN 30 . . . . . . . . . . . - - . . . - . . . . . . R . . . . . . . . . . . . . . . . . . . . . . .
17E-Fr      AN 6  . . . . . . . . . . . - - . . . - . . . . . . R . . . . . . . . . . . . . . . . . . . . . . .
            AN 8  . . . . . . . . . . . - - . . . - . . . . . . R . . . . . . . . . . . . . . . . . . . . . . .
            AN 23 . . . . . . . . . . . - - . . . - . . . . . . R . . . . . . . . . . . . . . . . . . . . . . .
            AN 34 . . . . . . . . . . . - - . . . - . . . . . . R . . . . . . . . . . . . . . . . . . . . . . .
            AN 40 . . . . . . . . . . . - - . . . - . . . . . . R . . . . . . . . . . . . . . . . . . . . . . .
            AN 25 . . . . . . . . . . . - - . . . - . . . . . . R . . . . . . . . . . . . . . . . . . . . . . .
            AN 31 . . . . . . . . . . . - - . . . - . . . . . . R . N . . . . . . . . . . * . . . . . . . . . .
B670-Cl 2   AN 17 . . . . G N V P . . T - - V P . - A . P S K E T A N V . . . . . . . V K N N . . T . . . . . P
B670-Cl 3   AN 28 . . . . G N V P . . T - - A . . - . . . T P K E R N V . . . . G . . V K N N . . T . . . P . P
B670-Cl 13  AN 7  . . . . G N A A . . T T T T . . A . . . T P K E T N V . . . . . . . V K N N . . T . . . P . P
B670-Cl 12  AN 18 . . . . G N A A . . T T T T . A S T . . P K G R A . V . . . . . . . V K N N . . T . . . . . P
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FIG. 2—*Continued.*

E Monkey M692

Provirus

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B670-Cl 2   —G17  W G L T G N V - P T T T V P - T A T P - T K E - T A N V V N E T S S C V K N N N C T G L E Q E P
            ┌G19  . . . . . . . - . . . . A T - . S . T - . P K E - T . . . . . . . . . . . . . . . . . . . P . .
B670-Cl 3   │G14  . . . . . . . - . . . . A T - . S . T - . P K E - T . . . . . . . . . . . . . . . . . . . P . .
            └G30  . . . . . . . - . . . . A T - . S . T - . P K E - T . . . . . . . . . . . . . . . . . . . P . .
B670-Cl 17  —G21  . . . . . . . - . . . . A T - A S . T - . P K G R . . . . . . . . . . . . . . . . . . . . . . .
B670-Cl 15  —G4   . . . . . . A - T I . . A A - P T . A - . P K G K . D . . . . . . . . . . . . . . . . . . P . .
B670-Cl 14  —G3   . . . . . . . - T . . . T T - A S . T - . P K G K . D . . . . . . . . . . . . . . . . . . P . .
B670-Cl 5   —G12  . . . . R . . E A . . . T T T . . . T - . T T P K . D . . . . . . . . . N . . . . . . . . . . .
            ┌G5   . . . . . . A A T . . . T T T A S . T - . P K G R . D . . . . . . . . . . . . . . . . . . P . .
            │G18  . . . . . . A A T . . . T T T A S . T - . P K G R . D . . . . . . . . . . . . . . . . . . . . .
B670-Cl 12  │G15  . . . . . . A A T . . . T T T A S . T - . P K G R . D . . . . . . . . . . . . . . . . . . . . .
            │G7   . . . . . . A A T . . . T T T . . . T T . P K G K . D . . . . . . . . . . . . . . . . . . . . .
            │G24  . . . . . . A A T . . . T T T . . S T T . P K G K . D . . . . . . . . . . . . . . . . . . . . .
            └G22  . . . . . . A A T . . . T T T . . S T T . P K G K . . . . . . . . . . . . . . . . . . . . . . .
B670-Cl 6   ┌G11  . . . . . . A T T . . A T T A A P P T R . P K V . . . . . . . . . . . . . . . . . . . . . . .
            └G16  . . . . . . A T T . . A T T A A P S T R . P . V . . . . . . . S . . . . . . . . . . . . . . . .
B670-Cl 16  —G8   . . . . . . A T T . . A T T A A T L A T . P K A . . A . . . . . . . . . . D . S . . . . . P . .
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Viral RNA (virion associated)

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            ┌N6   W G L T G N A T P T P T T T T T A S T T T P K G R A D V V N E T S S C V K N N N C T G L E Q E P
B670-Cl 12  │N1   . . . . . . . A T . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
            └N3   . . . . . . . A T . T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
B670-Cl 6   —N16  . . . . . . . . T . T A . . A A P . . R . . . V T . N . . . . . . . . . . . . . . . . . . . . .
B670-Cl 18  ┌N8   . . . . . . V . T . T A P . . A - T . . P K E . K . . . . . . . . . . . . . . . . . . . . . . .
            └N10  . . . . . . V . T . T A P . . S - A . . P K E - T . . . . . . . . . . . . . . . . . . . . P . .
B670-Cl 4   —N20  . . . . . . V E A . T K . . . . - V S . . T P - K . . . . . . . . . . . . . . . . . . . . . . .
B670-Cl 19  —N17  . . . . . . . . T . T . . . A A - T P . . K E - T . K . . . . S . P . . I . . T . A . . . P . .
            ┌N4   . . . . . . V P T . T A . . - S - T . . L K E - T - N . . . . . . . . . . . . . . . . . . P . .
B670-Cl 3   │N13  . . . . . . V P T . T A . . - S - T . . P K E - T - N . . . . . . . . . . . . . . . . . . R . .
            └N15  . . . . . . V P T . T A . . - S - T . . P K E - T - N . . . . . . . . . . . . . . . . . . R . .
B670-Cl 2   —N7   . . . . . . V P T . T V P . - A - T P S K E T - A - N . . . . . . . . . . . . . . . . . . . . .
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FIG. 2—*Continued*.

concordance with findings on humans (15, 20). Moreover, like that found in humans, the virus identified in the infants was either a major or a minor form found in the mothers at parturition. In both humans and macaques, the viral variants found in the infants were similar in some cases to those expressed in the mothers, whereas in other instances they were similar to the viral DNA population (15, 20).

The presence of a second viral genotype in the thymus of one infant monkey may also be consistent with recent observations made by Rossi et al. on humans (13). In those studies, an infant identified as HIV infected at birth by analysis of PBMC was found to harbor a single genotype also present in the mother. At 2 years of age, however, a second genotype emerged in the infant circulation that could also be identified in the maternal quasispecies. The significance of these observations is unclear, but they suggest cotransport of additional variants to the fetus that may be sequestered in fetal organs early in life only to reappear later.

In maternal-fetal transmission of HIV, several lines of evidence point toward the involvement of macrophage-tropic vi-

A

```
B670-Brain (B670-Cl 12)   W G L T G N A A T T T T T T T T A S T T T P K G R A D V V N E T S S C V K N N N C T G L E Q E
Monkey L535 consensus     . . . . . . . . . . . . . . . . . . . . . . . . . .   . . . . . . . . . . . . . . . . . . . .
Monkey M893 consensus     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Monkey R638 consensus     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
```

B

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17E-Fr                    W G L T K S I T T T A S T T S T T A S A R V D M V N E T S S C I A Q D N C I G L E Q E Q
Monkey R352 consensus     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
Monkey R644 consensus     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
```

FIG. 3. Amino acid consensus sequences derived from SIV envelope sequences obtained from tissues of infected infants. In these alignments, amino acids identical to those in the reference sequence are indicated by dots. (A) The amino acid consensus sequence derived from 28 envelope clones from spleen, lymph node, and thymus tissues of infant monkey L535, more than 40 envelope clones from the spleen, lymph node, and thymus tissues of infant monkey M893, and 12 clones from the spleen and lymph node tissues of infant monkey R638. These infant-derived sequences are compared with envelope sequences identified in the brains of macaques that developed encephalitis. (B) The amino acid consensus sequence derived from more than 30 clones obtained from the spleen, lymph node, and lung tissues of infant R352 and from direct sequencing of PCR-amplified SIV envelope sequences from the thymus of infant monkey R644. These infant-derived sequences are compared with the derived amino acid sequence from infectious molecular clone SIV/17E-Fr.

TABLE 2. Summary of viral genetic analysis in transmitting mothers

Monkey and viral variant	No. of clones identified/total sequenced[a]										
	32 days p.i. (at parturition)		79 days p.i. (at parturition)		6 days p.i. (at parturition)		Provirus[b] at 7 days p.i.	Viral RNA[d] at 28 days p.i.	Provirus[b] at 48 days p.i. (at parturition)	8 days p.i. (at parturition)	
	Provirus[b]	Viral RNA[c]	Provirus[b]	Viral RNA[c]	Provirus[b]	Viral RNA[d]				Provirus[b]	Viral RNA[d]
J110											
B670-Cl 6	7/11	20/20									
B670-Cl 12[e]	3/11	0									
B670-Cl 3	1/11	0									
M464											
B670-Cl 12[e]			9/14	0							
B670-Cl 3			3/14	18/18							
B670-Cl 11			1/14	0							
B670-Cl 21			1/14	0							
M820											
B670-Cl 3					6/14	2/10					
B670-Cl 6					3/14	3/10					
B670-Cl 4					2/14	2/10					
B670-Cl 8					1/14	0					
B670-Cl 9					1/14	0					
B670-Cl 12[e]					1/14	3/10					
N861											
B670-Cl 6							7/15	0	0		
B670-Cl 2							4/15	0	1/16		
B670-Cl 12							4/15	0	1/16		
17E-Fr[d]							0	4/13	12/16		
B670-Cl 3							0	9/13	1/16		
B670-Cl 13							0	0	1/16		
M692											
B670-Cl 12										6/17	3/12
B670-Cl 3										3/17	3/12
B670-Cl 6										2/17	1/12
B670-Cl 2										1/17	1/12
B670-Cl 5										1/17	0
B670-Cl 14										1/17	0
B670-Cl 15										1/17	0
B670-Cl 16										1/17	0
B670-Cl 17										1/17	0
B670-Cl 4										0	1/12
B670-Cl 18										0	2/12
B670-Cl 19										0	1/12
17E-Fr[e]										0	0

[a] p.i., postinoculation.
[b] Provirus was obtained from PBMC.
[c] Cell-associated viral RNA from PBMC.
[d] Viron-associated viral RNA from plasma.
[e] Viral variant transmitted to infant.

ruses. (i) A single genotype found in the diverse viral population of the mothers has been identified in infected neonates, suggesting some sort of selective mechanism (15, 20). (ii) In one study, macrophages were the only infected cell type identified in primary placental cell cultures (10). (iii) Most of the virus isolates from infected infants have demonstrated the slow-low replicative phenotype (isolates capable of replication in PBMC or Jurkat-tat cells only) (7, 14), which is an in vitro characteristic often associated with the macrophage-tropic phenotype.

Our studies provide additional support for the role of the macrophage-tropic viruses in fetal infection. Through the utilization of viral inocula that have been well characterized, we have shown that infection of infants involves specific variants contained within the quasispecies. The same genotype found within the SIV/DeltaB670 quasispecies was identified in three infants, and two other infants were infected with an infectious molecular clone with a known macrophage-tropic phenotype. Both variants have been shown to replicate well in rhesus macrophages in vitro and have been clonally identified in vivo in the central nervous systems of infected macaques which develop encephalitis.

It is interesting that both of the genotypes found in the infected fetuses were also identified in the central nervous system. Whether the viral factors which promote both neurotropism and fetotropism rely simply on the macrophage-tropic phenotype or whether additional factors are also necessary requires further study.

These findings provide compelling evidence for the role of macrophages in establishing fetal infection in utero and focus our future efforts on the development of strategies to block transplacental infection to those effective against the macrophage-tropic phenotype.

ACKNOWLEDGMENTS

We thank Lynn Fresh, Gail Plauche, Terese Theriot, Kim Burke, Patricia Phillippi, Eileen DeHaro, Calvin Lanclos, and Tami Unangest for technical assistance and Barbara Krieger for administrative assistance.

This work was supported by NIH grant A132302 to M. Murphey-Corb, principal investigator, and RR00164, base grant support for the Tulane Primate Center. A. M. Amedee is a Pediatric AIDS Foundation Scholar.

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