JOURNAL OF VIROLOGY, June 2011, p. 5757–5763 Vol. 85, No. 12
0022-538X/11/$12.00 doi:10.1128/JVI.02690-10

Mother-to-Infant Transmission of Simian Immunodeficiency Virus Is Rare in Sooty Mangabeys and Is Associated with Low Viremia▽

Ann Chahroudi,[1]† Tracy Meeker,[2]† Benton Lawson,[2] Sarah Ratcliffe,[3] James Else,[2] and Guido Silvestri[2]*

Division of Infectious Diseases, Department of Pediatrics, Emory University School of Medicine,[1] and Yerkes National Primate Research Center,[2] Emory University, Atlanta, Georgia, and Department of Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania[3]

Received 29 December 2010/Accepted 21 March 2011

Mother-to-child transmission of human immunodeficiency virus type 1 (HIV-1) occurs *in utero*, intrapartum, and through breastfeeding, with a cumulative rate of transmission of 35 to 40%. As a result, ~400,000 children become infected each year. Little is known about mother-to-infant transmission (MTIT) during natural simian immunodeficiency virus (SIV) infection of sooty mangabeys (SMs) that typically is nonpathogenic despite high viral loads. In this study, we retrospectively investigated the rates of MTIT in a large colony of naturally SIV-infected SMs using serological (anti-SIV antibody by enzyme-linked immunosorbent assay [ELISA] and Western blot analysis) and virological (SIV_{smm} real-time reverse transcription-PCR) methods. We examined 161 SM infants born to SIV-infected mothers and found that 150 (93.2%) were infected by non-MTIT ($n = 120$) or remained uninfected ($n = 30$). The remaining 11 SM infants (6.8%) were defined as acquiring SIV by presumptive MTIT based on (i) the presence of anti-SIV antibodies without seroreversion and (ii) a viral load of >500 copies/ml of serum in the first year of life. SM infants infected with SIV by presumptive MTIT did not show any increased morbidity or mortality, indicating that the infection is nonpathogenic even when acquired early in life. Interestingly, viral loads of SIV-infected SM infants with presumptive MTIT were 2-log lower than those of SIV-infected adult SMs living in the same colony (i.e., ~1,000 and 100,000 copies/ml, respectively). These results indicate that MTIT is substantially less frequent in naturally SIV-infected SMs than in HIV-1-infected humans and results in nonpathogenic infection associated with low SIV viremia. Evolutionary pressure to reduce MTIT may have contributed to the restriction of SIV pathogenesis in natural hosts.

The rate of mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV) in humans is estimated to be 35 to 40% without intervention (www.unaids.org/en/KnowledgeCentre/HIVData/EpiUpdate/EpiUpdArchive/2009/default.asp). Fortunately, in developed countries, strategies for the prevention of MTCT, including HIV testing of pregnant women, antiretroviral therapy for pregnant women and their infants, caesarian section, and formula feeding, are readily available (6). These interventions have the potential to reduce MTCT of HIV to less than 2%. In contrast, in developing countries with fewer resources, the rates of MTCT of HIV still are unacceptably high.

Sooty mangabeys (*Cercocebus atys*) are nonhuman primates that are natural hosts for simian immunodeficiency virus (SIV). Natural SIV hosts, including sooty mangabeys (SMs), African green monkeys (AGMs), mandrills, and numerous others in which primate lentiviral infections have been endemic for many thousands of years (32), display distinctive features of SIV infection that distinguish them from nonnatural or recent hosts, such as SIV_{mac}-infected rhesus macaques (RMs) and HIV-infected humans. Most characteristically, the infection of natural host species is nonpathogenic, with a lack of progression to AIDS.

During the past decade, much effort has been dedicated to understanding the mechanisms by which natural SIV hosts avoid progression to AIDS and maintain healthy $CD4^+$ T-cell counts in the face of high-level virus replication that is not controlled by the host cellular or humoral immunity (reviewed in references 5 and 20). These studies have shown that the acute SIV infection of natural hosts is characterized by the depletion of mucosal $CD4^+$ T cells and robust innate and adaptive immune responses to the virus (similar to those of SIV_{mac}-infected RMs and HIV-infected humans), whereas the chronic infection of natural hosts is distinguished from pathogenic infections of RMs and humans by the resolution of immune activation, the preservation of mucosal immune function, and preferential viral tropism for effector memory $CD4^+$ T cells (4, 8, 9, 12; G. Silvestri, unpublished data).

SIV infection rates in SMs and other natural hosts increase after the onset of sexual maturity (4 to 6 years of age) (11). Infection is thought to occur via the exchange of bodily fluids that occurs during sexual activity, as well as fighting, biting, and the licking of wounds. Earlier transmission may occur via aggressive grooming, mock sexual activity (that can begin before 1 year of age), or the premastication of food. Little is known about mother-to-infant transmission (MTIT) in natural hosts for SIV. Observations of AGMs and grivets in the wild suggest that infants of these species have low rates of SIV infection (18, 24). Similarly, MTIT of SIV was not observed in a colony of SIV-infected mandrills in Gabon (C. Apetrei and I. Pandrea,

* Corresponding author. Mailing address: Yerkes National Primate Research Center & Emory University School of Medicine, 3014 EVC Building, 954 Gatewood Rd. NE, Atlanta, GA 30329. Phone: (404) 727-9139. Fax: (404) 727-7768. E-mail: gsilves@emory.edu.
† These authors contributed equally to this work.
▽ Published ahead of print on 30 March 2011.

unpublished data). In SMs, phylogenetic and microsatellite analysis of SIV sequences isolated from fecal samples of wild animals in the Tai Forest in Cote d'Ivoire identified only two likely mother-daughter pairs (with nearly identical virus) in the context of an overall adult prevalence of SIV infection of 59% (26). In stark contrast, MTIT of SIV appears to be a frequent event in experimentally SIV-infected rhesus macaques (1, 2, 10, 15), thus suggesting a direct relationship between pathogenicity and MTIT in SIV-infected nonhuman primates.

In this work, we performed a retrospective analysis of all births to naturally SIV-infected SMs of the Yerkes colony from 1969 to 1999 to determine the rate of MTIT in this natural host species. We found that MTIT of SIV in SMs is relatively rare and is associated with a 2-log reduction in viremia compared to SMs that became SIV infected as adults. The clinical course of SIV infection was not altered by MTIT.

MATERIALS AND METHODS

Animals and serology. Two hundred forty-nine SMs born to naturally SIV-infected dams from the colony housed at the Yerkes National Primate Research Center of Emory University (YNPRC) were studied. These animals were selected from the 593 SMs born during the period 1969 to 1999 because one or more SIV tests had been performed on them. The breeding of SIV-infected SMs was halted in late 1999 due to budgetary constraints, thus no infants were available for study after this time. The SIV serological status of animals born to SIV-infected SMs was assessed by HIV-2 ELISA and Western blotting, as described previously (29). All animals were maintained in accordance with National Institutes of Health guidelines.

SIV_{smm} viral load. For SIV_{smm} RNA quantitation, RNA was extracted from plasma or serum and reverse transcribed as described previously (28). Real-time reverse transcription-PCR (RT-PCR) was performed by the amplification of 20 μl of cDNA in a 50-μl reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 4 mM $MgCl_2$, 0.2 μM forward primer, 0.3 μM reverse primer, 0.1 μM probe, and 5 U of AmpliTaq Gold DNA polymerase (reagents were from Applied Biosystems). Primer and probe sequences were targeted to the 5′-untranslated region of the SIV_{smm} genome; the forward primer sequence was 5′-GGC AGGAAAATCCCTAGCAG-3′, the reverse primer sequence was 5′-GCCCTT ACTGCCTTCACTCA-3′, and the probe sequence was 5′-6-carboxyfluorescein (FAM)-AGTCCCTGTTCRGGCGCCAA-6-carboxytetramethylrhodamine (TAMRA). Amplicon accumulation was monitored with an ABI PRISM 7500 sequence detection system (Applied Biosystems) with cycling conditions of 50°C for 2 min, 95°C for 10 min, and 40 cycles of 93°C for 30 s and 59.5°C for 1 min. The RNA copy number was determined by comparison to an external standard curve consisting of *in vitro* transcripts representing bases 216 to 2106 of the SIV_{mac239} genome. The lower limit of detection for this assay is 160 copies/ml. To confirm that the use of banked serum did not affect the level of SIV_{smm} RNA detected, we performed an analysis of SIV viral load comparing frozen and thawed serum to plasma from five SMs and found no significant differences in the results (data not shown). To determine the effect of prolonged cryopreservation on viral load measurement, we compared results from plasma frozen for approximately 7 years to those obtained at the time of sampling and found a relatively minor decline (by 0.3 log; range, 0.5 log to no change) in viral load in the cryopreserved samples (data not shown). While older (i.e., >10 years) paired cryopreserved samples were not available, these data suggest that even prolonged cryopreservation has a minor impact on the measured levels of viremia.

Lymphocyte studies and flow cytometry. Four- to seven-color flow-cytometric analysis was performed as described previously (8, 31) with whole-blood samples according to standard procedures using a panel of monoclonal antibodies (MAbs) that originally were designed to detect human molecules but that were shown to be cross-reactive with SMs (29). Flow-cytometric acquisition and analysis of samples was performed on a FACSCaliber (four-color) or an LSR-II (seven-color) flow cytometer driven by either the CellQuest or the DiVa software package, respectively (BD Biosciences). Analysis of the acquired data was performed using Flow Jo software (Tree Star).

Cytokine production by intracellular staining. Intracellular cytokine staining to detect SIV-specific T-cell responses was performed in SM peripheral blood mononuclear cells (PBMCs) as described in reference 8.

TABLE 1. Characteristics of SMs born to naturally SIV-infected dams at YNPRC

Transmission type	No. of SMs by characteristic			No. of serologic tests	
	≥1 SIV test	Male	Surviving for >1 yr	Total	At ≤1 yr of age
Presumptive MTIT	11	7	11	54	29
Non-MTIT	120	77	120	1,396	358
Nontransmission	30	7	27	476	96
Indeterminate	88	26	85	229	28
Total	249	117	243	2,155	511

CCR5 genetic analysis. The analysis of CCR5 gene polymorphisms to detect CCR5Delta2 and CCR5Delta24 alleles was conducted as described in reference 25.

Statistical analysis. For the comparison of viremia at different ages in SIV-infected SMs with presumptive MTIT, as well as the comparison between SIV-infected SMs with presumptive MTIT and SIV-infected SMs with non-MTIT, viral loads were log transformed for analysis. Mixed-effects models were used to test for differences between groups with a nested correlation structure. It was assumed that within the presumptive MTIT groups (ages ≤1 and ≥6 years), all observations are potentially correlated but that with a group the correlations would be stronger. Additionally, these models allow for various numbers of measurements being available for each animal. Values below the lower limit of detection for the assay were assigned a value of 80 copies/ml (half of the lower limit) for the purpose of statistical analyses. For the comparison of immune parameters between SIV-infected SMs with presumptive MTIT and SIV-infected SMs with non-MTIT, the nonparametric Mann-Whitney U-test or Fisher's exact test (for a mean equal to 0) was used. All analyses were conducted using Stata/MP 11.1 or GraphPad Prism 4.0c.

RESULTS

Sooty mangabey births at YNPRC. To ascertain the rate of MTIT in naturally SIV-infected sooty mangabeys (SMs), we conducted a retrospective analysis of mother-infant pairs housed at the Yerkes National Primate Research Center (YNPRC). During a 30-year time period (1969 to 1999), 593 SM infants were born. Of these, 569 were born to known or likely simian immunodeficiency virus (SIV)-infected mothers. Two hundred forty-nine progeny of SIV-infected SM dams were tested for the presence of anti-SIV antibodies at one or multiple time points, resulting in 2,155 total measurements of SIV serology available for review. In most animals, serial assessments of SIV serological status were conducted during the first year of life (resulting in 511 measurements), allowing for the documentation of the presence or absence of maternal antibody at birth and the subsequent evaluation of infection on a semimonthly basis (Table 1).

Definition of SIV transmission groups. The 249 infants with serologic data born to SIV-infected mothers were classified into four groups based on the results of SIV testing: (i) nontransmission; (ii) non-MTIT; (iii) presumptive MTIT; and (iv) indeterminate (Table 2). Serological testing was performed on all animals by ELISA and/or Western blotting. When MTIT was suspected, SIV viral load was assessed by SIV_{smm} quantitative real-time RT-PCR. Nontransmission was defined as (i) negative testing at all time points or (ii) persistent seroreversion at any time after birth. The non-MTIT group was comprised of the following SMs: (i) those who were seronegative at birth and then seroconverted after 1 year of life, and (ii) those

TABLE 2. SIV transmission in sooty mangabeys at the YNPRC

Transmission group	Characteristic(s)
Nontransmission	SIV negative at all time points SIV seropositive at birth with persistent seroreversion
Non-mother-to-infant transmission	SIV seronegative at birth with seroconversion after 1 year SIV seropositive at birth with seroreversion and then seroconversion after 1 year
Presumptive mother-to-infant transmission	SIV seropositive from birth with sample tested at ≥6 months and ≤1 yr, viral load of >500 copies/ml at ≤1 yr

who were seropositive at birth (due to maternal antibodies), seroreverted (loss of maternal antibodies), and subsequently seroconverted after 1 year of life. The SMs with presumptive MTIT were defined conservatively as those animals that met the following serologic and virologic criteria: SIV seropositivity from birth with a sample tested between 6 months and 1 year of life (to discount the contribution of passively transferred maternal antibodies that typically waned between 4 and 6 months after birth), and SIV_{smm} viral load of greater than 500 copies/ml of serum within the first year of life. The rationale for using this two-tiered approach was to account for the presence of maternal antibodies in the first 6 months of life and to provide secondary virologic confirmation of infection. Of note, late breastfeeding-related transmission events are virtually impossible to separate from early events of horizontal transmission resulting from mock sexual activity that can occur during the first year of life. As such, it is possible but basically nonprovable that a seroconversion occurring early in the second year of life is related to prolonged breastfeeding. On the other hand, it also is possible that some of the presumptive MTIT cases in fact do not reflect MTIT but rather early sexual activity. For these reasons, we believe that this combination of longitudinal serology and quantitative viral load analyses represents the best possible approach based on sample availability, and that our definition incorporates most, if not all, cases of *in utero* and intrapartum transmission, as well as the majority of cases of breastfeeding-related transmission.

Rate of MTIT of SIV is low in naturally SIV-infected SMs. There were 161 SM infants born to naturally SIV-infected dams that could be classified as nontransmission, non-MTIT, or presumptive MTIT. Of these 161 SMs, 150 (93.2%) were cases of nontransmission (30 animals) or non-MTIT (120 animals), and only 11 (6.8%) met the criteria for presumptive MTIT. Figure 1 shows the absolute number of births to naturally SIV-infected mothers at the YNPRC from 1976 to 1999 and illustrates the very infrequent occurrence of SIV transmission from mother to infant relative to overall births, particularly from 1991 to 1995. Data from prior to 1976 are not shown graphically, as no animals in the non-MTIT or presumptive MTIT groups were born before this date. This work is the first to comprehensively assess for SIV infection in a large number of infants of naturally SIV-infected SMs, and the rare incidence of MTIT in this natural host species appears to starkly contrast with the 35 to 40% mother-to-child transmission rate found in HIV infection.

Low levels of virus replication in SIV-infected SMs with presumptive MTIT. We performed SIV quantitative real-time RT-PCR in SIV-infected SMs with presumptive MTIT at multiple time points during the first year of life. The total number of samples assayed from these 11 infants was 26. As shown in Fig. 2A, the level of viremia in these infants was very low (geometric mean of 934 copies/ml of serum; range, below the limit of detection to 111,000 copies/ml of serum) compared to previously reported data on naturally SIV-infected adult SMs, which consistently show viremia in the range of 10^5 copies/ml of plasma (Fig. 2A) (31). Of note, three infants had viral loads below the lower limit of detection for the assay at one time point, and one infant was below the lower limit of detection at two time points during the first year of life. In only one instance was the undetectable viral load the first measurement obtained; the exclusion of this measurement from the analysis raised the geometric mean minimally (to 1,031 copies/ml of serum) in SM infants infected with SIV by presumptive MTIT.

We next measured viral loads in the presumptive MTIT group as adults (≥6 years of age, total of 19 samples available from 7/11 SMs) and found a geometric mean viral load of 12,055 copies/ml of serum or plasma (range, 177 to 187,000

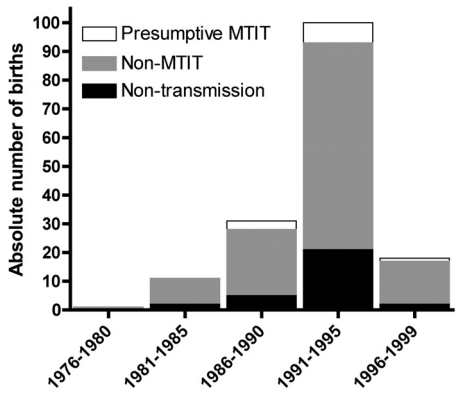
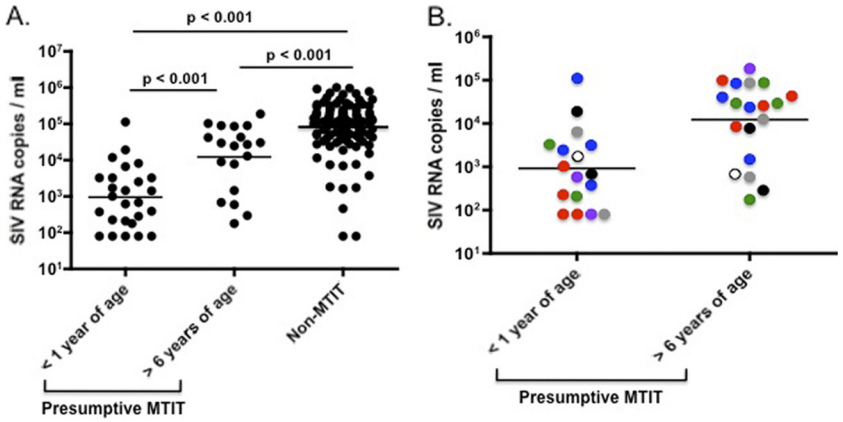


FIG. 1. SM births in the YNPRC colony from 1976 to 1999. The absolute number of SMs born to naturally SIV-infected dams is shown for each time period. Only SMs with ≥1 SIV antibody test are included ($n = 249$). SMs infected with SIV by presumptive MTIT are depicted by the white bars ($n = 11$); SMs infected with SIV by non-MTIT are depicted by the gray bars ($n = 120$); and uninfected SMs (nontransmission) are depicted by the black bars ($n = 30$). The presumptive MTIT group was SIV seropositive from birth (with a sample tested at ≥6 months and ≤1 year) and had an SIV_{smm} viral load of greater than 500 copies/ml of serum within the first year of life. The non-MTIT infected group was either seropositive at birth with seroreversion (loss of maternal antibody) and subsequent seroconversion after 1 year of life or seronegative at birth with seroconversion after 1 year of life. Nontransmission was defined as negative testing at all time points or persistent seroreversion at any time after birth. There were a further 88 SMs (not shown) who were classified as indeterminate.



C.

SMs SIV-Infected by Presumptive MTIT					
Animal Code	Age	ELISA	Western Blot	Viral Load[a]	CD4 count[b]
FDv	1 m	+	+	222	n.d.[c]
	4 m	+	+	1,001	n.d.
	7 m	+	+	< 160	n.d.
	10 m	+	+	< 160	n.d.
	8 y	+	+	102,000	1,330
	9 y	+	+	8,820	n.d.
	10 y	+	+	26,800	971
	12 y	+	+	42,100	822
FIn	1 m	+	+	207	n.d.
	9 m	+	+	3,274	n.d.
	12 y	+	+	177	n.d.
	13 y	+	+	29,600	1,715
	16 y	+	+	29,900	1,747
	17 y	+	+	88,200	919
SMs SIV-Infected by Non-MTIT					
Animal Code	Age	ELISA	Western Blot	Viral Load	CD4 count
FKr	1 m	+	+	n.d.	n.d.
	4 m	-	-	n.a.[d]	n.d.
	7 m	-	-	n.a.	n.d.
	10 m	-	-	n.a.	n.d.
	6 y	-	-	n.a.	n.d.
	7 y	+	+	n.d.	n.d.
	10 y	+	+	365,000	733
	13 y	+	+	64,366	472
	14 y	+	+	162,152	602
FNn	1 m	+	+	n.d.	n.d.
	4 m	+	+	n.d.	n.d.
	8 m	-	-	n.a.	n.d.
	10 m	-	-	n.a.	n.d.
	3 y	-	-	n.a.	n.d.
	4 y	+	+	n.d.	n.d.
	13 y	+	+	48,000	1,762
	16 y	+	+	102,608	947
	17 y	+	+	166,462	1,014

[a] SIV copies / ml of plasma or serum
[b] cells / mm^3
[c] n.d. = not done
[d] n.a. = not applicable

FIG. 2. Viral loads of SMs vary by mode of SIV transmission and age. (A) Viral loads in SMs infected with SIV by presumptive MTIT and non-MTIT were measured as SIV RNA copies per ml of serum or plasma. Serial measurements during the first year of life (≤1 year of age; n = 26) and as adults (≥6 years of age; n = 19) were performed for the 11 SIV-infected SMs with presumptive MTIT. For comparison, viral loads of 105 adult SMs infected with SIV by non-MTIT are shown. Measurements below the limit of detection for the assay are plotted at 80 SIV RNA copies/ml. Circles represent individual data points, and horizontal lines indicate geometric means. Mixed-effects models were used to determine statistical significance. (B) Direct comparison of the viral loads measured in seven SMs infected with SIV by presumptive MTIT for which samples were available at both ≤1 year of age and at ≥6 years of age. Individual animals are color coded. Measurements below the limit of detection for the assay are plotted at 80 SIV RNA copies/ml. Circles represent individual data points, and horizontal lines indicate geometric means. (C) Representative examples of SIV serology, viremia, and $CD4^+$ T-cell counts over time in two SMs infected with SIV by presumptive MTIT and two infected with SIV by non-MTIT.

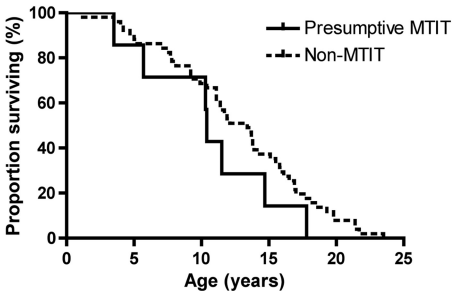


FIG. 3. Mortality is not affected by mode of SIV transmission. Kaplan-Meier survival curves are shown for SMs infected with SIV by presumptive MTIT ($n = 7$; filled line) and SMs infected with SIV by non-MTIT ($n = 51$; dashed line). The mortality rates of the two groups do not differ significantly ($P = 0.22$). The proportion of surviving SMs is shown in relation to their age in years.

copies/ml) (Fig. 2A). Statistical analysis using a mixed-effects model revealed that viral loads in SIV-infected SMs with presumptive MTIT tended to be 9.7 times higher at ≥6 years of age than at ≤1 year of age ($P < 0.001$). Interestingly, the viral loads of adult SMs infected with SIV by presumptive MTIT were approximately 1-log lower than those found in adult SIV-infected SMs with non-MTIT ($P < 0.001$) (Fig. 2A). We next performed a direct comparison of viral loads in seven SMs infected with SIV by presumptive MTIT, for which measurements were obtained at both ≤1 and ≥6 years of age, and found again that viral loads increased with age by approximately 1 log (Fig. 2B). Individual animals are color coded. Representative examples of SIV serology, viremia, and $CD4^+$ T-cell counts over time in two SMs infected with SIV by presumptive MTIT and two infected with SIV by non-MTIT are shown in Fig. 2C. These results reveal that SIV-infected SMs with presumptive MTIT have low viral loads in infancy, and that, while their levels of virus replication increase later in life, they remain lower than those of SIV-infected SMs with non-MTIT.

Mortality is not affected by mode of SIV transmission in SMs. Untreated HIV-1-infected infants have a more rapid and severe course of disease than their adult counterparts (17). To determine if a similarly rapid progression to AIDS was present in the SIV-infected SMs with presumptive MTIT described here, we compared their clinical course of infection to that observed in naturally SIV-infected SMs with non-MTIT. Four of the 11 SIV-infected SMs with presumptive MTIT are still living (14 to 19 years of age as of December 2010). The remaining seven SIV-infected SMs with presumptive MTIT died during the follow-up period, with a median age of death of 10.4 years. SIV-infected SMs in the non-MTIT group who died during follow-up (54 animals) had a median age of death of 13.4 years. We performed Kaplan-Meier survival analysis on these two groups and found no significant difference in the percent survival over time ($P = 0.22$) (Fig. 3). The fact that MTIT of SIV in SMs has no impact on their survival is indirectly confirmed by the observation that infant mortality in offspring of SIV-infected and uninfected mothers is the same (24 and 21%, respectively; data not shown).

Immune parameters are similar in SMs infected with SIV by presumptive MTIT and non-MTIT. In the first comprehensive immunological survey of 110 naturally infected SMs of the YNPRC colony (including a subset of the SIV-infected SMs with presumptive MTIT described here), correlates of preserved $CD4^+$ T-cell counts were described (30). To determine if MTIT of SIV in SMs is associated with specific immunological features, we compared a number of immune parameters in SIV-infected SMs with presumptive MTIT to those of SMs who became infected with SIV as adults (Table 3). Please note that, in this analysis, we used data that were obtained from SIV-infected SMs with presumptive MTIT as adults, since archived PBMC samples from their infancy were not available. Age ranges and medians for the two groups compared in Table 3 were not statistically different at the time of sampling. The SMs infected with SIV by presumptive MTIT described in this study showed an average $CD4^+$ T-cell count of 1,193 cells/mm^3 (Table 3), which is similar to the $CD4^+$ T-cell count (1,079 cells/mm^3) reported for SMs naturally infected with SIV by non-MTIT ($P = 0.336$) (30). Additionally, there was no difference between the percentages of $CD4^+$ and $CD8^+$ naïve, memory, and effector T cells (as defined by the markers CD28 and CD95), the activation/proliferation status of $CD4^+$ and $CD8^+$ T cells (as defined by Ki67 positivity), and $CD4^+$ and $CD8^+$ T-cell immune responses to SIV for SIV-infected SMs with presumptive MTIT compared to those of SIV-infected SMs with non-MTIT (Table 3). In a follow-up survey of 78 naturally SIV-infected SMs of the YNPRC colony (including a different subset of the SIV-infected SMs with presumptive MTIT described here), a progressive decline in $CD4^+$ T cells was found, with an average $CD4^+$ T-cell count of 679 cells/mm^3 (31). The SIV-infected SMs with presumptive MTIT did not show a steeper decline in $CD4^+$ T cells than SIV-infected SMs with non-MTIT, with average $CD4^+$ T-cell counts of 639 cells/mm^3 (Table 3). Numbers presented in Table 3 for the non-MTIT group differ slightly from those reported in the text and previously published, as they represent the sample of nat-

TABLE 3. Immune parameters in SIV-infected sooty mangabeys[a]

Parameter	Transmission group	
	Presumptive MTIT[b]	Non-MTIT[c]
$CD4^+$ T-cell count (2004)[d]	1,193	1,071
$CD4^+$ T-cell count (2009)[d]	639	674
$CD4^+$ $CCR5^+$ T cells (%)	2.1	2.9
$CD4^+$ $Ki67^+$ T cells (%)	4.1	3.1
$CD8^+$ $Ki67^+$ T cells (%)	6	3.2
$CD4^+$ $CD28^+$ $CD95^-$ (%)	43.6	37.4
$CD4^+$ $CD28^-$ $CD95^+$ (%)	0.15	0.6
$CD4^+$ $CD28^+$ $CD95^+$ (%)	56.2	61.8
$CD8^+$ $CD28^+$ $CD95^-$ (%)	24.8	20.9
$CD8^+$ $CD28^-$ $CD95^+$ (%)	21.6	21.6
$CD8^+$ $CD28^+$ $CD95^+$ (%)	52.5	57.0
Anti-SIV $CD4^+$ T-cell response: IFN-γ[e]	0	0.05
Anti-SIV $CD4^+$ T-cell response: TNF-α[e]	0.1	0.27
Anti-SIV $CD8^+$ T-cell response: IFN-γ[e]	0.08	0.27
Anti-SIV $CD8^+$ T-cell response: TNF-α[e]	0.07	0.36

[a] Means are shown for all parameters; *P* was not significant for all comparisons by Mann-Whitney U-test or Fisher's exact test (used for a mean equal to 0).
[b] $n = 5$. Unless stated otherwise, data are from 2004.
[c] $n = 105$ (2004) and 72 (2009). Unless otherwise stated, data are from 2004.
[d] In cells/mm^3.
[e] Percentage of $CD4^+$ or $CD8^+$ T cells secreting IFN-γ or TNF-α following stimulation with SIV Gag, Pol, Env, and Nef peptide pools.

urally SIV-infected SMs studied minus those animals subsequently identified as presumptively infected via MTIT. Statistical conclusions were not affected by these minor differences. Therefore, the MTIT of SIV in SMs did not differentially affect key immune parameters compared to the effects of non-MTIT of SIV.

We next investigated the possible role of CCR5 mutations in limiting MTIT of SIV in SMs. As recently described, 8% of the SMs at the YNPRC lack functional CCR5 due to homozygosity for a 2-bp deletion causing a frameshift mutation (CCR5Delta2) or double heterozygosity for CCR5delta2 and a 24-bp deletion (CCR5Delta24) (25). Of the 11 SM infants with presumptive MTIT, 6 were genotyped and none were homozygous for defective CCR5 alleles (data not shown). The frequency of homozygosity for defective CCR5 alleles in SMs belonging to the non-MTIT and nontransmission groups was 7 and 13%, respectively (data not shown). Of note, the number of genotyped animals in the presumptive MTIT and nontransmission groups was low (such that 0 and 13% frequency represent 0/6 and 1/7 animals, respectively). Thus, it does not appear that major differences in functional CCR5 expression distinguish the transmission groups described in this study.

DISCUSSION

In this study, we performed the first comprehensive longitudinal analysis of SIV acquisition in infants born to naturally SIV-infected SMs and observed that the presumptive rate of MTIT is 6.8%, i.e., much lower than the 35 to 40% MTCT rate found in HIV infection. In addition, we found that the SIV infection of infant SMs is associated with significantly lower levels of viremia than that of adult SMs. From the immunological and clinical point of view, those relatively rare instances of MTIT of SIV are associated with nonpathogenic infection with by-and-large preserved immune function, which is consistent with the phenotype observed in adult SMs.

The restriction of MTIT of SIV in this natural host species may reflect maternal and/or infant factors reducing the risk of acquisition of infection during the *in utero*, peripartum, and breastfeeding periods. A previous cross-sectional survey conducted in 1990 of a relatively small group of 27 infant SMs between 6 and 12 months of age (none of whom are included in the current work) found a prevalence of SIV_{smm} infection of 14.8%, higher than that in the current study (11). This discrepancy could be explained by the presence of non-MTIT of SIV in relatively older animals (i.e., closer to 1 year of age) via aggressive behavior and/or early mock sexual activity. Indeed, if a less stringent definition of presumptive MTIT is used in the current study, i.e., by including all SMs with a positive serology between 6 months and 1 year of life regardless of earlier seronegativity and without viral load determination, a possible rate of 13.6% MTIT is observed. However, given the generally limited availability of samples as well as the potentially problematic interpretation of serological data for these animals, we feel that the inclusion of positive viral load results in the definition of presumptive MTIT is essential to capture the biology of this type of transmission. In any event, the work described here extends that of Fultz et al. (11) by (i) increasing the number of studied animals from 27 to 249, (ii) longitudinally assessing the serological and virological status of the infants from or very close to birth, and (iii) providing data relative to the key clinical and immunological parameters of SIV infection in these infants. Importantly, our current results are consistent with the observation that MTIT of SIV is rare or absent in wild SMs, grivets, and African green monkeys (18, 24, 26). Similarly, no breastfeeding-related SIV transmission could be documented in six infant mandrills whose mothers were experimentally infected soon after giving birth (23).

We show here that SM infants infected with SIV by presumptive MTIT have 2-log less circulating virus than adults of the same colony who acquired SIV by non-MTIT. In addition, as adults, SMs in the presumptive MTIT group still have 1-log lower viral loads than SMs of similar ages infected with SIV by non-MTIT. These results are particularly intriguing given that untreated human infants with HIV infection acquired by MTCT typically have viral loads that exceed those of HIV-infected adults (7, 21, 27). In fact, asymptomatic HIV-infected infants have been reported to have levels of virus that are similar to those of HIV-infected adults with AIDS-defining illnesses (19). In a study of another natural host species, Beer et al. showed that neonatal AGMs infected either intravenously or intraperitoneally with SIV_{agm} had a delayed onset of viremia, and during chronic infection they tended to have viral loads that were lower than those of adult AGMs infected with the same virus (3). More recently, undetectable plasma viremia but the presence of replication-competent proviral DNA was observed in an infant AGM experimentally inoculated with SIV_{agm} (J. M. Brenchley, unpublished data).

We demonstrate that survival in SIV-infected SMs with presumptive MTIT was not different from that of SIV-infected SMs with non-MTIT. In addition, infant mortality was unchanged by the mother's SIV status. Previous work has shown that SIV infection does not affect mortality in adult SMs (14), which is consistent with the nonprogressive nature of SIV infection in natural hosts. MTCT of HIV, in contrast, has a significant impact on infant mortality, with more than half of all untreated children dying by the age of 2 (17). Our study also indicates that, as adults, SMs who acquire SIV by MTIT have immunological parameters similar to those of SMs who acquire SIV by non-MTIT.

The mechanisms underlying the restriction of MTIT and virus replication in infant natural hosts for SIV remain poorly understood and are likely to be complex. Conceivably, this lower level of viremia may be the result of more effective antiviral adaptive immune responses. This possibility, however, appears unlikely given the generally low levels of both SIV-specific cellular immune responses and SIV neutralizing antibody titers observed in SIV-infected SMs (8, 16). An alternative possibility, suggested by the previously quoted study of MTIT of SIV in mandrills (23), is that infant SMs have very low levels of activated $CD4^+$ T cells or $CD4^+$ $CCR5^+$ T cells that serve as targets for virus infection. Lower levels of CCR5 expression reflecting a higher fraction of CCR5-negative naïve $CD4^+$ T cells in infant SMs as well as an age-related increase in the fraction of antigen-experienced $CD4^+$ T cells may explain the relative rarity of MTIT in the offspring of SIV-infected SM dams and the low viral loads of the SIV-infected infants following MTIT. While in the current study the fraction of $CD4^+$ $CCR5^+$ T cells was not directly measured in infant SMs, this possibility is consistent with the known observation

that CCR5 expression on $CD4^+$ T cells is substantially lower in natural SIV hosts than in nonnatural hosts (22). Since SIV_{smm} appears to use additional coreceptors in SMs with CCR5 deletions (25), it also is possible that reduced susceptibility to MTIT reflects low expression of as-yet unidentified coreceptors for SIV in infant SMs.

At this time, we have not identified any specific virological, immunological, genetic, or behavioral feature that can provide a mechanistic explanation for the occurrence of MTIT of SIV in a relatively rare subset of infant SMs. An obvious possibility is that viremia during pregnancy was unusually high in the SIV-infected SM dams who successfully transmitted the virus to their infants. Unfortunately, due to the retrospective nature of the current study, samples were not available to address this hypothesis. The possibility that SIV-infected infants had higher levels of $CD4^+$ $CCR5^+$ target cells as a result of genetic factors is not supported by the observation that, as adults, the levels of these target cells were similarly low in SIV-infected SMs with both presumptive MTIT and non-MTIT. However, it is possible that environmental factors induced a transient increase of $CD4^+$ $CCR5^+$ T cells in the SIV-infected infants. Since the breeding of the SM colony at the YNPRC was reinstituted in 2010, future prospective studies will allow us to establish whether a threshold of virus replication or target cell availability is needed for MTIT of SIV in SMs.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants R01 AI066998 to G.S., RR00165 to the Yerkes National Primate Research Center, and P30 AI050409 to the Virology Core of the Emory Center for AIDS Research.

We thank the animal care and veterinary staff at the Yerkes National Primate Research Center.

REFERENCES

1. **Amedee, A. M., N. Lacour, and M. Ratterree.** 2003. Mother-to-infant transmission of SIV via breast-feeding in rhesus macaques. J. Med. Primatol. **32:**187–193.
2. **Amedee, A. M., J. Rychert, N. Lacour, L. Fresh, and M. Ratterree.** 2004. Viral and immunological factors associated with breast milk transmission of SIV in rhesus macaques. Retrovirology **1:**17.
3. **Beer, B., et al.** 1998. Simian immunodeficiency virus of African green monkeys is apathogenic in the newborn natural host. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. **18:**210–220.
4. **Bosinger, S. E., et al.** 2009. Global genomic analysis reveals rapid control of a robust innate response in SIV-infected sooty mangabeys. J. Clin. Investig. **119:**3556–3572.
5. **Brenchley, J. M., G. Silvestri, and D. C. Douek.** 2010. Nonprogressive and progressive primate immunodeficiency lentivirus infections. Immunity **32:** 737–742.
6. **Buchanan, A. M., and C. K. Cunningham.** 2009. Advances and failures in preventing perinatal human immunodeficiency virus infection. Clin. Microbiol. Rev. **22:**493–507.
7. **De Rossi, A., et al.** 1996. Dynamics of viral replication in infants with vertically acquired human immunodeficiency virus type 1 infection. J. Clin. Investig. **97:**323–330.
8. **Dunham, R., et al.** 2006. The AIDS resistance of naturally SIV-infected sooty mangabeys is independent of cellular immunity to the virus. Blood **108:**209–217.
9. **Estes, J. D., et al.** 2008. Early resolution of acute immune activation and induction of PD-1 in SIV-infected sooty mangabeys distinguishes nonpathogenic from pathogenic infection in rhesus macaques. J. Immunol. **180:**6798–6807.
10. **Fazely, F., et al.** 1993. Simian immunodeficiency virus infection via amniotic fluid: a model to study fetal immunopathogenesis and prophylaxis. J. Acquir. Immune Defic. Syndr. **6:**107–114.
11. **Fultz, P. N., T. P. Gordon, D. C. Anderson, and H. M. McClure.** 1990. Prevalence of natural infection with simian immunodeficiency virus and simian T-cell leukemia virus type I in a breeding colony of sooty mangabey monkeys. AIDS **4:**619–625.
12. **Gordon, S. N., et al.** 2007. Severe depletion of mucosal CD4+ T cells in AIDS-free simian immunodeficiency virus-infected sooty mangabeys. J. Immunol. **179:**3026–3034.
13. Reference deleted.
14. **Keele, B. F., et al.** 2009. Increased mortality and AIDS-like immunopathology in wild chimpanzees infected with SIVcpz. Nature **460:**515–519.
15. **Klumpp, S. A., et al.** 1993. Clinical and pathologic findings in infant rhesus macaques infected with SIVsmm by maternal transmission. J. Med. Primatol. **22:**169–176.
16. **Li, B., et al.** 2010. Nonpathogenic simian immunodeficiency virus infection of sooty mangabeys is not associated with high levels of autologous neutralizing antibodies. J. Virol. **84:**6248–6253.
17. **Newell, M. L., et al.** 2004. Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. Lancet **364:**1236–1243.
18. **Otsyula, M. G., et al.** 1995. Apparent lack of vertical transmission of simian immunodeficiency virus (SIV) in naturally infected African green monkeys, Cercopithecus aethiops. Ann. Trop. Med. Parasitol. **89:**573–576.
19. **Paediatric European Network for Treatment of AIDS.** 1998. HIV-1 viral load and CD4 cell count in untreated children with vertically acquired asymptomatic or mild disease. Paediatric European Network for Treatment of AIDS. AIDS **12:**F1–F8.
20. **Paiardini, M., I. Pandrea, C. Apetrei, and G. Silvestri.** 2009. Lessons learned from the natural hosts of HIV-related viruses. Annu. Rev. Med. **60:**485–495.
21. **Palumbo, P. E., et al.** 1995. Viral measurement by polymerase chain reaction-based assays in human immunodeficiency virus-infected infants. J. Pediatr. **126:**592–595.
22. **Pandrea, I., et al.** 2007. Paucity of CD4+CCR5+ T cells is a typical feature of natural SIV hosts. Blood **109:**1069–1076.
23. **Pandrea, I., et al.** 2008. Paucity of CD4+ CCR5+ T cells may prevent transmission of simian immunodeficiency virus in natural nonhuman primate hosts by breast-feeding. J. Virol. **82:**5501–5509.
24. **Phillips-Conroy, J. E., C. J. Jolly, B. Petros, J. S. Allan, and R. C. Desrosiers.** 1994. Sexual transmission of SIVagm in wild grivet monkeys. J. Med. Primatol. **23:**1–7.
25. **Riddick, N. E., et al.** 2010. A novel CCR5 mutation common in sooty mangabeys reveals SIVsmm infection of CCR5-null natural hosts and efficient alternative coreceptor use in vivo. PLoS Pathog. **6:**e1001064.
26. **Santiago, M. L., et al.** 2005. Simian immunodeficiency virus infection in free-ranging sooty mangabeys (Cercocebus atys atys) from the Tai Forest, Cote d'Ivoire: implications for the origin of epidemic human immunodeficiency virus type 2. J. Virol. **79:**12515–12527.
27. **Shearer, W. T., et al.** 1997. Viral load and disease progression in infants infected with human immunodeficiency virus type 1. Women and Infants Transmission Study Group. N. Engl. J. Med. **336:**1337–1342.
28. **Silvestri, G., et al.** 2005. Divergent host responses during primary simian immunodeficiency virus SIVsm infection of natural sooty mangabey and nonnatural rhesus macaque hosts. J. Virol. **79:**4043–4054.
29. **Silvestri, G., et al.** 2003. Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. Immunity **18:**441–452.
30. **Sumpter, B., et al.** 2007. Correlates of preserved CD4(+) T cell homeostasis during natural, nonpathogenic simian immunodeficiency virus infection of sooty mangabeys: implications for AIDS pathogenesis. J. Immunol. **178:** 1680–1691.
31. **Taaffe, J., et al.** 2010. A five-year longitudinal analysis of sooty mangabeys naturally infected with simian immunodeficiency virus reveals a slow but progressive decline in CD4+ T-cell count whose magnitude is not predicted by viral load or immune activation. J. Virol. **84:**5476–5484.
32. **Worobey, M., et al.** 2010. Island biogeography reveals the deep history of SIV. Science **329:**1487.