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In Vivo Cellular Tropism of Gorilla Simian Foamy Virus in Blood of Infected Humans

Rejane Rua,[a,b,c] Edouard Betsem,[a,b,d] Thomas Montange,[a,b] Florence Buseyne,[a,b] Antoine Gessain[a,b]

Unit of Epidemiology and Physiopathology of Oncogenic Viruses, Department of Virology, Institut Pasteur, Paris, France[a]; Centre National de la Recherche Scientifique (CNRS), UMR 3569, Paris, France[b]; Université Paris Diderot, Cellule Pasteur, Paris, France[c]; Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon[d]

ABSTRACT

Simian foamy viruses (SFV) are retroviruses that are widespread among nonhuman primates. SFV can be transmitted to humans, giving rise to a persistent infection. Only a few data are available concerning the distribution of SFV in human blood cells. Here we purified blood mononuclear cell subsets from 11 individuals infected with a *Gorilla gorilla* SFV strain and quantified SFV DNA levels by quantitative PCR. SFV DNA was detected in the majority of the $CD8^+$, $CD4^+$, and $CD19^+$ lymphocyte samples and rarely in $CD14^+$ monocyte and $CD56^+$ NK lymphocyte samples. The median (interquartile range [IQR]) SFV DNA counts were 16.0 (11.0 to 49.8), 11.3 (5.9 to 28.3), and 17.2 (2.0 to 25.2) copies/10^5 cells in $CD8^+$ T lymphocytes, $CD4^+$ T lymphocytes, and $CD19^+$ B lymphocytes, respectively. In the CD4 compartment, SFV DNA was detected in both memory and naive $CD4^+$ T lymphocytes. SFV DNA levels in $CD4^+$ T cells were positively correlated with the duration of the infection. Our study shows with a quantitative method that $CD8^+$, $CD4^+$, and B lymphocytes are major cellular targets of SFV in the blood of infected humans.

IMPORTANCE

Investigation of SFV infections in humans is important due to the origin of human immunodeficiency viruses (HIV) and human T cell lymphotropic viruses (HTLV) from cross-species transmission of their simian counterparts to humans. Surprisingly little is known about many aspects of the biology of SFV in infected humans, including quantitative data concerning the cellular targets of SFV *in vivo*. Here we show that the distribution of SFV DNA among the different leukocyte populations is not homogeneous and that viral load in $CD4^+$ T lymphocytes is correlated with the duration of infection. These new data will help in understanding the biology of retroviral infections in humans and can be useful in the growing field of SFV-based gene therapy.

Like simian immunodeficiency viruses (SIVs) and simian T cell lymphotropic viruses (STLVs), simian foamy viruses (SFVs) are complex retroviruses that can infect humans after cross-species transmission. No pathology has been reported so far in the natural hosts or in humans, although large-scale dedicated studies have not yet been performed (1, 2). Humans are not the natural hosts of foamy viruses, but more than 100 cases of SFV infection have been reported so far within individuals in close contact with nonhuman primates (NHP). In such persons, SFV infection is persistent, and the SFV viral load ranges from 1 to >1,000 SFV DNA copies/10^5 blood cells (3–6). While it is known that both retroviruses, human immunodeficiency virus type 1 (HIV-1) and human T cell lymphotropic virus (HTLV-1), can infect T cells (7, 8), little is known about the distribution of SFV in human blood cells (9). One pioneer study with chimpanzees and African green monkeys revealed a preferential infection of lymphocytes, especially $CD8^+$ T cells, in 13 SFV-infected animals (10). In humans, using semiquantitative PCR assays, von Laer et al. found SFV DNA exclusively in $CD8^+$ T cells in two tested individuals (10). Interestingly, SFV DNA was detected in monocytes and B lymphocytes, but not in $CD4^+$ and $CD8^+$ T cells, in one individual who was infected with an African green monkey viral strain containing deleterious mutations in the viral *bet* gene (11). However, partly due to the difficulty in obtaining, quickly processing, and accurately quantifying SFV DNA in samples from a series of SFV-infected individuals whose history of infection is known or presumed, no further data are available concerning SFV tropism in humans.

In this study, we aimed to quantify and analyze SFV viral load in the major populations of peripheral blood mononuclear cells (PBMCs) in a series of 11 hunters from Central Africa who were chronically infected with a gorilla strain of SFV (4). In contrast, the two previous studies were related to SFVs from African green monkeys and chimpanzees and comprise more isolated reports than this more systematic study (10, 11). Moreover, for the first time, quantitative assays were performed to better evaluate the levels of SFV DNA in the different cell populations.

MATERIALS AND METHODS

Participants. We studied 11 participants; all of them live in villages or settlements in the rain forest of South Cameroon (Table 1). All participants received detailed information about the study and gave their written consent. The study received administrative and ethical clearance in Cameroon from the National Committee of Ethics, and in France, from the Comité de Protection des Personnes and the Commission Nationale de l'Informatique et des Libertés.

SFV status of enrolled individuals. We enrolled in the present study 11 participants who had been shown to be infected with a gorilla SFV

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Address correspondence to Rejane Rua, rrua@phare.normalesup.org.
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TABLE 1 Epidemiological features of the 11 simian foamy virus-infected humans

Individual[a]	Ethnicity	Age at contact[b] (yr)	Age at sampling (yr)	Estimated duration of infection (yr)	Wound location	Severity[c]	Sampling date (mo/day/yr) (study)[d]	Complete SFV genome available[e] (GenBank accession no.)
AKO394	Bantu	53	58	5	Thigh	1	9/18/2012 (P)	No
BAD348	Bantu	19	32	13	Leg	3	3/14/2013 (P); 11/19/2013 (P + M)	No
BAD447	Bantu	40	60	20	Hand	2	3/10/2013 (P)	No
BAD456	Bantu	24	34	10	Leg	2	3/10/2013 (P)	No
BAD463	Bantu	37	47	10	Leg	3	11/19/2013 (P + M)	No
BAD468	Bantu	25	38	13	Several	3	9/04/2012 (P); 3/10/2012 (M); 11/15/2013 (P)	Yes (JQ867465.1)
BAK177	Pygmy	26	40	14	Leg	3	11/15/2013 (P)	No
BAK232	Pygmy	40	63	23	Hand	2	11/19/2013 (P + M)	No
BAK55	Pygmy	30	70	40	Arm	2	11/15/2013 (P)	No
BAK74	Pygmy	26	51	25	Foot	2	9/04/2012 (P); 03/14/2012 (M); 11/15/2013 (P)	Yes (JQ867464.1)
LOBAK2	Pygmy	37	64	27	Thigh	3	03/10/2013 (P)	No

[a] All individuals lived in South Cameroon rainforest villages/settlements.
[b] All individuals had been bitten by a gorilla during hunting activities and are infected with a gorilla SFV (3). Age at contact was assumed, based on interview.
[c] Wound bite severity (based on interview and physical injury): 1 = low, 2 = medium, 3 = high.
[d] P corresponds to sampling for study of the general tropism of SFV in PBMCs, and M corresponds to sampling for study of the tropism of SFV in naive and memory cells.
[e] Complete sequences of isolated viral strains from the indicated individuals are available in our previous study (14), and their GenBank accession numbers are provided.

strain in our previous work (4) (Table 1). Briefly, the 11 participants were determined to be infected with SFV based on both serological results (Western blot positivity) and molecular studies on PBMCs (SFV detection by nested PCR on the *pol-in* fragment [integrase-coding sequence of the polymerase gene] and/or the long terminal repeat [LTR] fragment). All of the participants have been bitten by a gorilla, which is very probably the cause of SFV infection, as suggested previously (4). Of note, the strain of SFV infecting each individual always matched the NHP species they were bitten by (herein, gorillas), as declared in the interview.

Purification of PBMC subpopulations. Fresh blood samples were collected in EDTA tubes and shipped at room temperature from the Pasteur Institute of Yaoundé to the Pasteur Institute of Paris for processing. Within 20 h following sampling, fresh PBMCs were isolated by a Ficoll-Hypaque gradient. PBMC subpopulations were purified by sequential separation using magnetic beads; $CD19^+$ B lymphocytes, $CD14^+$ monocytes, $CD4^+$ T cells, $CD56^+$ NK cells, and $CD8^+$ T cells were isolated using the corresponding positive-selection kits (Miltenyi Biotec, Paris, France). For the "memory study," $CD4^+$ T cells were first selected by T cell isolation kits followed by positive selection of $CD45RO^+$ memory cells (Miltenyi Biotec, Paris). The flow-through contained the naive $CD4^+$ T cell-enriched fraction. The purity of the separated cells was checked by flow cytometry using a BD FacsCanto system for data acquisition and FlowJo V8.7.1 software for data analysis after labeling with a composition of the following antibodies: anti-CD3-fluorescein isothiocyanate (FITC), anti-CD45RA-V450 (BD Biosciences, Rungis, France), anti-CD8-allophycocyanin (APC), anti-CD14-phycoerythrin (PE) (Beckman Coulter, Villepinte, France), anti-CD16-V450, anti-CD56-PC7, anti-CD20-PC5 (BD Pharmingen, Rungis, France), and anti-CD4-APC-H7 (eBioscience, Paris, France). Live/Dead Fixable Dead Cell Stain kits (Life Technologies, Saint Aubin, France) were used to identify viable cells. A gating strategy among live cells identified $CD3^+$ $CD4^+$ cells as $CD4^+$ T lymphocytes, $CD3^+$ $CD8^+$ cells as $CD8^+$ T lymphocytes, $CD3^-$ $CD19^+$ cells as B lymphocytes, $CD3^-$ $CD14^+$ as monocytes, and both $CD3^-$ $CD56^+$ and $CD3^-$ $CD16^+$ cells as NK lymphocytes. The purities of $CD4^+$, $CD8^+$, and $CD19^+$ lymphocytes, $CD14^+$ monocytes, and $CD56^+$ NK cells were 94.0% ± 3.1%, 87.9% ± 8.1%, 79.9% ± 7.5%, 73.2% ± 15.2%, and 61.4% ± 16.4%, respectively (see Table S1 in the supplemental material). The purity of the $CD4^+$ naive cells was 77.1% ± 18.5%, and the purity of the $CD4^+$ memory cells was 86.5% ± 4.1% (data not shown).

Quantification of SFV DNA viral loads. Quantitative PCRs (qPCRs) targeting the SFV polymerase gene (*pol*) were performed mainly as previously described (5). Briefly, the primers used (GF5qpcr-TAGACCTGAA GGAACCAAAATAATTCC, and GR5qpcr-TCCTTCCTCATATTAGGC CACC) were designed to detect a 144-bp nucleic acid region of the gorilla SFV polymerase gene. We used the Eppendorf Mastercycler *realplex* gradient detection system and Quantitect SYBR green (Qiagen) in a 20-μl-volume reaction mixture containing 10 μl of SYBR green buffer, 150 nM each primer, and 500 ng of a DNA sample (an approximately 75,000-cell equivalent) quantified by a NanoDrop 8000 spectrophotometer (Nanodrop Technologies). The optimized qPCR conditions used were as follows: 95°C for 15 min and 40 cycles at 95°C for 15s, 60°C for 30s, and 72°C for 30s. Samples were tested in triplicate in two independent assays, and the mean and standard deviation (SD) were calculated.

To standardize the qPCR, we cloned a 465-bp region that included the PCR target sequence from one primary isolate (BAK74, a gorilla strain of SFV) into a PCR cloning plasmid using a TOPO TA cloning kit (Invitrogen) and diluted it in 500 ng of human genomic DNA (Promega). All SFV qPCRs included a standard curve (from 3 to 3^7 = 2,187 SFV DNA copies; 3-fold serial dilutions) and were performed in triplicate. In each assay, SFV DNA copies from 3 to 3^7 were in the range of linear detection, and the correlation coefficient of SFV standard curves was >0.95. The limit of detection (LOD) was determined as the number of SFV DNA copies corresponding to the threshold cycle at which at most 5% of true positive samples scored arbitrarily negative. Using this definition, we determined the LOD to be equivalent to 3 SFV DNA copies/75,000 cells (equivalent to 4 SFV DNA copies/10^5 cells). qPCRs below the LOD were arbitrarily set up at half the LOD (equivalent to 2 SFV DNA copies/10^5 cells).

A 141-bp cellular albumin qPCR was performed on a 250× dilution of the samples (2 ng) to normalize the result with that for cellular DNA content (albF-AAACTCATGGGAGCTGCTGGTT, albR-GCTGTCATC TCTTGTGGGCTGT) using the same PCR conditions as for SFV quantification. All albumin qPCR assays included an albumin standard curve generated by adding 5-fold dilutions of human genomic DNA (Promega), ranging from 0.08 ng of DNA to 50 ng of DNA, to the PCR reagents. The correlation coefficient of albumin standard curves was >0.95.

We checked in every individual assay the specificity of the primers by using a melting curve.

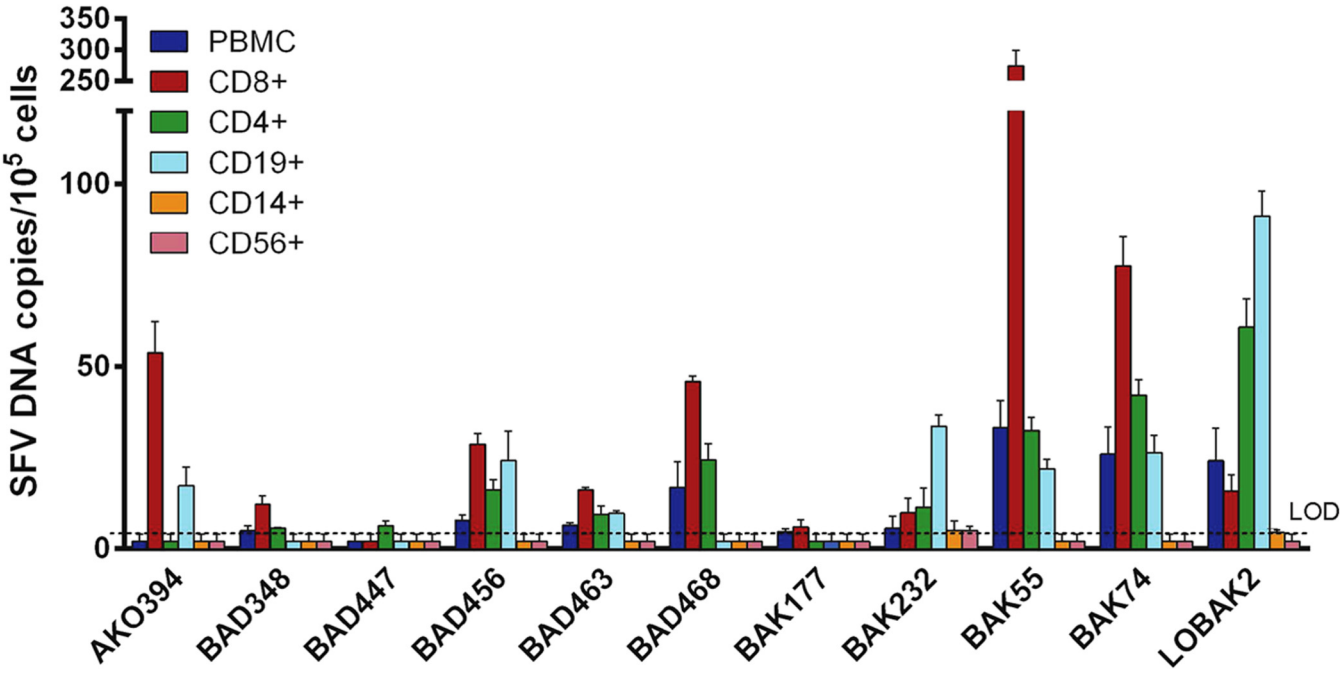

FIG 1 SFV DNA loads in PBMC populations of 11 hunters infected with a *Gorilla gorilla* SFV strain. PBMC populations from 11 SFV-infected individuals were isolated, and the SFV DNA load was quantified in each PBMC population. Each quantification was performed twice in triplicate (e.g., $n = 6$). Means ± standard deviations are shown. Values below the limit of detection (LOD) were arbitrarily set as half the LOD, 2 SFV DNA copies/10^5 cells.

SFV *bet* DNA coding sequences. We performed strain-specific nested PCR to sequence the entire SFV *bet* coding DNA sequence (CDS) from genomic DNA of PBMCs of SFV-infected individuals. We used nested PCR to generate three PCR products, A (572-bp *bet open reading frame 1* [*orf-1*]), B (761-bp 5′ fragment of *bet orf-2*), and C (861-bp 3′ fragment of *bet orf-2*, overlapping with B).

The primers used to generate PCR product A were primers A1 (5′-ACTGGAGAGAGCTAAAGCAG-3′) and A2 (5′-CCAGACACCAACATCAGCA-3′) for the outer PCR and primers A3 (5′-ATTGGTAACTTCTTAACTGG-3′) and A4 (5′-GTAACAGGCATAGGTTCATG-3′) for the inner PCR. The reaction conditions were 95°C for 15 min and 40 cycles at 95°C for 15 s, 50°C for 1 min, and 72°C for 1 min, followed by 72° for 7 min.

The primers used to generate PCR product B were primers B1 (5′-CATGAACCTATGCCTGTTAC-3′) and B2 (5′-CTGATCTGAAAGATTTGCAGC-3′) for the outer PCR and primers B3 (5′-TGCTGATGTTGGTGTCTGG-3′) and B4 (5′-CTTGATAAGCATATTGGAGCC-3′) for the inner PCR. The reaction conditions were 95°C for 15 min and 40 cycles at 95°C for 15s, 65°C for 1 min, and 72°C for 1 min, followed by 72° for 7 min.

The primers used to generate PCR product C were primers C1 (5′-CAGAACCTGATGTATGGTG-3′) and C2 (5′-CCTCTCTAGGGATTATACAG-3′) for the outer PCR and primers C3 (5′-GGTGTAGTGCAGCACTTTG-3′) and C4 (5′-CTCTTCTTACACTACTTCC-3′) for the inner PCR. The reaction conditions were 95°C for 15 min and 40 cycles at 95°C for 15s, 50°C for 1 min, and 72°C for 1 min, followed by 72° for 7 min.

Inner primers A3 and A4, B3 and B4, or C3 and C4 were used to sequence the PCR products A, B, and C, respectively. For each donor, one of each PCR product A, B, and C was sequenced by direct sequencing of the PCR product (this implies sequencing of the major variants). Each base was sequenced twice, using forward and reverse primers. Sequences were aligned using CLC Genomics Workbench software (Gap open cost, 10.0; Gap extension cost, 1.0).

Statistical analysis. For qualitative analysis, SFV DNA detection rates across the 5 cell subsets were compared using Cochran's Q test and two-by-two comparisons were performed using McNemar's test. For quantitative analysis, the Friedman test was used to compare SFV DNA levels across T and B lymphocytes. Spearman's rank test was used to assess correlations between SFV DNA levels and variables related to SFV infection. GraphPad Prism software was used for statistical analysis.

Nucleotide sequence accession numbers. The GenBank accession numbers for the sequences of *bet orf-1* are KF982250 to KF982260, and for *bet orf-2*, KF982262 to KF982272.

RESULTS

SFV DNA was quantified in selected subsets of peripheral blood cells from 11 individuals (Fig. 1). SFV DNA was detected in $CD8^+$ T lymphocytes from 10 of 11 (91%) donors, in $CD4^+$ T lymphocytes from 9 (82%) of them, and in $CD19^+$ B lymphocytes from 7 (64%) of them. In contrast, SFV DNA levels were above the limit of detection in only two (18%) $CD14^+$ monocyte samples and one (9%) $CD56^+$ NK lymphocyte sample. The frequencies of SFV DNA detection across the 5 mononuclear cell subsets were significantly different (Cochran's Q test; $P < 0.001$). The frequency of SFV DNA detection in $CD8^+$, $CD4^+$, and $CD19^+$ B lymphocytes was significantly higher than in $CD56^+$ NK lymphocytes and in $CD8^+$ and $CD4^+$ T lymphocytes than in $CD14^+$ monocytes (McNemar's test; $P < 0.05$ for each two-by-two comparison). There was a trend for a higher frequency of SFV DNA detection in B lymphocytes than in monocytes, although it did not reach statistical significance (McNemar's test; $P = 0.07$).

We then performed quantitative analysis on $CD8^+$, $CD4^+$, and $CD19^+$ lymphocytes, as SFV DNA was generally above the limit of detection in these subsets. The mean (±SD) SFV DNA loads were 49.2 ± 78.1, 19.3 ± 18.9, and 21.1 ± 25.9 copies/10^5 cells in $CD8^+$ T lymphocytes, $CD4^+$ T lymphocytes, and $CD19^+$ B lymphocytes, respectively. The median SFV DNA values were 16.0 (interquartile

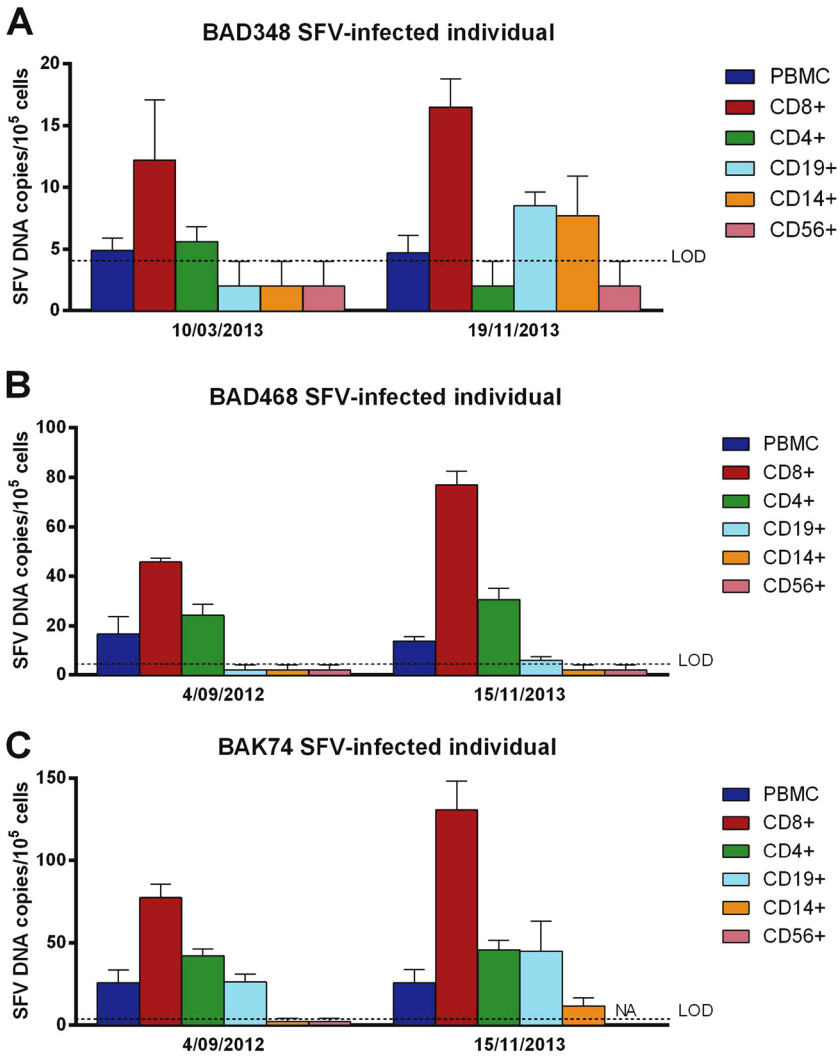

FIG 2 SFV DNA loads in PBMC populations of 3 hunters infected with a *Gorilla gorilla* SFV strain at two time points. PBMC populations from 3 SFV-infected individuals were isolated, and the SFV DNA load was quantified in each PBMC population at two different time points. Each quantification was performed twice in triplicate (e.g., $n = 6$). Means ± standard deviations are shown. NA, not available. Values below the limit of detection (LOD) were arbitrarily set as half the LOD, 2 SFV DNA copies/10^5 cells.

range [IQR], 11.0 to 49.8), 11.3 (IQR, 5.9 to 28.3), and 17.2 (IQR, 2.0 to 25.2) copies/10^5 cells in $CD8^+$ T lymphocytes, $CD4^+$ T lymphocytes, and B lymphocytes, respectively. SFV DNA levels in $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, and B lymphocytes were not statistically different (Friedman's test; $P = 0.12$). SFV DNA loads in $CD4^+$ T lymphocytes and B lymphocytes were correlated (Spearman's $\rho = 0.646$, $P = 0.04$) but did not correlate with the SFV DNA load in $CD8^+$ T lymphocytes (for $CD4^+$ T lymphocytes, Spearman's $\rho = 0.469$, $P = 0.15$; for B lymphocytes, Spearman's $\rho = 0.312$, $P = 0.35$).

We repeated peripheral blood sampling, cell subset separation, and SFV DNA quantification 8 to 14 months later for three donors (BAD348, BAD468, and BAK74) (Fig. 2). For these three individuals, the $CD8^+$ T lymphocytes contained the highest SFV DNA levels at both time points. In $CD4^+$ T cells from BAD468 and in both $CD4^+$ and $CD19^+$ cells from BAK74, SFV DNA was detected at both time points. Finally, samples with an undetectable level of SFV DNA at one time point ($CD14^+$ monocytes and $CD56^+$ NK cells from the 3 donors, as well as $CD4^+$ and $CD19^+$ cells from BAD348 and $CD19^+$ cells from BAD468) displayed undetectable or low levels (<10 copies/10^5 cells) of SFV DNA at the other time point. In conclusion, distribution of SFV DNA across blood cell subsets appeared to be mainly stable over an 8- to 14-month period.

We quantified SFV DNA load in naive and memory $CD4^+$ T cells from five individuals (Fig. 3). The SFV DNA load was above the limit of detection in memory $CD4^+$ T cells from all donors and in naive $CD4^+$ T cells from 3 donors. The mean (±SD) SFV DNA load was 21.8 ± 18.7 copies/10^5 cells in memory $CD4^+$ T cells and 10.1 ± 11.3 copies/10^5 cells in naive $CD4^+$ T cells; medians were 15.0 (IQR, 6.2 to 40.9) and 2.0 (IQR, 2.0 to 18.7) SFV DNA copies/10^5 cells in memory and naive $CD4^+$ T cells, respectively. Thus, SFV targets both $CD4^+$ naive and $CD4^+$ memory cells. Due to limitations in the biological material available, analyses of SFV DNA loads in naive and memory CD8 T lymphocytes could not be performed.

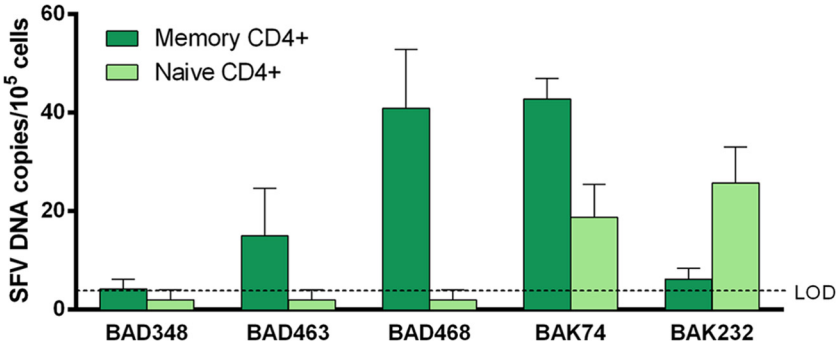

FIG 3 SFV DNA loads in memory and naive $CD4^+$ T cells of 5 hunters infected with a *Gorilla gorilla* SFV strain. $CD4^+$ cells were separated into naive and memory cells before quantification of the SFV DNA load in 5 SFV-infected individuals. Each quantification was performed twice in triplicate (e.g., $n = 6$). Means ± standard deviations are shown. Values below the limit of detection (LOD) were arbitrarily set as half the LOD, 2 SFV DNA copies/10^5 cells.

We then studied the association between SFV DNA load and the characteristics of infection. The SFV DNA load in PBMCs tended to be positively correlated with the duration of infection without reaching statistical significance (Spearman's $\rho = 0.563$, $P = 0.07$) (Fig. 4A). The SFV DNA load in $CD4^+$ T cells was positively correlated with the duration of infection (Spearman's $\rho = 0.632$, $P = 0.04$) (Fig. 4B). In contrast, such a correlation was not found for $CD8^+$ and $CD19^+$ lymphocytes (Spearman's $\rho = 0.032$, $P = 0.93$ for $CD8^+$ lymphocytes; Spearman's $\rho = 0.425$, $P = 0.19$ for $CD19^+$ lymphocytes) (Fig. 4C and D). These data suggest a selective increase in viral load in $CD4^+$ T lymphocytes over time when long periods of time (decades) are considered. The number of SFV DNA copies in the different cell subsets examined did not correlate with age at infection or age at sampling (data not shown).

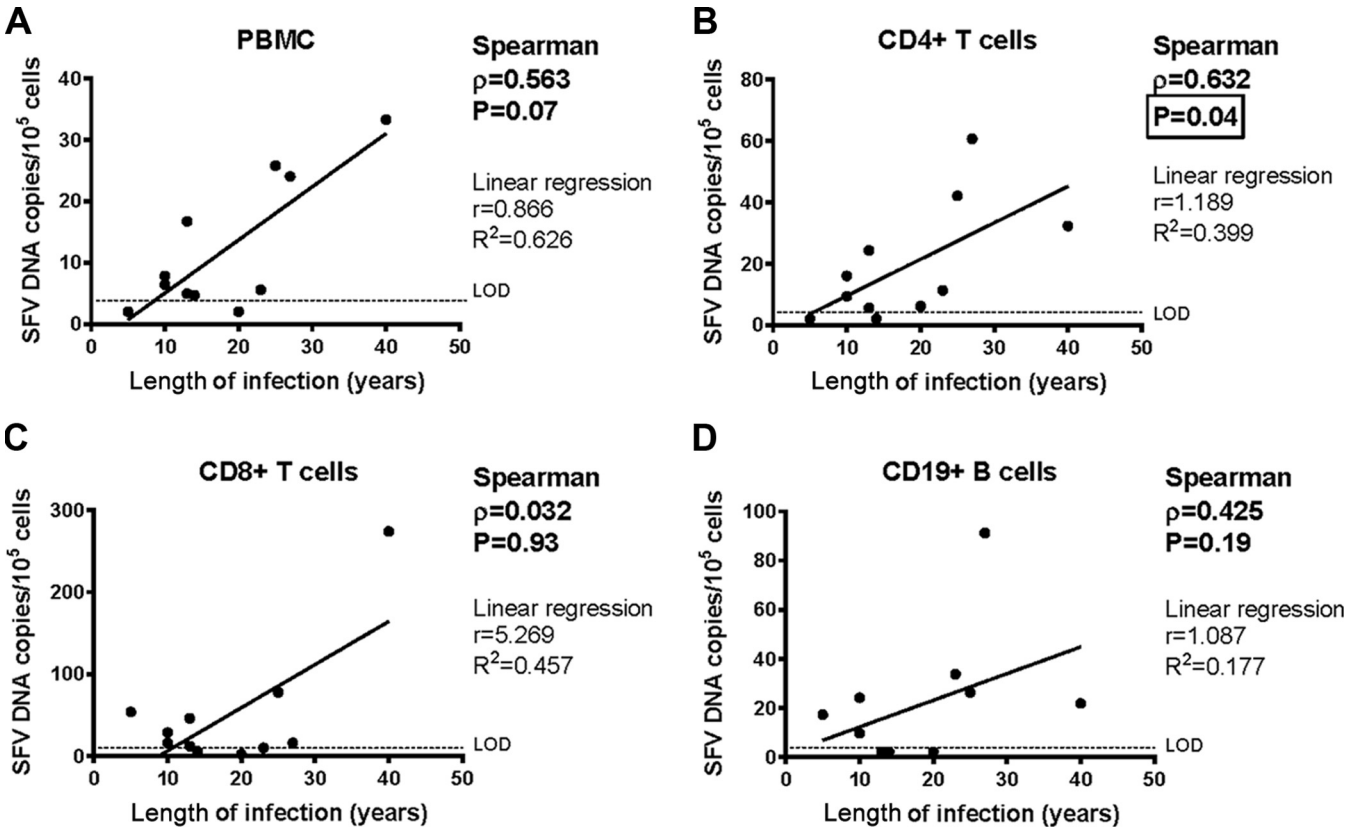

FIG 4 SFV DNA loads in PBMCs, $CD4^+$ T cells, $CD8^+$ T cells, and $CD19^+$ B cells as a function of the duration of infection. PBMC populations from 11 individuals infected with a *Gorilla gorilla* SFV strain were isolated, and the SFV DNA load was quantified in each PBMC population. Each quantification was performed twice in triplicate (e.g., $n = 6$) and plotted as a function of the duration of infection. Time of infection was based on interviews, as described previously (4). Means are shown as well as Spearman's test results (bold characters). Of note, Spearman's test is nonparametric and thus appropriate for our set of data (there were no assumptions about the probability distributions of the variables, here viral loads). Linear regression (parametric) is provided for visualization but has no statistical relevance, as the correlation existing between viral load and the duration of infection is not linear. The values below the limit of detection (LOD) were arbitrarily set as half the LOD, 2 SFV DNA copies/10^5 cells.

Interestingly, premature stop codons in the viral accessory gene *bet* were reported for one SFV-infected individual (11). For this individual, SFV DNA was found in $CD19^+$ B lymphocyte and $CD14^+$ monocyte cells, but it was not detected in $CD4^+$ T cells and $CD8^+$ T cells, and the study's authors suggested that this may be linked to SFV *bet* mutations. Therefore, we sequenced the entire SFV *bet* CDS (1,443 bp) from the 11 individuals. We did not find deletion events or stop codons in the 11 *bet* gene sequences (see Fig. S1 and S2 in the supplemental material).

DISCUSSION

Here, we quantified SFV DNA load in the major PBMC subpopulations of 11 individuals infected with a *Gorilla gorilla* SFV strain. Previous reports on three infected persons showed that B and T lymphocytes as well as monocytes harbor SFV DNA (10, 11). Semiquantitative analysis of SFV DNA loads in the different PBMC populations of 2 individuals revealed that 10^4 to 10^5 CD8 T cells were sufficient to amplify SFV DNA, indicating a viral load of 1 to 10 SFV DNA copies/10^5 cells, which is slightly lower than our results and might be related to PCR sensitivity, the infecting strain of SFV, and/or the sample size. Our study is the first that accurately quantifies SFV DNA loads in PBMC populations in a series from SFV-infected individuals. We observed that the frequency of SFV detection in T and B lymphocytes was higher than in NK lymphocytes and monocytes. No difference in SFV DNA levels was observed between T and B lymphocytes. Among $CD4^+$ T cells, SFV DNA was found in both naive and memory $CD4^+$ T lymphocytes. Interestingly, HIV-1 (12) and HTLV-1 (8) preferentially infect memory $CD4^+$ lymphocytes. More data would have been necessary to conclude whether there was a preferential infection of memory $CD4^+$ T lymphocytes. Defining naive and memory $CD8^+$ T lymphocytes and B lymphocytes relies on the coexpression of two molecules, and their purification requires a cell sorter in a biosafety level 2 laboratory, which was not available at the time of the study. Further experiments will be needed to determine if the observed cellular targets of SFV in humans are linked to biological properties of SFV, such as replication in the different cell subsets, as was previously suggested by a study *in vitro* (13). It would also be interesting to know whether infection of the T and B cell progenitor, clonal expansion of lymphocytes, and/or homeostatic $CD8^+$ proliferation accounts for SFV DNA levels.

We observed that the distribution of SFV DNA in peripheral blood cell subsets was stable over several months. However, our cross-sectional study suggests that SFV cellular targets may evolve over several decades. We found that the SFV DNA load in $CD4^+$ T cells was positively correlated with the duration of infection, whereas this correlation was not observed for $CD19^+$ B cells or $CD8^+$ T cells. Further longitudinal studies will be needed to better understand the evolution of SFV DNA load and cellular target in the course of infection (5). Finally, it would be interesting to have quantitative data on the natural host of SFV infections, gorillas in our case, but blood sampling of a large series of SFV-infected gorillas in this area is extremely challenging.

In humans, SFV DNA was found exclusively in $CD8^+$ T cells from two tested individuals (10) and in monocytes and B lymphocytes from one individual who was infected with a viral strain containing deleterious mutations in the SFV *bet* accessory gene (11). Of note, we have also found a premature stop codon in the *bet* gene of the *Pan troglodytes troglodytes* strain of SFV (from 3 chimpanzees and 4 humans infected with SFV) resulting in a predicted Bet protein that is shortened by 2% (14). These observations led us to sequence the *bet* coding sequence from the 11 individuals. No sequences harbored stop codons or major deletions, suggesting that at least in our series of individuals infected with a gorilla foamy virus, deleterious mutations in *bet* are not required for infection of monocytes and B lymphocytes. Of note, 2 of 11 *bet* sequences were obtained after virus isolation in our previous study (14) and showed >99.5% similarity with the ones presented here. The number of cell fractions with detectable SFV DNA varied between one and five, and we did not observe a bimodal distribution of the virus across the mononuclear cell subsets. The variations across the two previous studies and our results may be related to the small size of the study populations, the differences in assay sensitivity, the interindividual variations, or distinct specificities of SFV from different clades. Indeed, we have studied individuals infected with SFV from gorillas, whereas studies reported by Callahan et al. and van Laer et al. focused on individuals infected with SFV from African green monkeys or chimpanzees (10, 11).

The sample size is small, but this comes as a result of the relative rarity of SFV-infected individuals and the difficulty in obtaining and preserving high-quality samples in the field. For most donors, SFV DNA loads in purified CD8, CD4, and CD19 subsets were similar or higher than in whole PBMCs, in line with the preferential infection of these lymphocyte subsets. For some samples, a fraction of PBMCs partially lost the expression of CD markers after overnight shipment or was undergoing apoptosis. As a consequence, the SFV DNA load could be higher in enriched viable subsets than in whole PBMCs (as for donor AKO394). Importantly, SFV DNA loads in different PBMCs and/or buffy coat samples from these same individuals were reported by our team in previous studies, and they were in the same range (4, 5). Moreover, the lower level of purity of certain cell subsets (especially NK cells) is another limitation of our study, although SFV DNA in NK cell subsets is generally undetectable. These technical issues do not change our major conclusion that $CD8^+$ and $CD4^+$ T lymphocytes and B lymphocytes are preferentially infected in individuals infected with a gorilla SFV strain.

Our findings raise concerns about the consequences of *in vivo* retroviral coinfections, because we found that SFV, like HIV-1 and HTLV-1, shows long-term persistence in leukocytes, including T lymphocytes. Furthermore, SFV can increase HIV binding at the cell surface via heparan sulfate glycosaminoglycans and can upregulate HIV-1 LTR transactivation (15, 16). Cases of HIV-1/SFV (17) or HTLV-1/SFV (unpublished data) coinfections have been found in humans. In macaques, experimental SIV infection in either non-SFV-infected or naturally SFV-infected animals revealed that SFV infection increases the pathology of SIV infection (18). Given the ongoing risk of exposure to these three primate retroviruses, especially in Central Africa, microbiological surveillance is required to better evaluate the potential clinical and physiopathological impact of such retroviral interactions. Furthermore, our findings indicate the cellular distribution of SFV *in vivo*. This could have important implications for the development of SFV-based viral vectors for use in human gene therapy. For instance, recent approaches for treating blood-related diseases consist of direct intravenous administration of SFV viral vectors. Identifying the cellular targets of SFV is critical to better estimate the efficiency and safety of such vectors (19, 20).

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