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Impact of Viral Factors on Very Early In Vivo Replication Profiles in Simian Immunodeficiency Virus SIVagm-Infected African Green Monkeys

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To better understand which factors govern the levels of viral loads in early lentiviral infections of primates, we developed a model that allows distinguishing between the influences of host and viral factors on viremia. Herein we report that two species of African green monkeys (*Chlorocebus sabaeus* and *C. pygerythrus*) infected with their respective wild-type simian immunodeficiency virus SIVagm viruses (SIVagm.sab92018 and SIVagm.ver644) consistently showed reproducible differences in viremia during primary infection but not at later stages of infection. Cross-infections of SIVagm.sab92018 and SIVagm.ver644 into, respectively, *C. pygerythrus* and *C. sabaeus* revealed that the dynamics of viral replication during primary infection were dependent on the viral strain used for the infection but not on the host. Hence, the kinetics of SIVagm.sab92018 and SIVagm.ver644 were similar in both sabaeus and vervet animals, indicating that the difference in viremia levels between the two groups during the early phase of infection was not associated with the host. Coreceptor usage for these two strains showed a larger coreceptor repertoire for SIVagm.sab92018, which is able to efficiently use CXCR4 in addition to CCR5, than for SIVagm.ver644, which showed a classical CCR5 coreceptor usage pattern. These differences could not be explained by different charges of the V3 loop for SIVagm.sab92018 and for SIVagm.ver644. In conclusion, our study showed that the extent of virus replication during the primary infection is primarily dependent on viral determinants.

African green monkeys (AGMs), together with many other African nonhuman primate species, are natural hosts of simian immunodeficiency virus (SIV). Each of the four AGM species, i.e., vervet (*Chlorocebus pygerythrus*), grivet (*C. aethiops*), sabaeus (*C. sabaeus*), and tantalus (*C. tantalus*), carries its species-specific SIVagm subtype, referred to as SIVagm.ver, SIVagm.gri, SIVagm.sab, and SIVagm.tan, respectively (1, 26, 29, 49). SIVagm infections in AGMs were repeatedly reported to be nonpathogenic (6, 9, 18, 22, 23), similar to what has been reported for other SIV infections in natural hosts (32). Immunodeficiency is rare in African nonhuman primate hosts (2, 40, 43, 57, 68). This is in striking contrast to the outcome of human immunodeficiency virus (HIV) and SIVmac infections in humans and macaques, in which infection almost always progresses to AIDS. SIVagm, as well as SIVsm, SIVlhoest, and SIVsun are pathogenic in pig-tailed macaques and lead to the development of immunodeficiency in this species (20, 24, 25). In contrast, cross-species transmissions of SIVagm to baboons, patas monkeys, and white-crowned mangabeys occurring in the wild were not reported to cause AIDS (30). However, in these cases, the follow-up was too short for a proper evaluation of these cross-species transmissions.

The reasons for the rarity of disease progression in natural hosts have not yet been elucidated. In pathogenic HIV-1/SIVmac infections, plasma viral RNA (vRNA) levels are closely related to disease progression, and the set-point level of viremia (occurring several weeks postinfection [p.i.]) is predictive for the subsequent disease course (46, 54, 66). Some studies reported that SIV replication levels in macaques as early as day 7 p.i. predict postacute plasma vRNA levels and thereby disease progression (39, 63). In nonpathogenic SIV infection in the natural host, neither early nor set-point viremia levels are predictive for the outcome of SIV infection. AGMs can display high plasma viral load (VL) levels during primary infection (up to 2×10^8 vRNA copies/ml) (18). During the chronic phase of infection, plasma vRNA levels show a wide range in naturally SIVagm-infected AGMs. Some AGMs have low VLs (below 10^3 copies/ml plasma), whereas in others, viral replication during the chronic phase is high (3.5×10^6 to 1.1×10^7 RNA copies/ml) (9, 18, 22, 51). Thus, viral replication in AGMs reproduces situations observed in pathogenic infections (9, 18, 22). Wide ranges of viremia were also described in chronically SIVsm-infected sooty mangabeys (12, 41, 60, 61), SIVmnd-1-infected mandrills (55, 56), and SIVcpz.ant-infected chimpanzees (65). Thus, significant differences in the degrees of viremia have been reported during SIV infection in the natural hosts, similar to HIV-1 and SIVmac infections in humans and macaques, respectively, but the relationship between these differences in the con-

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TABLE 1. Description of animals infected with SIVagm.sab9218 or SIVagm.ver644

Animal[a]	Sex[b]	Age (yr)[c]	SIVagm strain, inoculum	CD4 (cells/mm^3)[d]	P27 (ng/ml) at peak	RNA VL at peak	RNA VL a t set point
sabaeus 96001	M	2.5	sab92018	894	4	4.1×10^7	6.0×10^3
sabaeus 96008	M	2.5	sab92018	413	1.25	4.0×10^6	3.0×10^3
sabaeus 96011	F	2.5	sab92018	745	5	3.9×10^6	6.0×10^3
sabaeus 96023	F	2.5	sab92018	1,337	1.8	3.8×10^7	6.0×10^3
sabaeus 96010	M	2.5	sab92018, 30 TCID50	n.d.	0.64	n.d.	n.d.
sabaeus 98007	M	3–4	sab92018, 100 TCID50	1,487	19.8	3.4×10^7	8.5×10^4
sabaeus 98008	M	3–4	sab92018, 100 TCID50	3,414	0.72	2.1×10^7	5.6×10^3
sabaeus 98011	F	3–4	sab92018, 100 TCID50	1,739	4.96	2.5×10^7	3.7×10^3
sabaeus 97008	F	3–4	sab92018, 300 TCID50	1,242	n.d.	1.0×10^7	9.3×10^4
sabaeus 98013	M	3–4	sab92018, 300 TCID50	686	n.d.	7.1×10^7	8.5×10^4
sabaeus 00020	F	>5	sab92018, 300 TCID50	828	n.d.	9×10^7	3×10^4
sabaeus 01013	F	1.5–2	sab92018, 300 TCID50	1,701	n.d.	8.8×10^7	7.8×10^4
sabaeus 01015	M	1.5–2	sab92018, 300 TCID50	2,004	n.d.	1.0×10^7	6.2×10^4
sabaeus 02003	M	1.5–2	sab92018, 300 TCID50	1,143	n.d.	3.7×10^7	3.7×10^5
vervet 728	M	15	ver644, 100 TCID50	n.d.	<0.05	5.0×10^4	1.0×10^3
vervet 805	M	14	ver644, 100 TCID50	n.d.	0.22	5.0×10^4	1.1×10^3
vervet 511B	F	1.5	ver644, 100 TCID50	819	0.11	5.9×10^5	5.0×10^3
vervet 1421C	M	2.5	ver644, 100 TCID50	905	0.08	1.5×10^6	2.3×10^4
vervet 1517	F	8	ver644, 100 TCID50	n.d.	0.56	n.d.	n.d.
vervet 1426	F	9	ver644, 100 TCID50	358	0.18	2.4×10^4	3.4×10^3
vervet 1465	F	9	ver644, 100 TCID50	261	0.14	2.4×10^5	4.4×10^3
sabaeus 96029	F	6–7	ver644, 100 TCID50	601	0.05	$<1.0 \times 10^3$	$<1.0 \times 10^3$
sabaeus 97005	M	6–7	ver644, 100 TCID50	718	0.36	1.3×10^5	1.2×10^3
vervet 511A	M	4	sab92018, 100 TCID50	n.d.	10.86	7.2×10^6	1.6×10^3
vervet 535B	F	3–4	sab92018, 100 TCID50	n.d.	9.28	1.5×10^8	3.4×10^4

[a] Data for four AGMs (96001, 96008, 96011, and 96023) are derived from previous experiments (18) and published elsewhere (18).
[b] M, male; F, female.
[c] Age and CD4 values are from the time of infection.
[d] n.d. not determined.

text of natural infections is still poorly understood. To better understand which factors govern the level of VL, we developed a virus-host system that allows distinguishing between the influences of host and/or viral factors on SIV viremia. This system is constituted by AGMs belonging to two species, sabaeus (*C. sabaeus*) and vervet (*C. pygerythrus*), and two SIVagm strains, SIVagm.sab92018 and SIVagm.ver644. Infections of the two AGM species with these two SIV strains consistently showed reproducible differences in peak viremia. We have performed cross-infections between the two groups of animals to examine if the differences in VLs could be attributed rather to host and/or viral factors. Our study showed that the extent of early virus replication is primarily dependent on viral determinants and not on the host, whereas the host environment has a greater impact on the viremia starting from the postacute infection phase on.

MATERIALS AND METHODS

Animals and infections. The animals in this study consisted of 25 AGMs (16 sabaeus and 9 vervet monkeys) housed at the Institut Pasteur in Senegal and the Centre International de Recherche Medicale Franceville, Franceville, Gabon, respectively (Table 1), according to national and international guidelines. All monkeys included in this study were tested prior to their inclusion and shown to be seronegative for simian T-cell lymphotropic virus (STLV) and SIV (Bio-Rad, Marne la Coquette, France). Negativity for SIV was also confirmed by PCR on the day of infection. The virus stocks used for the infections consisted of SIVagm.sab92018 and SIVagm.ver644. To avoid selection of viral variants in vitro, all inocula used in this study consisted of blood derivatives (i.e., plasma) obtained from SIVagm.ver644- or SIVagm.sab92018-infected vervet and sabaeus monkeys, respectively, during primary infection and were prepared as described elsewhere (18). Titers of plasma stocks were determined on SupT1 cells as previously described (18). Inoculations were performed intravenously. Four sabaeus already reported elsewhere (96001, 96008, 96011, and 96023) received plasma and peripheral blood mononuclear cells (PBMC) (18). All other viral stocks were cell free; the infectious doses of these inocula are presented in Table 1.

Specimen collection. Whole EDTA blood was collected from every monkey four times before infection (days −60, −30, −15, and 0), twice weekly throughout the first 3 weeks p.i., weekly at the end of primary infection and at the beginning of the postacute phase (days 28 and 35 p.i.), and finally, every 3 months during chronic infection (between 2 and 12 months p.i.). EDTA-treated whole blood was used for flow cytometry. Plasma aliquots were stored at −70°C and used to quantify VL.

Antibody detection. Antibody responses to SIV infection were monitored by enzyme-linked immunosorbent assay (ELISA) using either Genscreen (Bio-Rad, France) or Elavia II (Sanofi-Pasteur, France) and SIV-specific enzyme immunoassay based on peptides mapping the conserved Gp41 immunodominant region and the highly variable V3 loop (62). Serological reactivities have been confirmed by Western blotting for all the monkeys included in this study (New Lav Blot II; Bio-Rad, Marne-la-Coquette, France).

Quantification of viral P27 antigenemia. Plasma samples were assayed for P27 antigen by using an antigen capture ELISA for SIV Gag P27 (Coulter Corporation, Hialeah, FL) with the standards provided in the kit. Quantification was done according to the manufacturer's instructions. This ELISA kit was initially designed to detect P27 antigens of SIVmac, but it was reported to detect also Gag antigens of SIVagm (37). In addition, in previous studies we confirmed its cross-reactivity for SIVagm Gag by testing culture supernatants of cells infected by SIVagm.ver and SIVagm.sab as well as plasma samples from acutely infected AGMs. Supernatants from uninfected cells and plasma from uninfected AGMs were used as negative controls (18).

Viral RNA quantification. Viral RNA was extracted from 540 μl of plasma using the QIAamp viral RNA extraction kit (QIAGEN, Courtaboeuf, France). Real-time (RT) PCR assays specific for each virus were developed for SIVagm RNA quantification. Briefly, total RNA was retrotranscribed into cDNA by use of the TaqMan Gold RT PCR kit and random hexamers (PE, Applied Biosystems, Foster City, CA). PCRs were carried out in a spectrofluorometric thermal cycler (ABI PRISM 7700; PE). For both viral strains, the quantifications were based on the amplification of a 180-bp-located long terminal repeat (LTR)

region. This region is highly conserved within different SIVagm types. Different sets of primers have been designed for each viral strain, due to significant nucleotide diversity among SIVagm subtypes. The primers and probe for SIVagm.sab were J15S (5′-CTG GGT GTT CTC TGG TAA G-3′), 5′ J15S (5′-CAA GAC TTT ATT GAG GCA AT-3′), and J15P (6-carboxyfluorescein-CGA ACA CCC AGG CTC AAG CTG G-6-carboxytetramethylrhodamine) as previously described (18). SIVagm.sab cDNA was added to the universal master mix (PE, Applied Biosystems), containing 10 μM of each primer and 10 μM of the probe. The LTR primers used for the SIVagm.ver quantification were SIVagm.verFW2 (5′-TTG AGC CTG GGT GTT C-3′) and SIVagm.verREV1 (5′-CAA GAC TTT ATT GAG GCA AT-3′). The PCR mix contained the SIVagm.ver cDNA, 2× universal SybrGreen master mix buffer (Applied Biosystems, Courtaboeuf, France), and primers in a final concentration of 0.3 μM. All PCRs were carried out in duplicate in parallel to a negative non-reverse transcription control reaction. The PCR cycling conditions were identical for all assays: a first cycle of denaturation (95°C, 10 min) was followed by 45 cycles of denaturation (95°C, 10 s), annealing (50°C, 30 s), and extension (72°C, 30 s). Absolute viral RNA copy numbers were deduced by comparing the relative signal strengths to corresponding values obtained for five 10-fold dilutions of standard RNAs that were reverse transcribed and amplified in parallel. In order to construct these RNA standards, larger LTR regions of SIVagm.sab and SIVagm.ver were PCR amplified. The SIVagm.sab LTR PCR product was obtained by amplifying a SIVagm.sab reference virus (plasmid psab-1) in a PCR with primers LTR2A (5′-AAC TAA GGC AAG ACT TTA TTG AGG-3′) and LTR4S (5′-ACT GGG CGG TAC TGG GAG TGG CTT-3′). For SIVagm.ver644 RNA, a DNA fragment was PCR generated using the primer pair J15V (5′-TGC ATA AAA GCA GAT GCT-3′) and VervetLTR (5′-AGA GAG AAC CCA GTA AG-3′). The PCR products were cloned into a pCR 2.1 vector (Invitrogen, Groningen, Netherlands). In vitro transcription was then performed using the MEGAscript kit (Ambion, Austin, TX). Known amounts of the SIVagm LTR standard RNAs were used to determine the target copy numbers. The detection limit of the SIVagm quantification assays was 5×10^2 RNA copies/0.5 ml of plasma.

SIVagm *nef* sequencing. In order to sequence SIVagm.ver644 *nef*, DNA was extracted from PBMCs collected early after infection (day 10) from an SIVagm.ver644-infected monkey (1421C). The *nef* coding region was PCR amplified using the following primers located in *env* and the LTR (TAR): forward primer envB (29) and reverse primer TAR (5′-CCA GCG AAC ACC CAG GCT C-3′). The PCR product was purified (Qiaex II gel extraction kit, QIAGEN) and sequenced using BigDye Terminator reagents (Applied Biosystems). Additional primers were used for the sequencing as follows: ver.NefFW2 (5′-GGA AAC AGG CAC AAA GAC AAC TCA A-3′), ver.NefRV2 (5′-CCA CGC ATT CCA ATC ATC AAT AAT TC-3′), ver.NefFW4 (5′-GA ATT ATT GAT GAT TGG AAT GCG TGG-3′), and ver.NefRV4 (5′-T TGA GTT GTC TTT GTG CCT GTT TCC-3′).

SIVagm *env* V3 loop sequencing. We sequenced genomic RNA from SIVagm.sab92018, SIVagm.ver644, and SIVagm.sabD46. For SIVagm.sab92018, the RNA was that of the inoculum (corresponding to plasma collected at the peak in one infected AGM [00008]). For SIVagm.ver644, the RNA was extracted from a plasma sample collected at the peak in another infected AGM (511b). Viral RNA was extracted by using a QIAamp viral RNA mini kit (QIAGEN). Then, viral RNA was retrotranscribed by using SuperScript II RNase H$^-$ reverse transcriptase (Invitrogen) according to the manufacturer's instructions with primers EnvASab and EnvBSab (18). Reverse transcription was done at 25°C for 10 min, 50°C for 30 min, and 70°C for 15 min. Resultant cDNA was amplified by PCR using the proof-reading DNA polymerases TAKARA Ex *Taq* (Takara Bio) or Platinum *Taq* (QIAGEN) following the manufacturer's instructions. The PCR conditions consisted of 40 cycles of denaturation (95°C for 10 s), hybridization (55°C for 30 s), and elongation (72°C for 1 min). Due to low copy numbers, we submitted amplicons obtained from SIVagm.ver644 cDNA to a seminested PCR by using the same conditions as those for the PCR along with the following primers: EnvAsab and EnvBsab for the first round and EnvAsab and NS3asVerTYO (5′ GAA GCC TAA GAA CCC TAG CAC AAA 3′) for the seminested reaction. Purified amplicons were sequenced (BigDye Terminator Ready Reaction mix, Applied Biosystems). For sequencing, primers EnvAsab, EnvBsab, NS3asSab (5′ AAG CCT AGG AAC CCT AGC ACG AA 3′; reverse), NS3asVerTYO, MMas (5′ TTC AGA TAA TTT AGA AAC CAA TC 3′; reverse), and MMasSab92018 (5′ TTG AGG TAA TTC AAA AAC CAA TC 3′; reverse) were used.

Flow cytometry analysis. From a subset of 11 sabaeus and 4 vervets, CD4$^+$ and CD8$^+$ lymphocyte subsets from AGMs were analyzed by flow cytometry. One hundred microliters of EDTA-treated whole blood was stained with 5 μl of anti-CD4 (Leu-3a; Becton Dickinson, Mountain View, CA) or anti-CD8 (Leu-2a; Becton Dickinson), together with anti-CD3 (FN18; Biosource) using appropriate negative controls in parallel (immunoglobulin G1 [IgG1] fluorescein isothiocyanate [PN0639; Immunotech] and IgG2a phycoerythrin and IgG1 phycoerythrin [Becton Dickinson]). Cells were incubated with the antibody for 20 min at 4°C. This was followed by an incubation of 5 min in a lysing solution containing NH_4CO_3H (0.9 mM) and NH_4Cl (131 mM) to lyse the erythrocytes. Cells were then washed twice with phosphate-buffered saline and fixed with 1% paraformaldehyde. Samples were analyzed on a FACScalibur flow cytometer using Cell Quest software (Becton Dickinson).

Coreceptor usage. The coreceptor usage of SIVagm.sab92018 and SIVagm.ver644 was determined on U87 and GHOST cells as previously described (7, 47, 48). The U87 cells expressed CD4 alone or along with CXCR4 or CCR5. The GHOST cells expressed CD4 alone or CD4 in combination with the following coreceptors: CCR2b, CCR3, CCR5, CXCR4, BOB/GPR15, and Bonzo/STRL33. Cells were cultured in complete Dulbecco's minimal essential medium containing G418 (5 μg/ml), hygromycin (1 μg/ml), and puromycin (1 μg/ml). GHOST cells expressing only CD4 were used as controls; they were cultured in the same medium except that puromycin was omitted. Expression of the coreceptors on these cells was verified at the day of infection. 2×10^5 cells were exposed to at least three distinct infectious titers (between 2 and 150 times the 50% tissue culture infective dose [$TCID_{50}$]) of each virus. Productive viral replication was monitored by P27 measurement in the culture supernatants on days 0, 2, 4, 6, and 9 or subjected to fluorescence-activated cell sorter analysis to detect green fluorescence protein expression 3 days after infection. For P27 Ag measurements, supernatants were clarified from cells by short-term centrifugation. P27 Ag tests were performed as described above. Several positive controls were included: HIV-1_{B117} (previously called virus isolate 25), which uses the coreceptors CXCR4, CCR5, Bonzo, and Bob (8), and two SIVagm.sab isolates, i.e., SIVagm.sabD46 (59) and SIVagm.sab2 (1). We previously reported that the latter uses CCR5 and CXCR6 (36), as do most SIVagm strains analyzed thus far. SIVrcm (21) was used as a CCR5 CXCR4 negative control because it has been previously reported to specifically use CCR2 as its main coreceptor (13). Uninfected cells were used as a negative control.

Statistical analysis. Differences in viral replication between different groups of AGMs were analyzed for significant differences ($P < 0.05$) by the Mann-Whitney test and Spearman rank analysis with Statview software.

Nucleotide sequence accession numbers. The nucleotide sequence of the *nef* gene of SIVagm.ver644 was deposited in GenBank under accession number AY773283. *env* gene fragments encompassing the V3 regions of SIVagm.sab92018, SIVagm.sabD46, and SIVagm.ver644 were deposited in GenBank under the accession numbers AY766078, AY766079, and AY766080, respectively.

RESULTS

Transient early T CD4$^+$ cell decline in SIVagm.sab92018-infected monkeys. All sabaeus and vervet monkeys inoculated with SIVagm.sab92018 and SIVagm.ver644, respectively, became infected and seroconverted between 28 and 45 days p.i. (data not shown). The dynamics of the CD4$^+$ and CD8$^+$ T-cell counts were measured in a subset of 15 AGMs (11 sabaeus and 4 vervets). The SIVagm.sab92018-infected monkeys which had high (above 300 cells/μl) CD4$^+$ T-cell counts at the time of infection consistently demonstrated a significant drop in CD4$^+$ T-cell numbers between days 6 and 21 p.i., reaching a minimum between days 8 and 10 p.i. (Fig. 1a). This decline was also observed in the CD8$^+$ T-cell compartment, although the duration was shorter and the rebound more pronounced (data not shown). Thus, similar to macaques with SIVmac251 infection, SIVagm.sab92018-infected AGMs demonstrated declines in blood CD4/CD8 ratios during primary infection. However, peripheral CD4$^+$ and CD8$^+$ lymphocyte counts in SIVagm.sab92018-infected AGMs readily rebounded to close-to-preinfection values. This resulted in an only transient decrease of the peripheral CD4$^+$ T cells during acute SIVagm.sab920198 infection. After day 28 p.i., no significant changes in the pe-

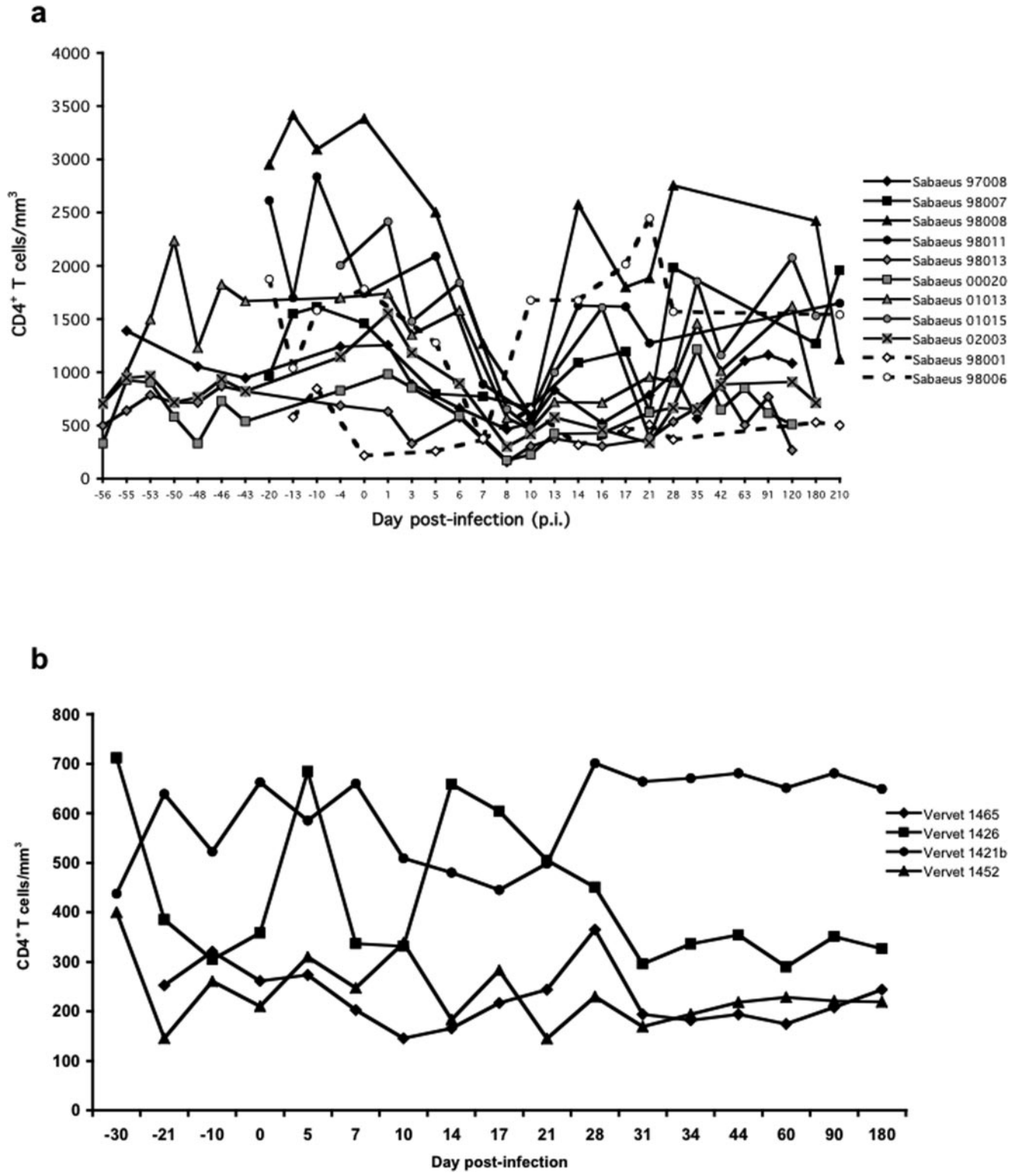

FIG. 1. $CD4^+$ T-cell numbers (/mm^3) before and after infection in 11 sabaeus infected with SIVagm.sab92018 (a) and 4 vervets infected with SIVagm.ver644 (b). Only SIVagm.sab92018-infected sabaeus showed constant significant declines in CD4 T-cell counts at the peak of plasma viral load. The $CD4^+$ T cell counts rebounded to normal values after the viremia set point was reached.

ripheral T $CD4^+$ cell counts were observed in the infected AGMs.

In contrast to SIVagm.sab92018-infected AGMs, SIVagm.ver644-infected monkeys showed only moderate depletions of $CD4^+$ T cells (Fig. 1b) not resulting in significant changes in peripheral $CD4^+$ or $CD8^+$ cell counts subsequent to the infection. Thus, $CD4^+$ and $CD8^+$ T-cell dynamics did not alter blood CD4/CD8 ratios throughout the courses of infection in SIVagm.ver644-infected vervets (data not shown).

High levels of P27 antigenemia in SIVagm.sab92018-infected AGMs contrasts with low levels of P27 antigenemia in SIVagm.ver-infected monkeys. To evaluate systemic viral replication, we measured two viral parameters: plasma P27 antigenemia and plasma vRNA. We first quantified P27 antigenemia in a subset of eight SIVagm.sab92018-infected sabaeus monkeys. All eight animals were positive for P27 during primary infection. The peak P27 values varied from 0.72 to 19.8 ng/ml of plasma (Table 1). Therefore, we confirm our previous observation that the peak P27 values in SIVagm.sab92018-infected sabaeus monkeys are high and comparable to levels previously described for pathogenic SIVmac infections (10). No significant association was found between the P27 antigenemia levels at their peaks and the inoculum sizes used (30, 100, and 300 times the $TCID_{50}$). After primary infection, P27 antigenemia became undetectable, similar to what was shown during the asymptomatic phase of chronic SIVmac and HIV-1 infections.

In contrast to the high peak antigenemia levels in SIVagm.sab92018-infected sabaeus monkeys, the P27 peak values for seven SIVagm.ver644-infected vervet monkeys (728, 805, 511B, 1421C, 1517, 1426, and 1465) were all significantly lower ($<$0.05 to 0.56 ng/ml) (Table 1), suggesting that although there were noteworthy interindividual variations among AGMs infected with a same virus, the patterns of early SIVagm replication in the two subspecies (as reflected by P27 antigenemia levels) were significantly different.

Higher plasma VLs during primary infection with SIVagm.sab92018 than with SIVagm.ver644. The kinetics of SIVagm.sab92018 vRNA levels in sabaeus monkeys are shown in Fig. 2a. For all 13 animals, the peak plasma viremia levels were high (between 3.9×10^6 and 10^8 vRNA copies/ml) (Fig. 2a and Table 1). The peak was reached at the latest at day 10 p.i. in all animals. Plasma viremia levels in the six SIVagm.ver644-infected vervets analyzed (728, 805, 1426, 1465, 511B, and 1421C) peaked later than those in sabaeus monkeys, i.e., at day 12 or 14 p.i (Fig. 2b) and were always lower than those observed during SIVagm.sab92018 infection of sabaeus monkeys, ranging from 3×10^4 to 1.5×10^6 vRNA copies/ml at the peak (Fig. 2b and Table 1). Thus, plasma vRNA quantification confirmed the high antigenemia levels detected in SIVagm.sab92018-infected animals and showed that the differences observed between the two groups based on P27 quantification are not due to a better cross-reactivity of the anti-SIVmac P27 antibodies for SIVagm.sab than for SIVagm.ver P27. Furthermore, the plasma vRNA levels in vervet and sabaeus monkeys significantly differed from each other during primary infection (Mann-Whitney $P = 0.0002$). Following primary infection, plasma viremia levels in vervets and sabaeus declined to the set-point levels, which ranged between 3×10^3 and 2×10^5 vRNA copies/ml in SIVagm.sab92018-infected AGMs (Fig. 2a) and between $<10^3$ and 5.2×10^4 vRNA copies/ml in SIVagm.ver644-infected AGMs (Fig. 2b). The vRNA plasma levels during the postacute and chronic phases were not significantly different between the two groups and were in the same range as those reported in naturally infected AGMs (22).

No correlation between VLs and preinfection $CD4^+$ T-cell counts. Analyses of plasma VL thus led to the identification of a consistent difference in early viremia levels between sabaeus and vervets infected with their species-specific SIVagm strains. This difference might be due to viral and/or host factors. Dissimilarities between the two groups of monkeys with regard to their basal peripheral $CD4^+$ counts and ages were considered as potential host factors responsible for such a difference, since studies of SIVsm infection in sooty mangabeys reported that age-related changes in T-cell homeostasis may impact VL values (11). The sabaeus and vervet monkeys for which we examined plasma vRNA did not, however, differ significantly with regard to their ages ($P = 0.11$) or basal CD4 counts ($P = 0.26$). More importantly, the vRNA peak values were not associated with the basal $CD4^+$ counts of the infected animals (Spearman rank correlation, $P = 0.38$), whereas there was a trend for an association of the vRNA peak levels with the ages of the animals (Spearman rank correlation, $P = 0.067$). This correlates with the observation that within the same group, the highest values were often observed in the younger animals. However, differences in the ages of the animals alone were not sufficient to explain differences in the levels of viremia between the two groups, as young SIVagm.ver644-infected vervets (511B, 1421C) showed lower viremia levels than SIVagm.sab92018-infected sabaeus monkeys of the same age, thus suggesting only a minor influence of the age of the respective animal on the height of its initial peak vRNA level during primary infection.

Cross-infections. To study whether the observed differences in early viremia between the two groups could be attributed to intrinsic species-specific host factors and to differentiate between host and viral factors, we infected two sabaeus monkeys (96029 and 97005) with 100 times the $TCID_{50}$ of SIVagm.ver644 and two vervets (511A and 535B) with 100 times the $TCID_{50}$ of SIVagm.sab92018. Follow-up of P27 levels in blood revealed an association between antigenemia and the virus strain used for the infection but not with the host species. Thus, high plasma P27 levels were observed in SIVagm.sab92018-infected vervets during primary infection (9.3 and 10.9 ng/ml at day 10 p.i.) (Table 1). In contrast, P27 antigenemia levels in SIVagm.ver644-infected sabaeus monkeys were very low (0.36 ng/ml in 97005 and undetectable in 96029). We cannot exclude the possibility that these low viremia levels were due to host-specific barriers in sabaeus monkeys exposed to the SIVagm.ver644 virus. However, in vervets we observed high antigenemia levels with SIVagm.sab92018 and low antigenemia levels with SIVagm.ver644. Therefore, species barriers could not explain the observed differences.

The differences in antigenemia levels between SIVagm.ver644-infected sabaeus monkeys and SIVagm.sab92018-infected vervets were confirmed by quantification of plasma VL. The two vervets infected with SIVagm.sab92018 (511A and 535B) showed high numbers of vRNA copies during primary infection, peaking at 10 days p.i. and ranging from 7.2×10^6 to

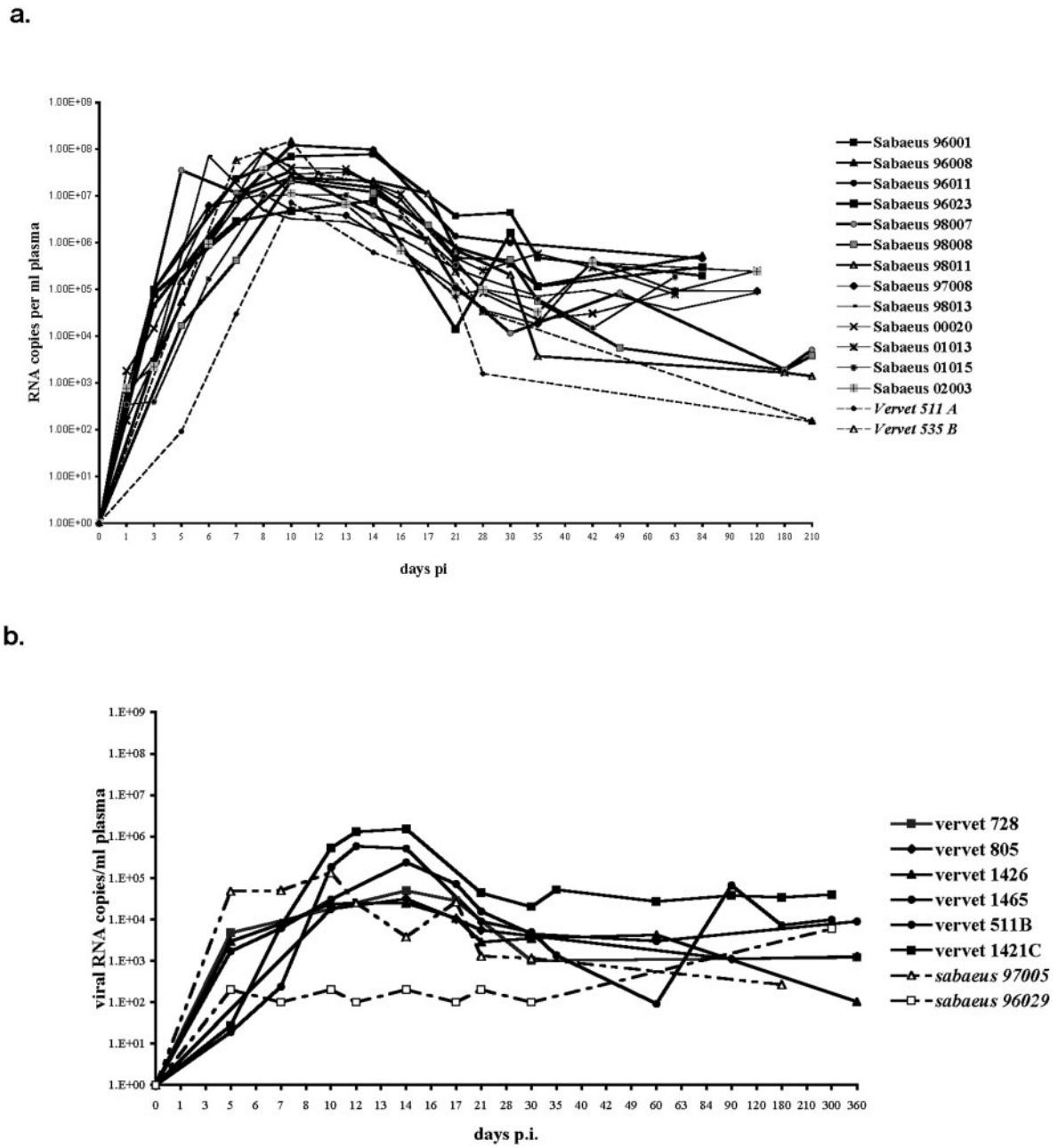

FIG. 2. Viral RNA copy numbers in plasma samples from SIVagm.sab92018- and SIVagm.ver644-infected AGMs. The detection limit of the assays was 10^3 copies/ml and is indicated by the dashed horizontal lines. Values below the detection limit were arbitrarily set to 5.10^2 copies. (a) Plasma RNA load in SIVagm.sab92018-infected AGMs. The solid lines stand for sabaeus monkeys and the dashed lines for vervet monkeys infected with SIVagm.sab92018. RNA levels in sabaeus monkeys 96001, 96008, 96011, and 96023 are not shown since they have already been published elsewhere (18). (b) Plasma RNA levels in SIVagm.ver644-infected AGMs. The solid lines stand for vervets and the dashed lines for sabaeus monkeys infected with SIVagm.ver644. In animal 96029, viral RNA was detected only once at day 360 p.i. (7×10^3 RNA copies/ml).

1.5×10^8 copies/ml (Fig. 2a). In contrast, the two sabaeus monkeys (97005 and 96029) infected with SIVagm.ver644 showed lower vRNA levels during primary infection (Fig. 1b). Thus, in monkey 96029, the plasma vRNA level was below the detection limit, and monkey 97005 showed only 1.35×10^5 viral RNA copies at the peak, similar to the peak levels monitored in vervets infected with SIVagm.ver644. Hence, the kinetics of SIVagm.sab92018 and SIVagm.ver644 were similar in both sabaeus and vervet monkeys, indicating that the difference in viremia levels between the two groups during the early phase of infection was not determined by their species.

No deletion in SIVagm.ver644 *nef*. The cross-infection studies pointed out the predominant role of viral determinants for early viremia levels. Among viral factors, the presence of an intact *nef* gene is known to be associated with higher VL in vivo (17). To study whether deletions in the *nef* gene of SIVagm.

ver644 could be responsible for the lower early viremia levels, we sequenced the *nef* gene of SIVagm.ver644. We did not find deletions or premature stop mutations (data not shown). Amino acid stretches previously described as conserved in all SIVagm isolates (31) were also readily identified in SIVagm.ver644 *nef*. The myristoylation signals (GXXXS) as well as both domains essential for the interactions of Nef with GTP were found to be identical to the amino acid sequence of the SIVagm.verTYO prototype strain (19). The putative interacting sites with GTP and the Pro-rich sequence (PXXPXXP) which binds to the SH3 domain of Src kinases were conserved in SIVagm.ver644 (3).

Wild-type SIVagm.sab92018 uses CXCR4 as a coreceptor. We tested if differences in coreceptor usage exist between wild-type SIVagm.sab92018 and SIVagm.ver644. GHOST and U87 cells expressing various chemokine receptors were infected with the two viruses to investigate whether results observed for SIVagm.sab92018 are characteristic for SIVagm.sab or are strain specific. We also tested two other SIVagm.sab strains: SIVagm.sabD46 (59) and SIVagm.sab2 (1). We also included HIV-1_{B117} as a positive control for CCR5, CXCR4, Bob, and Bonzo usage, as well as SIVrcm (13) as a CCR5 CXCR4 negative control. All tested viruses were able to use efficiently CCR5 as a coreceptor, with the exception of SIVrcm, which mainly used CCR2 as previously described (13). Interestingly, we observed that two analyzed SIVagm.sab strains (SIVagm.sab92018 and SIVagm.sabD46) were capable of using CXCR4, especially SIVagm.sab92018, which showed a preferential CXCR4 usage. In contrast, SIVagm.ver644 was able to efficiently use CCR5 but not CXCR4, and therefore displayed a more classical SIV tropism pattern.

SIVagm.sab92018 *env* V3 sequencing. The V3 sequence impacts SIVmac and SIVagm tropism (34, 35, 64). Therefore, we investigated whether a more-positively-charged V3 loop region in Env of SIVagm.sab92018 and of a second SIV.agm.sab strain (D46) in comparison to the net charge of the V3 loop of SIVagm.ver644 could explain the usage of CXCR4. The obtained SIVagm V3 sequences were aligned with known SIVagm sequences from the database (Fig. 3). The three sequences are all different from each other, but the V3 loops exhibited the same net charge (+7). This analysis also confirms our previous observation that the V3 loop region is relatively highly conserved among all SIVagm lineages (49). No significant differences in the potential N glycosylation sites located in the V3 region could account for the differences in the coreceptor usage reported here (Fig. 3).

DISCUSSION

Our study lead to the identification of two wild-type SIVagm strains that show a consistent difference in their initial in vivo replication characteristics. SIVagm.sab92018 induced high levels of VL during primary infection, whereas plasma virus levels associated with primary SIVagm.ver644 infection were significantly lower. The differences in VL during primary infection cannot be explained by differences in the sensitivities of the assays used for the quantification of SIVagm.sab and SIVagm.ver replication. First of all, we measured two distinct viral parameters (P27 antigenemia levels and RNA plasma load), and the same replication pattern was determined by both methods. Furthermore, PCR amplifications were also carried out for each virus in other genomic regions, such as *env* and *nef*, and SIVagm.sab92018 was always readily amplified, whereas SIVagm.ver644 needed at least one if not two rounds of nested PCR (data not shown). Finally, only the SIVagm.sab92018-infected animals showed significant decreases in peripheral $CD4^+$ and $CD8^+$ T-cell numbers during primary infection, whereas SIVagm.ver644 induced no significant $CD4^+$ T-cell depletion during the primary infection. The peak levels of VL were not predictive for the degrees of subsequent control of viral replication in the host. This is in contrast to what was initially reported in macaques infected with SIVsm (39, 63) but corroborates other reports on SIV infections in macaques showing no association between early peak viremia levels and subsequent viral set-point levels (27, 72). In contrast to the case with primary infection, vRNA levels after the set point of viremia were not correlated with the virus strains. Therefore, our study suggests that the extent of early virus replication in these animals was primarily dependent on viral determinants and not the host species or individual parameters of the infected monkeys, whereas the host environment had a greater impact on viremia beginning at the postacute infection phase. Previous studies of macaques infected with SIVmac have shown that levels of VL in vivo, both at the peak and later at the set-point stage, can be correlated with the intrinsic susceptibility of the individual PBMC to infection in vitro (22). However, in our study we did not observe differences in in vivo replication levels between animals infected with the same virus strain. One explanation could be that AGMs included in this study showed similar intrinsic susceptibilities to SIVagm infection. This cannot be excluded for the animals of the same AGM species, since all sabaeus were from the same commercial source in Senegal, and all vervets originated or were born from animals from the same center in Kenya. We detected moderate variations within a group of monkeys with the same origin that might be related to variations in the susceptibilities of target cells to infection. However, sabaeus and vervets are genetically distinct, having been separated in different geographical regions tens of thousands of years ago. The cross-infection experiments demonstrated that the differences in peak viremia levels between the two viruses were not associated with intrinsic factors related to the hosts.

The route of infection and the dose of inoculum have been proposed as potential viral factors that exert an influence on viremia during both acute and chronic infections (33, 42, 52, 67). In our experiments, the route of inoculation was identical for all monkeys included. With regard to the inoculum size, titers of both SIVagm.ver644 and SIVagm.sab92018 virus stocks were determined on the same cells (SupT1), and the monkeys were infected with similar doses. Recent studies on SIVmac infections indicated that the size of inoculum is not predictive of the levels of either peak or set-point viremia (27). Furthermore, if SIVagm.sab92018 was able to eventually better replicate in SupT1 cells than SIVagm.ver644 because of its CXCR4 usage, we would then have underestimated the infectious titer in comparison to that of SIVagm.ver644, which then again could not explain the higher VLs observed with SIVagm.sab92018.

Another potential viral factor that could have influenced the level of viremia is the subtype of the infecting SIVagm virus.

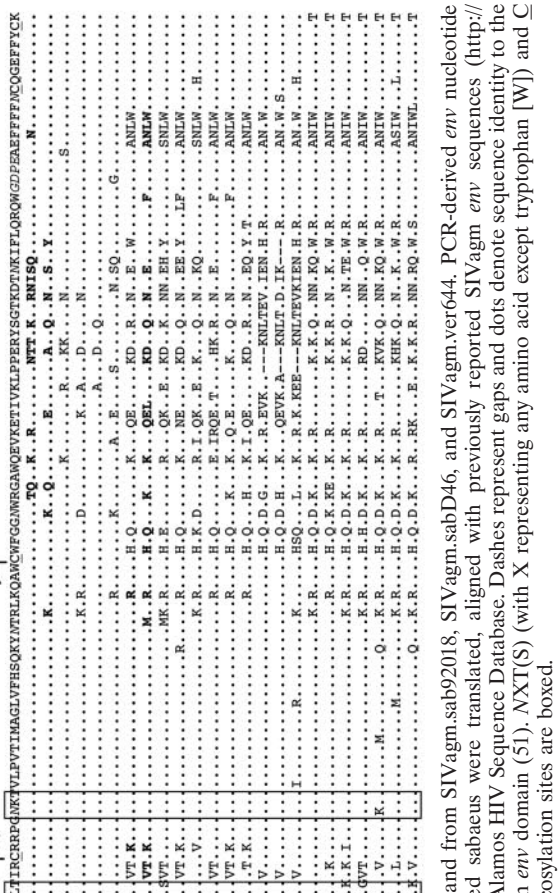

FIG. 3. Alignment of partial envelope protein sequences from multiple SIVagm isolates and from SIVagm.sab92018, SIVagm.sabD46, and SIVagm.ver644. PCR-derived *env* nucleotide sequences from SIVagm.ver644-infected vervet, and SIVagm.sab (92018 and D46)-infected sabaeus were translated, aligned with previously reported SIVagm *env* sequences (http://www.hiv.lanl.gov/content/hiv-db), and compared with a consensus sequence available on Los Alamos HIV Sequence Database. Dashes represent gaps and dots denote sequence identity to the SIVagm.sab consensus sequence. V3 designates a previously described hypervariable SIVagm *env* domain (51). *N*XT(S) (with X representing any amino acid except tryptophan [W]) and C represent putative N-linked glycosylation sites and cysteine residues, respectively. The N glycosylation sites are boxed.

SIVagm.sab, in contrast to the other three SIVagm subtypes, has a mosaic genome, produced by a recombination event between SIVagm and SIVrcm ancestors (4, 29). The recombinant SIVagm.sab seems to have replaced the pure, ancestral form of SIVagm in the natural habitat, suggesting indeed a higher fitness of this virus in the monkeys. Several circulating recombinant forms of HIV are apparently also able to spread more efficiently than their parents (44, 50, 58). Our results thus suggest that the differences observed between the two SIVagm strains used in our study are subtype related. However, one should note that these results are obtained based on the investigation of only one strain of each subtype. Therefore, a definitive conclusion can be drawn only after more strains of the two SIVagm subtypes have been investigated in vivo. These investigations are currently in progress (Vanessa Hirsch, personal communication). Differences between the in vivo pathogenicities of a given viral strain were in some cases associated with genomic changes, such as mutations and deletions in the viral accessory genes (16, 17). In HIV-1 and SIVmac infections, mutations in the *nef* gene are responsible for significant differences in viral virulence (33). The in vivo replication of a SIVagm3 with a deletion of *nef* in AGMs is indeed severely impaired (5). However, we found no deletions, premature stop codons, or amino acid substitutions in known functional domains of the SIVagm.ver644 *nef* gene. Although this does not totally rule out a role for Nef, it is also possible that mutations in other genes are responsible for the differences between the two viruses regarding their early in vivo replicative capacities. In vitro, both viruses readily replicate on SupT1 cells, and both induce a cytopathic effect when cultured on cells expressing the "adequate" coreceptors (data not shown). One should, however, note that multiple viral genes are involved in SIV virulence, which is often the result of a complex interplay between more than one viral determinant (14, 53, 71). Here we show for the first time that a primary SIV (SIVagm.sab92018) can be dually tropic. The association between higher VLs and a broader coreceptor usage by SIVagm.sab92018 corroborates our observation that AGMs naturally express low levels of $CD4^+CCR5^+CD45RA^-$ cells (I. Pandrea et al., submitted), which were reported to be the target cells of SIV (70). This reduced target cell expression in AGMs mainly concerns the major site of SIV replication, the intestine (69). The ability of SIVagm.sab92018 to use CXCR4 will thus increase the availability of target cells and thus explain the higher VLs. Differences in viral tropism were already reported to impact the levels of peak viremia in humans, in which higher percentages of CD4-positive lymphocytes express CXCR4 than express CCR5 (38). A large body of work on HIV-1 has established that isolates with relatively low positive net charges (+3 to +6) in the V3 loop commonly utilize CCR5, whereas isolates with more basic regions (usually +7 to +9) more effectively use CXCR4 (reviewed in reference 45). For HIV-2 it has been reported that an increase in the net positive charge of the V3 region allows efficient utilization of CXCR4 (28). In a different model, increases in the net positive charge of the V3 region up to +10 were not associated with an efficient use of CXCR4 as an entry cofactor in SIVmac239 infections (45). In our study, the use of CXCR4 as an entry cofactor was not associated with a higher positive net charge of the V3 loop of SIVagm.sab92018. Therefore, our results are in agreement with the

hypothesis that other determinants of viral tropism besides charge should be taken into account in studies of SIV infection (45).

In contrast to the impact of viral factors during the early phase of infection, the level of viremia starting from the postacute phase was more influenced by the host, as indicated by the fact that we were unable to observe any significant difference in chronic-phase viremia levels between SIVagm. sab92018- and SIVagm.ver644-infected animals. This also implies that these host factors were not constitutive but were induced subsequently to SIV infection, without excluding a critical role for the innate immune system in priming the adaptive host responses.

Combined, these data provide further insights into the influences of viral versus host factors on the establishment of viremia levels. We show that differences in capacities of viral replication between wild-type strains might be visible only during the early phase of infection and can be masked by host responses starting from the postacute phase on. This is relevant for studies designed to identify those HIV-1 parameters responsible for differences in the efficiency of viral transmission in vivo. It is known that some viruses are more successful in dissemination than others (i.e., HIV-1 versus HIV-2 or HIV-1 M CRF02-A/G versus other group M subtypes in west central Africa [15]). It is possible that more-transmissible viruses have a better capacity to replicate to higher levels very early on. This could not be analyzed so far, since studies on early infection in humans are limited to individuals that are generally detected as HIV positive only after the viral peak. Furthermore, only a few cohorts exist that allow the study of primary infection with viruses other than subtype B. Early peak levels with eventually better-transmissible viruses might thus have gone undetected.

Our data provide additional clues on viral replication during primary infection. They do not explain why AGMs are resistant to disease, but they strengthen previous observations that a high VL level in plasma alone does not account for pathogenesis (9, 18, 22). Indeed, previous data by us and others have already shown that AGMs do not progress to AIDS despite plasma RNA levels similar to those in HIV-1 and SIVmac infections (9, 18, 22). The high plasma VLs that can be observed in vivo for some SIVagm strains suggest that damage caused during virus-host interplay, such as chronic abnormal signaling to T cells, may be a determining factor in the outcomes of infections.

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REFERENCES

1. **Allan, J. S., M. Short, M. E. Taylor, S. Su, V. M. Hirsch, P. R. Johnson, G. M. Shaw, and B. H. Hahn.** 1991. Species-specific diversity among simian immunodeficiency viruses from African green monkeys. J. Virol. **65:**2816–2828.
2. **Apetrei, C., B. Gormus, I. Pandrea, M. Metzger, P. ten Haaft, L. N. Martin, R. Bohm, X. Alvarez, G. Koopman, M. Murphey-Corb, R. S. Veazey, A. A. Lackner, G. Baskin, J. Heeney, and P. A. Marx.** 2004. Direct inoculation of simian immunodeficiency virus from sooty mangabeys in black mangabeys (*Lophocebus aterrimus*): first evidence of AIDS in a heterologous African species and different pathologic outcomes of experimental infection. J. Virol. **78:**11506–11518.
3. **Arold, S. T., and A. S. Baur.** 2001. Dynamic Nef and Nef dynamics: how structure could explain the complex activities of this small HIV protein. Trends Biochem. Sci. **26:**356–363.
4. **Bailes, E., F. Gao, F. Bibollet-Ruche, V. Courgnaud, M. Peeters, P. A. Marx, B. H. Hahn, and P. M. Sharp.** 2003. Hybrid origin of SIV in chimpanzees. Science **300:**1713.
5. **Beer, B., M. Baier, J. zur Megede, S. Norley, and R. Kurth.** 1997. Vaccine effect using a live attenuated *nef*-deficient simian immunodeficiency virus of African green monkeys in the absence of detectable vaccine virus replication in vivo. Proc. Natl. Acad. Sci. USA **94:**4062–4067.
6. **Beer, B., J. Denner, C. R. Brown, S. Norley, J. zur Megede, C. Coulibaly, R. Plesker, S. Holzammer, M. Baier, V. M. Hirsch, and R. Kurth.** 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.
7. **Begaud, E., G. Feindirongai, P. Versmisse, J. Ipero, J. Leal, Y. Germani, J. Morvan, H. Fleury, M. Muller-Trutwin, F. Barre-Sinoussi, and G. Pancino.** 2003. Broad spectrum of coreceptor usage and rapid disease progression in HIV-1-infected individuals from Central African Republic. AIDS Res. Hum. Retrovir. **19:**551–560.
8. **Bjorndal, A., H. Deng, M. Jansson, J. R. Fiore, C. Colognesi, A. Karlsson, J. Albert, G. Scarlatti, D. R. Littman, and E. M. Fenyo.** 1997. Coreceptor usage of primary human immunodeficiency virus type 1 isolates varies according to biological phenotype. J. Virol. **71:**7478–7487.
9. **Broussard, S. R., S. I. Staprans, R. White, E. M. Whitehead, M. B. Feinberg, and J. S. Allan.** 2001. Simian immunodeficiency virus replicates to high levels in naturally infected African green monkeys without inducing immunologic or neurologic disease. J. Virol. **75:**2262–2275.
10. **Chakrabarti, L., M. C. Cumont, L. Montagnier, and B. Hurtrel.** 1994. Variable course of primary simian immunodeficiency virus infection in lymph nodes: relation to disease progression. J. Virol. **68:**6634–6643.
11. **Chakrabarti, L. A., S. R. Lewin, L. Zhang, A. Gettie, A. Luckay, L. N. Martin, E. Skulsky, D. D. Ho, C. Cheng-Mayer, and P. A. Marx.** 2000. Age-dependent changes in T cell homeostasis and SIV load in sooty mangabeys. J. Med. Primatol. **29:**158–165.
12. **Chakrabarti, L. A., S. R. Lewin, L. Zhang, A. Gettie, A. Luckay, L. N. Martin, E. Skulsky, D. D. Ho, C. Cheng-Mayer, and P. A. Marx.** 2000. Normal T-cell turnover in sooty mangabeys harboring active simian immunodeficiency virus infection. J. Virol. **74:**1209–1223.
13. **Chen, Z., D. Kwon, Z. Jin, S. Monard, P. Telfer, M. S. Jones, C. Y. Lu, R. F. Aguilar, D. D. Ho, and P. A. Marx.** 1998. Natural infection of a homozygous delta24 CCR5 red-capped mangabey with an R2b-tropic simian immunodeficiency virus. J. Exp. Med. **188:**2057–2065.
14. **Courgnaud, V., F. Laure, P. N. Fultz, L. Montagnier, C. Brechot, and P. Sonigo.** 1992. Genetic differences accounting for evolution and pathogenicity of simian immunodeficiency virus from a sooty mangabey monkey after cross-species transmission to a pig-tailed macaque. J. Virol. **66:**414–419.
15. **De Cock, K. M., G. Adjorlolo, E. Ekpini, T. Sibailly, J. Kouadio, M. Maran, K. Brattegaard, K. M. Vetter, R. Doorly, and H. D. Gayle.** 1993. Epidemiology and transmission of HIV-2. Why there is no HIV-2 pandemic? JAMA **270:**2083–2086.
16. **Desrosiers, R. C.** 1992. HIV with multiple gene deletions as a live attenuated vaccine for AIDS. AIDS Res. Hum. Retrovir. **8:**1457.
17. **Desrosiers, R. C., J. D. Lifson, J. S. Gibbs, S. C. Czajak, A. Y. Howe, L. O. Arthur, and R. P. Johnson.** 1998. Identification of highly attenuated mutants of simian immunodeficiency virus. J. Virol. **72:**1431–1437.
18. **Diop, O. M., A. Gueye, M. Dias-Tavares, C. Kornfeld, A. Faye, P. Ave, M. Huerre, S. Corbet, F. Barre-Sinoussi, and M. C. Muller-Trutwin.** 2000. High levels of viral replication during primary simian immunodeficiency virus SIVagm infection are rapidly and strongly controlled in African green monkeys. J. Virol. **74:**7538–7547.
19. **Fukasawa, M., T. Miura, A. Hasegawa, S. Morikawa, H. Tsujimoto, K. Miki, T. Kitamura, and M. Hayami.** 1988. Sequence of simian immunodeficiency virus from African green monkey, a new member of the HIV/SIV group. Nature **333:**457–461.
20. **Fultz, P. N., H. M. McClure, D. C. Anderson, and W. M. Switzer.** 1989. Identification and biologic characterization of an acutely lethal variant of simian immunodeficiency virus from sooty mangabeys (SIV/SMM). AIDS Res. Hum. Retrovir. **5:**397–409.
21. **Georges-Courbot, M. C., C. Y. Lu, M. Makuwa, P. Telfer, R. Onanga, G. Dubreuil, Z. Chen, S. M. Smith, A. Georges, F. Gao, B. H. Hahn, and P. A. Marx.** 1998. Natural infection of a household pet red-capped mangabey (*Cercocebus torquatus torquatus*) with a new simian immunodeficiency virus. J. Virol. **72:**600–608.
22. **Goldstein, S., I. Ourmanov, C. R. Brown, B. E. Beer, W. R. Elkins, R.**

Plishka, A. Buckler-White, and V. M. Hirsch. 2000. Wide range of viral load in healthy African green monkeys naturally infected with simian immunodeficiency virus. J. Virol. **74:**11744–11753.
23. **Gueye, A., O. M. Diop, M. J. Ploquin, C. Kornfeld, A. Faye, M. C. Cumont, B. Hurtrel, F. Barre-Sinoussi, and M. C. Muller-Trutwin.** 2004. Viral load in tissues during the early and chronic phase of non-pathogenic SIVagm infection. J. Med. Primatol. **33:**83–97.
24. **Hirsch, V. M., B. J. Campbell, E. Bailes, R. Goeken, C. Brown, W. R. Elkins, M. Axthelm, M. Murphey-Corb, and P. M. Sharp.** 1999. Characterization of a novel simian immunodeficiency virus (SIV) from L'Hoest monkeys (*Cercopithecus l'hoesti*): implications for the origins of SIVmnd and other primate lentiviruses. J. Virol. **73:**1036–1045.
25. **Hirsch, V. M., G. Dapolito, P. R. Johnson, W. R. Elkins, W. T. London, R. J. Montali, S. Goldstein, and C. Brown.** 1995. Induction of AIDS by simian immunodeficiency virus from an African green monkey: species-specific variation in pathogenicity correlates with the extent of in vivo replication. J. Virol. **69:**955–967.
26. **Hirsch, V. M., C. McGann, G. Dapolito, S. Goldstein, A. Ogen-Odoi, B. Biryawaho, T. Lakwo, and P. R. Johnson.** 1993. Identification of a new subgroup of SIVagm in tantalus monkeys. Virology **197:**426–430.
27. **Holterman, L., H. Niphuis, W. Koornstra, R. Dubbes, P. ten Haaft, and J. L. Heeney.** 2000. The rate of progression to AIDS is independent of virus dose in simian immunodeficiency virus-infected macaques. J. Gen. Virol. **81:**1719–1726.
28. **Isaka, Y., A. Sato, S. Miki, S. Kawauchi, H. Sakaida, T. Hori, T. Uchiyama, A. Adachi, M. Hayami, T. Fujiwara, and O. Yoshie.** 1999. Small amino acid changes in the V3 loop of human immunodeficiency virus type 2 determines the coreceptor usage for CXCR4 and CCR5. Virology **264:**237–243.
29. **Jin, M. J., H. Hui, D. L. Robertson, M. C. Muller, F. Barre-Sinoussi, V. M. Hirsch, J. S. Allan, G. M. Shaw, P. M. Sharp, and B. H. Hahn.** 1994. Mosaic genome structure of simian immunodeficiency virus from west African green monkeys. EMBO J. **13:**2935–2947.
30. **Jin, M. J., J. Rogers, J. E. Phillips-Conroy, J. S. Allan, R. C. Desrosiers, G. M. Shaw, P. M. Sharp, and B. H. Hahn.** 1994. Infection of a yellow baboon with simian immunodeficiency virus from African green monkeys: evidence for cross-species transmission in the wild. J. Virol. **68:**8454–8460.
31. **Jubier-Maurin, V., P. Sarni-Manchado, F. Veas, N. Vidal, F. Bibollet-Ruche, J. P. Durand, A. Galat-Luong, and G. Cuny.** 1995. Regulatory genes of simian immunodeficiency viruses from west African green monkeys (*Cercopithecus aethiops sabaeus*). J. Virol. **69:**7349–7353.
32. **Kaur, A., R. M. Grant, R. E. Means, H. McClure, M. Feinberg, and R. P. Johnson.** 1998. Diverse host responses and outcomes following simian immunodeficiency virus SIVmac239 infection in sooty mangabeys and rhesus macaques. J. Virol. **72:**9597–9611.
33. **Kestler, H. W., III, D. J. Ringler, K. Mori, D. L. Panicali, P. K. Sehgal, M. D. Daniel, and R. C. Desrosiers.** 1991. Importance of the *nef* gene for maintenance of high virus loads and for development of AIDS. Cell **65:**651–662.
34. **Kirchhoff, F., H. G. Morrison, and R. C. Desrosiers.** 1995. Identification of V3 mutations that can compensate for inactivating mutations in C4 of simian immunodeficiency virus. Virology **213:**179–189.
35. **Konig, R. R., E. Flory, S. Steidl, J. Neumann, C. Coulibaly, E. Holznagel, S. Holzammer, S. Norley, and K. Cichutek.** 2002. Engineered CD4- and CXCR4-using simian immunodeficiency virus from African green monkeys is neutralization sensitive and replicates in nonstimulated lymphocytes. J. Virol. **76:**10627–10636.
36. **Kuhmann, S. E., N. Madani, O. M. Diop, E. J. Platt, J. Morvan, M. C. Muller-Trutwin, F. Barre-Sinoussi, and D. Kabat.** 2001. Frequent substitution polymorphisms in African green monkey CCR5 cluster at critical sites for infections by simian immunodeficiency virus SIVagm, implying ancient virus-host coevolution. J. Virol. **75:**8449–8460.
37. **Lairmore, M. D., D. E. Hofheinz, N. L. Letvin, C. S. Stoner, S. Pearlman, and G. P. Toedter.** 1993. Detection of simian immunodeficiency virus and human immunodeficiency virus type 2 capsid antigens by a monoclonal antibody-based antigen capture assay. AIDS Res. Hum. Retrovir. **9:**565–571.
38. **Lee, B., M. Sharron, L. J. Montaner, D. Weissman, and R. W. Doms.** 1999. Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages. Proc. Natl. Acad. Sci. USA **96:**5215–5220.
39. **Lifson, J. D., M. A. Nowak, S. Goldstein, J. L. Rossio, A. Kinter, G. Vasquez, T. A. Wiltrout, C. Brown, D. Schneider, L. Wahl, A. L. Lloyd, J. Williams, W. R. Elkins, A. S. Fauci, and V. M. Hirsch.** 1997. The extent of early viral replication is a critical determinant of the natural history of simian immunodeficiency virus infection. J. Virol. **71:**9508–9514.
40. **Ling, B., C. Apetrei, I. Pandrea, R. S. Veazey, A. A. Lackner, B. Gormus, and P. A. Marx.** 2004. Classic AIDS in a sooty mangabey after an 18-year natural infection. J. Virol. **78:**8902–8908.
41. **Ling, B., M. L. Santiago, S. Meleth, B. Gormus, H. M. McClure, C. Apetrei, B. H. Hahn, and P. A. Marx.** 2003. Noninvasive detection of new simian immunodeficiency virus lineages in captive sooty mangabeys: ability to amplify virion RNA from fecal samples correlates with viral load in plasma. J. Virol. **77:**2214–2226.
42. **Marthas, M. L., R. A. Ramos, B. L. Lohman, K. K. Van Rompay, R. E. Unger, C. J. Miller, B. Banapour, N. C. Pedersen, and P. A. Luciw.** 1993. Viral determinants of simian immunodeficiency virus (SIV) virulence in rhesus macaques assessed by using attenuated and pathogenic molecular clones of SIVmac. J. Virol. **67:**6047–6055.
43. **McClure, H. M., D. C. Anderson, T. P. Gordon, A. A. Ansari, P. N. Fultz, S. A. Klumpp, P. Emau, and M. Isahakia.** 1992. Natural simian immunodeficiency virus infection in nonhuman primates. Top. Primatol. **3:**425–438.
44. **McCutchan, F. E., J. K. Carr, M. Bajani, E. Sanders-Buell, T. O. Harry, T. C. Stoeckli, K. E. Robbins, W. Gashau, A. Nasidi, W. Janssens, and M. L. Kalish.** 1999. Subtype G and multiple forms of A/G intersubtype recombinant human immunodeficiency virus type 1 in Nigeria. Virology **254:**226–234.
45. **Meister, S., C. Otto, A. Papkalla, M. Krumbiegel, S. Pohlmann, and F. Kirchhoff.** 2001. Basic amino acid residues in the V3 loop of simian immunodeficiency virus envelope alter viral coreceptor tropism and infectivity but do not allow efficient utilization of CXCR4 as entry cofactor. Virology **284:**287–296.
46. **Mellors, J. W., C. R. Rinaldo, Jr., P. Gupta, R. M. White, J. A. Todd, and L. A. Kingsley.** 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science **272:**1167–1170.
47. **Menu, E., J. M. Reynes, M. C. Muller-Trutwin, L. Guillemot, P. Versmisse, M. Chiron, S. An, V. Trouplin, P. Charneau, H. Fleury, F. Barre-Sinoussi, and F. F. Sainte Marie.** 1999. Predominance of CCR5-dependent HIV-1 subtype E isolates in Cambodia. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. **20:**481–487.
48. **Morner, A., A. Bjorndal, J. Albert, V. N. Kewalramani, D. R. Littman, R. Inoue, R. Thorstensson, E. M. Fenyo, and E. Bjorling.** 1999. Primary human immunodeficiency virus type 2 (HIV-2) isolates, like HIV-1 isolates, frequently use CCR5 but show promiscuity in coreceptor usage. J. Virol. **73:**2343–2349.
49. **Muller, M. C., N. K. Saksena, E. Nerrienet, C. Chappey, V. M. Herve, J. P. Durand, P. Legal-Campodonico, M. C. Lang, J. P. Digoutte, and A. J. Georges.** 1993. Simian immunodeficiency viruses from central and western Africa: evidence for a new species-specific lentivirus in tantalus monkeys. J. Virol. **67:**1227–1235.
50. **Muller-Trutwin, M. C., M. L. Chaix, F. Letourneur, E. Begaud, D. Beaumont, A. Deslandres, B. You, J. Morvan, C. Mathiot, F. Barre-Sinoussi, and S. Saragosti.** 1999. Increase of HIV-1 subtype A in Central African Republic. J. Acquir. Immune Defic. Syndr. **21:**164–171.
51. **Muller-Trutwin, M. C., S. Corbet, M. D. Tavares, V. M. Herve, E. Nerrienet, M. C. Georges-Courbot, W. Saurin, P. Sonigo, and F. Barre-Sinoussi.** 1996. The evolutionary rate of nonpathogenic simian immunodeficiency virus (SIVagm) is in agreement with a rapid and continuous replication in vivo. Virology **223:**89–102.
52. **Nielsen, C., C. Pedersen, J. D. Lundgren, and J. Gerstoft.** 1993. Biological properties of HIV isolates in primary HIV infection: consequences for the subsequent course of infection. AIDS **7:**1035–1040.
53. **Novembre, F. J., P. R. Johnson, M. G. Lewis, D. C. Anderson, S. Klumpp, H. M. McClure, and V. M. Hirsch.** 1993. Multiple viral determinants contribute to pathogenicity of the acutely lethal simian immunodeficiency virus SIVsmmPBj variant. J. Virol. **67:**2466–2474.
54. **Nowak, M. A., A. L. Lloyd, G. M. Vasquez, T. A. Wiltrout, L. M. Wahl, N. Bischofberger, J. Williams, A. Kinter, A. S. Fauci, V. M. Hirsch, and J. D. Lifson.** 1997. Viral dynamics of primary viremia and antiretroviral therapy in simian immunodeficiency virus infection. J. Virol. **71:**7518–7525.
55. **Onanga, R., C. Kornfeld, I. Pandrea, J. Estaquier, S. Souquiere, P. Rouquet, V. P. Mavoungou, O. Bourry, S. M'Boup, F. Barre-Sinoussi, F. Simon, C. Apetrei, P. Roques, and M. C. Muller-Trutwin.** 2002. High levels of viral replication contrast with only transient changes in CD4+ and CD8+ cell numbers during the early phase of experimental infection with simian immunodeficiency virus SIVmnd-1 in *Mandrillus sphinx*. J. Virol. **76:**10256–10263.
56. **Pandrea, I., R. Onanga, C. Kornfeld, P. Rouquet, O. Bourry, S. Clifford, P. T. Telfer, K. Abernethy, L. T. White, P. Ngari, M. Muller-Trutwin, P. Roques, P. A. Marx, F. Simon, and C. Apetrei.** 2003. High levels of SIVmnd-1 replication in chronically infected *Mandrillus sphinx*. Virology **317:**119–127.
57. **Pandrea, I., R. Onanga, P. Rouquet, O. Bourry, P. Ngari, E. J. Wickings, P. Roques, and C. Apetrei.** 2001. Chronic SIV infection ultimately causes immunodeficiency in African non-human primates. AIDS **15:**2461–2462.
58. **Pandrea, I., D. L. Robertson, R. Onanga, F. Gao, M. Makuwa, P. Ngari, I. Bedjabaga, P. Roques, F. Simon, and C. Apetrei.** 2002. Analysis of partial pol and env sequences indicates a high prevalence of HIV type 1 recombinant strains circulating in Gabon. AIDS Res. Hum. Retrovir. **18:**1103–1116.
59. **Ploquin, M. J., O. M. Diop, N. Sol-Foulon, L. Mortara, A. Faye, M. A. Soares, E. Nerrienet, R. Le Grand, Y. Van Kooyk, A. Amara, O. Schwartz, F. Barre-Sinoussi, and M. C. Muller-Trutwin.** 2004. DC-SIGN from African green monkeys is expressed in lymph nodes and mediates infection in *trans* of simian immunodeficiency virus SIVagm. J. Virol. **78:**798–810.
60. **Rey-Cuille, M. A., J. L. Berthier, M. C. Bomsel-Demontoy, Y. Chaduc, L. Montagnier, A. G. Hovanessian, and L. A. Chakrabarti.** 1998. Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease. J. Virol. **72:**3872–3886.

61. **Silvestri, G., D. L. Sodora, R. A. Koup, M. Paiardini, S. P. O'Neil, H. M. McClure, S. I. Staprans, and M. B. Feinberg.** 2003. Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. Immunity **18:**441–452.
62. **Simon, F., S. Souquiere, F. Damond, A. Kfutwah, M. Makuwa, E. Leroy, P. Rouquet, J. L. Berthier, J. Rigoulet, A. Lecu, P. T. Telfer, I. Pandrea, J. C. Plantier, F. Barre-Sinoussi, P. Roques, M. C. Muller-Trutwin, and C. Apetrei.** 2001. Synthetic peptide strategy for the detection of and discrimination among highly divergent primate lentiviruses. AIDS Res. Hum. Retrovir. **17:**937–952.
63. **Staprans, S. I., P. J. Dailey, A. Rosenthal, C. Horton, R. M. Grant, N. Lerche, and M. B. Feinberg.** 1999. Simian immunodeficiency virus disease course is predicted by the extent of virus replication during primary infection. J. Virol. **73:**4829–4839.
64. **Steidl, S., J. Stitz, I. Schmitt, R. Konig, E. Flory, M. Schweizer, and K. Cichutek.** 2002. Coreceptor switch of [MLV(SIVagm)] pseudotype vectors by V3-loop exchange. Virology **300:**205–216.
65. **ten Haaft, P., K. Murthy, M. Salas, H. McClure, R. Dubbes, W. Koornstra, H. Niphuis, D. Davis, G. van der Groen, and J. Heeney.** 2001. Differences in early virus loads with different phenotypic variants of HIV-1 and SIV(cpz) in chimpanzees. AIDS **15:**2085–2092.
66. **Ten Haaft, P., B. Verstrepen, K. Uberla, B. Rosenwirth, and J. Heeney.** 1998. A pathogenic threshold of virus load defined in simian immunodeficiency virus- or simian-human immunodeficiency virus-infected macaques. J. Virol. **72:**10281–10285.
67. **Tersmette, M., J. M. Lange, R. E. de Goede, F. de Wolf, J. K. Eeftink-Schattenkerk, P. T. Schellekens, R. A. Coutinho, J. G. Huisman, J. Goudsmit, and F. Miedema.** 1989. Association between biological properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. Lancet **1:**983–985.
68. **Traina-Dorge, V., J. Blanchard, L. Martin, and M. Murphey-Corb.** 1992. Immunodeficiency and lymphoproliferative disease in an African green monkey dually infected with SIV and STLV-I. AIDS Res. Hum. Retrovir. **8:**97–100.
69. **Veazey, R. S., M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L. Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers, and A. A. Lackner.** 1998. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science **280:**427–431.
70. **Veazey, R. S., I. C. Tham, K. G. Mansfield, M. DeMaria, A. E. Forand, D. E. Shvetz, L. V. Chalifoux, P. K. Sehgal, and A. A. Lackner.** 2000. Identifying the target cell in primary simian immunodeficiency virus (SIV) infection: highly activated memory CD4+ T cells are rapidly eliminated in early SIV infection in vivo. J. Virol. **74:**57–64.
71. **Wagener, S., M. T. Dittmar, B. Beer, R. Konig, R. Plesker, S. Norley, R. Kurth, and K. Cichutek.** 1998. The U3 promoter and the *nef* gene of simian immunodeficiency virus (SIV) smmPBj1.9 do not confer acute pathogenicity upon SIVagm. J. Virol. **72:**3446–3450.
72. **Watson, A., J. Ranchalis, B. Travis, J. McClure, W. Sutton, P. R. Johnson, S. L. Hu, and N. L. Haigwood.** 1997. Plasma viremia in macaques infected with simian immunodeficiency virus: plasma viral load early in infection predicts survival. J. Virol. **71:**284–290.