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In vivo cellular tropism of gorilla simian foamy virus in blood of infected humans

Running title: Tropism of simian foamy virus in humans

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Abstract

Simian foamy viruses (SFV) are retroviruses that are widespread among non-human primates. SFV can be transmitted to humans, giving rise to a persistent infection. Only 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 in rare CD14+ monocyte and CD56+ NK lymphocyte samples. The median (Interquartile range, IQR) SFV DNA were 16.0 (11.0-49.8), 11.3 (5.9-28.3) and 17.2 (2.0-25.2) copies/10^5 cells in CD8+ T lymphocytes, CD4+ T lymphocytes and CD19+ B lymphocytes, respectively. Looking at 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 simian foamy virus (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 counterpart 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

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the biology of retroviral infections in humans and can be useful in the growing field of SFV-based gene therapy.

Introduction

Like simian immunodeficiency virus (SIV) and simian T cell lymphotropic virus (STLV), simian foamy viruses (SFV) 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 been performed yet (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 SFV viral load ranges from 1 to >1000 SFV DNA copies/10^5 blood cells (3-6). While it is known that both retroviruses, HIV-1 and HTLV-1, can infect T cells (7,8), little is known about the distribution of SFV in human blood cells (9). One pioneer study in chimpanzees and African green monkeys revealed a preferential infection of lymphocytes, especially CD8+ T cells, in 13 SFV-infected animals (10). In humans, using semi-quantitative PCR assays, SFV DNA was found 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 to obtain, quickly process, and accurately quantify 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.

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In this study, we aimed to quantify and analyze SFV viral load in the major populations of peripheral blood mononuclear cells (PBMC) 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 compared to 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 Liberté'.

SFV status of enrolled individuals

We enrolled in the present study 11 participants who had been shown to be infected with a gorilla SFV strain in our previous work (4) (Table 1). Briefly, the 11 participants were determined infected with SFV based on both serological results (Western-Blot positivity) and molecular studies on PBMCs (SFV detection by nested- polymerase chain reaction PCR on the pol-in fragment (integrase coding sequence of the polymerase gene) and/or the LTR fragment). All of them have been bitten by a gorilla, which is very probably the cause of SFV infection, as suggested previously

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(4). Of note, the strain of SFV infecting each individual always matched with the NHP species they were bitten by, as declared in the interview (herein, gorillas).

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 hours following sampling, fresh PBMC 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). For the 'memory study', CD4+ T cells were first selected by the T cell isolation kit 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 BDFacsCanto for data acquisition and FlowJo V8.7.1 software for data analysis, after labeling with a composition of the following antibodies: anti-CD3-FITC, anti-CD45RA-V450 (BD Biosciences, Rungis, France), anti-CD8-APC, anti-CD14-PE (Beckman Coulter, Villepinte, France), anti-CD16-V450, anti-CD56-PC7, anti-CD20-PC5 (BD Pharmingen, Rungis, France), anti-CD4-APC-H7 (eBioscience, Paris, France). LIVE/DEAD® Fixable Dead Cell Stain Kit (Life Technologies, Saint Aubin, France) was used to identify viable cells. 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+ as NK lymphocytes. The purity of CD4+, CD8+ and CD19+ lymphocytes, CD14+ monocytes, and CD56+ NK cells was 94.0±3.1%, 87.9±8.1%,

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79.9±7.5%, 73.2±15.2%, and 61.4±16.4%, respectively (Supplementary Table S1). 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 polymerase chain reaction assays (qPCR) targeting SFV polymerase gene (*pol*) were performed mainly as previously described (5). Briefly, the primers used (GF5qpcr-TAGACCTGAAGGAACCAAAATAATTCC, and GR5qpcr-TCCTTCCTCATATTAGGCCACC) are designed to detect a 144 bp nucleic acid region of the gorilla SFV polymerase gene. We used the Eppendorf realplex master gradient detection system and SYBR Green Quantitect (Qiagen) in a 20 µl volume reaction containing 10 µl of SYBR Green buffer, 150 nM of each primer and 500 ng DNA sample (approximately 75 000 cells equivalent) quantified by ND 8000 (Nanodrop Technologies). The optimized qPCR conditions used were as follows: 95°C for 15min, 40 cycles of: 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 were calculated.

To standardize quantitative PCR (qPCR), a 465 bp region that included the PCR target sequence from one primary isolate (BAK74, gorilla strain of SFV) was cloned into a PCR cloning plasmid, TOPO TA cloning kit (Invitrogen), and diluted in 500ng of human genomic DNA (Promega). All SFV qPCR assays included a standard curve (from 3 to 3^7 =2187 SFV DNA copies, 3-fold serial dilutions), performed in triplicate. In each assay, SFV DNA copies from 3 to 3^7 were in the range of linear detection, and coefficient correlation of SFV standard curves was >0.95. The limit of detection (LOD) was determined as the amount of SFV DNA copies corresponding to the

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threshold cycle at which at most 5% of true-positive samples scored arbitrarily negative. Using this definition, the LOD was determined as 3 SFV DNA copies/75 000 cells equivalent (4 SFV DNA copies/10^5 cells equivalent). qPCR below the LOD were arbitrarily set up at half the LOD (2 SFV DNA copies/10^5 cells equivalent).

A 141bp-cellular albumin qPCR was performed on a 250x dilution of the samples (2ng) to normalize with cellular DNA content (albF-AAACTCATGGGAGCTGCTGGTT, albR- GCTGTCATCTCTTGTGGGCTGT), 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) to the PCR reagents, ranging from 0.08ng DNA to 50ng DNA. Coefficient correlation of albumin standard curves was >0.95.

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

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 PBMC of SFV-infected individuals. We used nested-PCR to generate three PCR products, A (572 bp, *bet* open reading frame orf-1), B (761 bp, 5'-fragment of *bet* orf-2), and C (861bp, 3'-fragment of *bet* orf-2, overlapping with B).

The primers used to generate PCR-product A were primers A1 (5'-AACTGGAGAGAGCTAAAGCAG-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

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95°C for 15min, 40 cycles of: 95°C for 15s, 50°C for 1min and 72°C for 1min, 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 15min, 40 cycles of: 95°C for 15s, 65°C for 1min, and 72°C for 1min, 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 15min, 40 cycles of: 95°C for 15s, 50°C for 1min and 72°C for 1min, followed by 72° for 7 min.

The inner primers A3 and A4, B3 and B4, or C3 and C4 were used to sequence the PCR products A, B, or 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 the software CLC Genomics workbench (Gap open cost: 10.0, Gap extension cost: 1.0). GenBank accession numbers are: KF982250-KF98226 (*bet* orf-1) and KF982262-KF982272 (*bet* orf-2).

Statistical analysis

Concerning qualitative analysis, SFV DNA detection rates across the 5 cell subsets were compared using the Cochran's Q test, and two-by-two comparisons were

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198 performed by the McNemar's test. Concerning quantitative analysis, the Friedman
199 test was used to compare SFV DNA levels across T and B lymphocytes. Spearman's
200 rank test was used to assess correlations between SFV DNA levels and variables
201 related to SFV infection. Graphpad Prism software was used for statistical analysis.
202
203 **Results**
204 SFV DNA was quantified in selected subsets of peripheral blood cells from 11
205 individuals (Figure 1). SFV DNA was detected in CD8+ T lymphocytes for 10 out of
206 11 (91%) donors, in CD4+ T lymphocytes for 9 (82%) of them and in CD19+ B
207 lymphocytes from 7 (64%) of them. In contrast, SFV DNA levels were above the limit
208 of detection in only two (18%) CD14+ monocyte samples and one (9%) CD56+ NK
209 lymphocyte samples. The frequency of SFV DNA detection across the 5
210 mononuclear cell subsets was significantly different (Cochran's Q test $P < 0.001$). The
211 frequency of SFV DNA detection was significantly higher in CD8+, CD4+ and CD19+
212 B lymphocytes than in CD56+ NK lymphocytes, and in CD8+ and CD4+ T
213 lymphocytes than in CD14+ monocytes (McNemar's test $P<0.05$ for each two-by-two
214 comparisons). There was a trend for a higher frequency of SFV DNA detection in B
215 lymphocytes than in monocytes, although not reaching statistical significance
216 (McNemar's test $P=0.07$).
217 We then performed quantitative analysis on CD8+, CD4+ and CD19+ lymphocytes
218 as SFV DNA was generally above the limit of detection in these subsets. The mean
219 (±standard deviation, SD) SFV DNA loads were 49.2 ±78.1, 19.3 ±18.9, and
220 21.1±25.9 copies/10^5 cells in CD8+ T lymphocytes, CD4+ T lymphocytes and CD19+
221 B lymphocytes, respectively. The median (Interquartile, IQR) SFV DNA values were
222 16.0 (11.0-49.8), 11.3 (IQR, 5.9-28.3) and 17.2 (IQR, 2.0-25.2) copies/10^5 cells in

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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 load in CD4+ T lymphocytes and B lymphocytes were correlated (Spearman's ρ=0.646, P=0.04) but neither correlated with SFV DNA load in CD8+ T lymphocytes (CD4+ T lymphocytes: Spearman's ρ=0.469, P=0.15; B lymphocytes: Spearman's ρ=0.312, P=0.35).

We repeated peripheral blood sampling, cell subset separation and SFV DNA quantification 8-14 months later for three donors (BAD348, BAD468 and BAK74) (Figure 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 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 appears mainly stable over a 8-14 months period.

We quantified SFV DNA load in naive and memory CD4+ T cells from five individuals (Figure 3). 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 in naive CD4+ T cells; medians (IQR) were 15.0 (6.2-40.9) and 2.0 (IQR, 2.0-18.7) 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

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biological material available, analysis of SFV DNA load in naive and memory CD8 T lymphocytes could not be performed.

We then studied the association between SFV DNA load and characteristics of infection. SFV DNA load in PBMC tended to be positively correlated with the duration of infection without reaching statistical significance (Spearman's ρ=0.563, P=0.07, Figure 4A). SFV DNA load in CD4+ T cells was positively correlated with the duration of the infection (Spearman's ρ=0.632, P=0.04, Figure 4B). In contrast, such correlation was not found for CD8+ and CD19+ lymphocytes (Spearman's ρ=0.032, P=0.93 for CD8+ lymphocytes, and Spearman's ρ=0.425, P=0.19 for CD19+ lymphocytes, Figure 4C and 4D respectively). These data suggest a selective increase in viral load in CD4+ T lymphocytes over time, when considering long periods of time (over decades). 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).

Interestingly, premature stop codons in the viral accessory gene *bet* were reported for one SFV-infected individual (11). In this individual, SFV DNA was found in CD19+ B lymphocytes and CD14+ monocytes cells but it was not detected in CD4+ T cells and CD8+ T cells and the authors suggested that this may be linked to SFV *bet* mutations. Therefore, we sequenced the entire SFV *bet* CDS (1443 bp) from the 11 individuals. We did not find deletion events nor stop codons in the 11 *bet* gene sequences (Supplementary Figures S1 and S2).

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

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infected persons showed that B and T lymphocytes as well as monocytes harbor SFV DNA (10,11). Semi-quantitative analysis of SFV DNA loads in the different PBMC populations of 2 individuals revealed that 10^4-10^5 CD8 T cells were sufficient to amplify SFV DNA, indicating viral loads of 1-10 SFV DNA copies/10^5 cells, which is slightly lower than our results, and might be related to PCR sensitivity, infecting strain of SFV and/or samples size. Our study is the first that accurately quantifies SFV DNA loads in PBMC populations in a series of SFV-infected individuals. We observed that the frequency of SFV detection was higher in T and B lymphocytes than in NK lymphocytes and monocytes. No difference in SFV DNA levels were 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 for a preferential infection of memory CD4+ T lymphocytes if any. Definition of naive and memory CD8+ T lymphocytes and B lymphocytes relies on coexpression of two molecules and their purification requires a cell-sorter in biosafety level 2 laboratory, that was not available at the time of the study. Further experiments will be needed to understand 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 previously suggested *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 account 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 SFV DNA load in CD4+ T cells was positively correlated with the duration of the infection whereas this

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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 of 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 in two tested individuals (10), and in monocytes and B lymphocytes in 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 (in 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, 2/11 *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 study populations, the differences in assay sensitivity, the inter-individual variations, or to 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

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Laer et al. focused on individuals infected with SFV from African green monkey or chimpanzee (10,11).

Sample size is small but this comes as a result of the relative rarity of SFV-infected individuals, and the difficulty to obtain and preserve high-quality samples in the field. In most donors, SFV DNA load in purified CD8, CD4 and CD19 subsets were similar or higher than in whole PBMC, in line with the preferential infection of these lymphocyte subsets. For some samples, a fraction of PBMC has partially lost the expression of CD markers after overnight shipment, or was undergoing apoptosis. As a consequence, SFV DNA load could be higher in enriched viable subsets than in whole PBMC (as for donor AKO394). Importantly, SFV DNA loads in different PBMC 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 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 of the preferential infection of CD8+ and CD4+ T lymphocytes and B lymphocytes in individuals infected with a gorilla SFV strain.

Our findings raise concerns about the consequences of *in vivo* retroviral co-infections, because we find that SFV, as HIV-1 and HTLV-1, show long-term persistence in leukocytes including T lymphocytes. Furthermore, SFV can increase HIV binding at the cell surface *via* heparan sulfate glycosaminoglycans, and can up-regulate HIV-1 LTR transactivation (15,16). Cases of HIV-1/SFV (17) or HTLV-1/SFV (unpublished data) co-infections 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

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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).

Authorship Contributions

R.R., E.B., T.M. and A.G. performed experiments; R.R. and F.B. analyzed data; R.R., F.B. and A.G. wrote the manuscript. A.G. supervised the research project.

Conflict of interest disclosure

The authors declare no competing financial interests.

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Table 1: Epidemiological features of the 11 simian foamy virus (SFV)-infected humans.

Individual[a]	Ethnicity	Age at contact[b] (years)	Age at sampling (years)	Estimated duration of infection (years)	Wound location	Severity[c]	Sampling date and study[d]	Complete SFV genome available[e]
AKO394	Bantu	53	58	5	Thigh	1	18/09/2012 (P)	No
BAD348	Bantu	19	32	13	Leg	3	14/03/2013 (P); 19/11/2013 (P+M)	No
BAD447	Bantu	40	60	20	Hand	2	10/03/2013 (P)	No
BAD456	Bantu	24	34	10	Leg	2	10/03/2013 (P)	No
BAD463	Bantu	37	47	10	Leg	3	19/11/2013 (P+M)	No
BAD468	Bantu	25	38	13	Several	3	04/09/2012 (P); 10/03/2012 (M); 15/11/2013 (P)	Yes (JQ867465.1)
BAK177	Pygmy	26	40	14	Leg	3	15/11/2013 (P)	No
BAK232	Pygmy	40	63	23	Hand	2	19/11/2013 (P+M)	No
BAK55	Pygmy	30	70	40	Arm	2	15/11/2013 (P)	No
BAK74	Pygmy	26	51	25	Foot	2	04/09/2012 (P); 14/03/2012 (M); 15/11/2013 (P)	Yes (JQ867464.1)
LOBAK2	Pygmy	37	64	27	Thigh	3	10/03/2013 (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.

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[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 PBMC and (M) corresponds to sampling for study of the tropism of SFV in naive and memory cells.

[e] Complete sequence of isolated viral strains from the indicated individuals are available in our previous study, and their GenBank accession numbers is provided (14).

Figure legends

Figure 1: SFV DNA load in PBMC populations of 11 hunters infected with a *Gorilla gorilla* SFV strain

PBMC populations from 11 SFV-infected individuals were isolated and 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. The values below the limit of detection (LOD) were arbitrary set as half the LOD, 2 SFV DNA copies/10^5 cells.

Figure 2: SFV DNA load 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 SFV DNA load was quantified in each PBMC population at two different time points. Each quantification was performed twice in triplicatetriplicate (e.g. n=6). Means ± Standard

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deviations are shown. NA: not available. The values below the limit of detection (LOD) were arbitrarily set as half the LOD, 2 SFV DNA copies/10^5 cells.

Figure 3: SFV DNA load 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 SFV DNA load in 5 SFV-infected individuals. Each quantification was performed twice in triplicate (e.g. n=6). Means ± Standard deviations are shown. The values below the limit of detection (LOD) were arbitrarily set as half the LOD, 2 SFV DNA copies/10^5 cells.

Figure 4: SFV DNA load in PBMC, CD4+ T cells, CD8+ T cells and CD19+ B cells as a function of duration of infection

PBMC populations from 11 individuals infected with a *Gorilla gorilla* SFV strain were isolated and 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 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 non-parametric and thus appropriate for our set of data (no assumptions about the probability distributions of the variables, here viral loads). The linear regression (parametric) is provided for visualization but has no statistical relevance, as the correlation existing between viral loads and duration of the 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.

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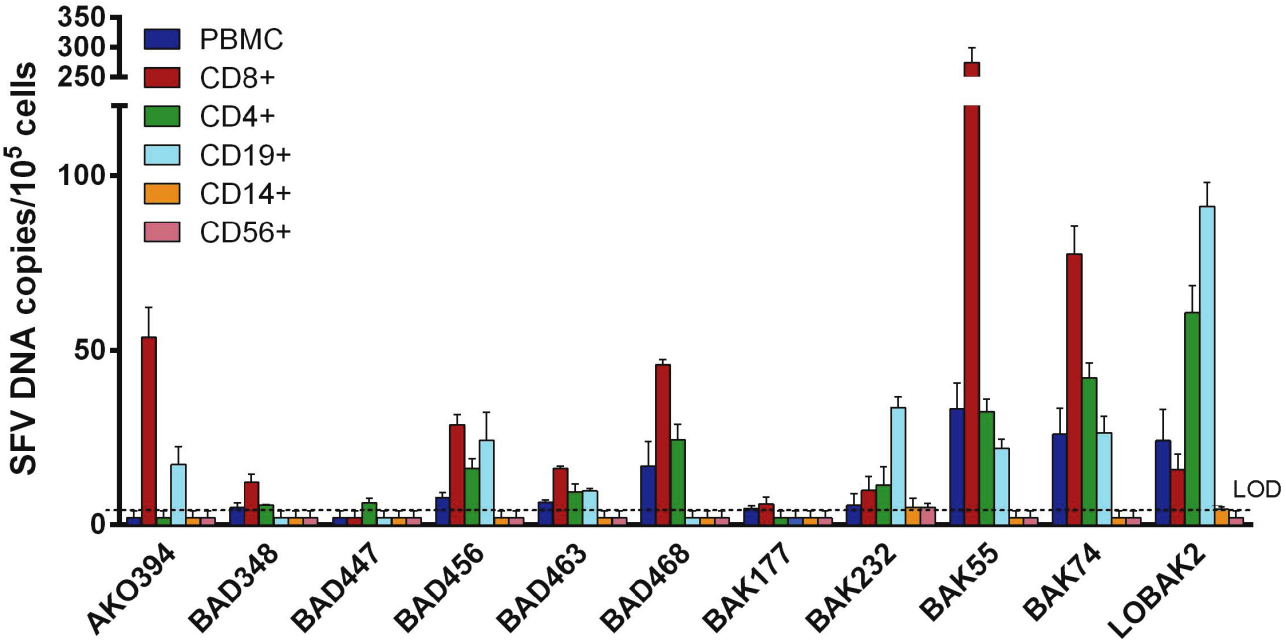


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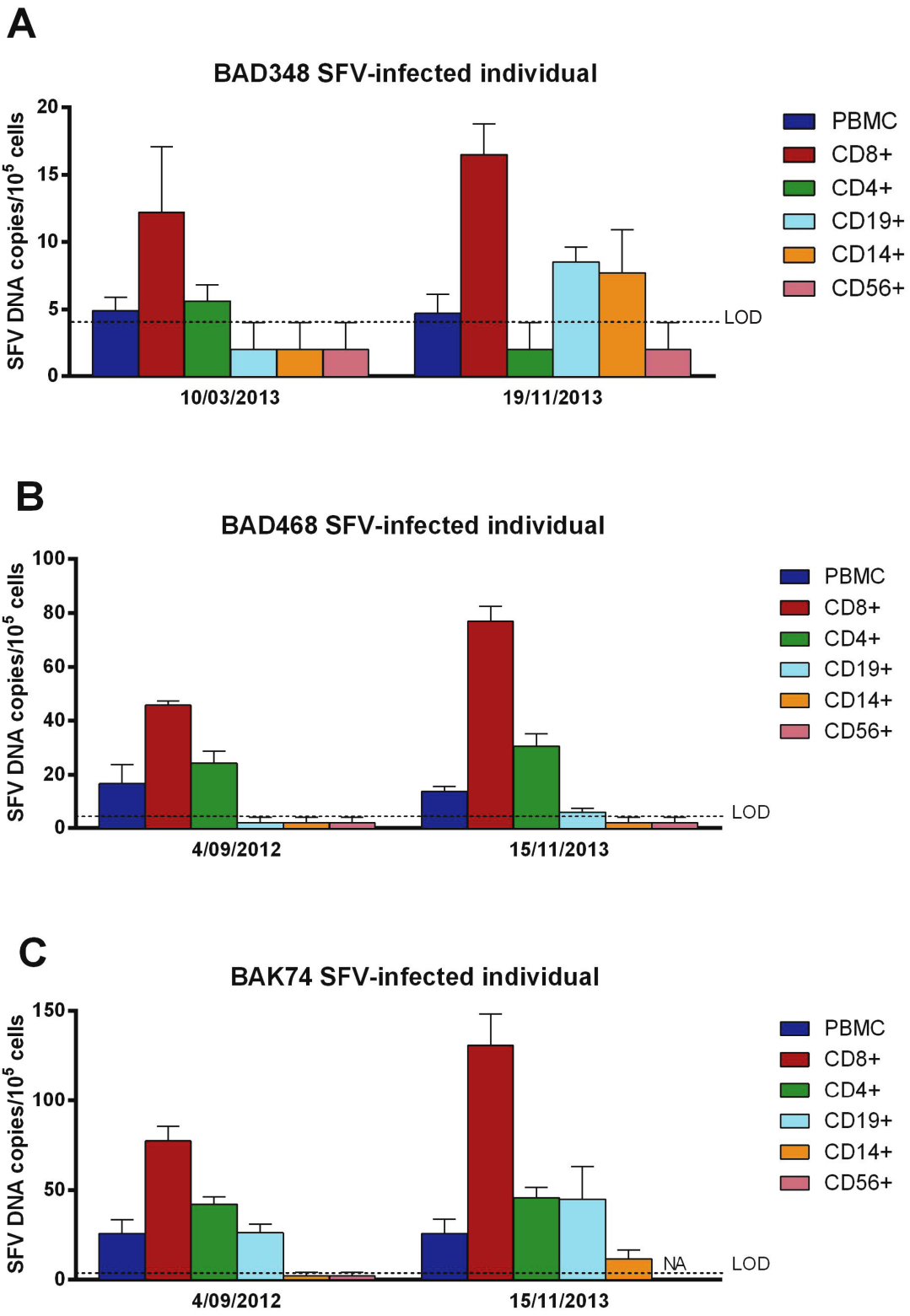


Figure 2: SFV DNA load in PBMC populations of 3 hunters infected with a *Gorilla gorilla* SFV strain, at two time points

PBMC populations from 3 SFV-infected individuals were isolated and 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. The values below the limit of detection (LOD) were arbitrarily set as half the LOD. 2 SFV DNA copies/10^5 cells.

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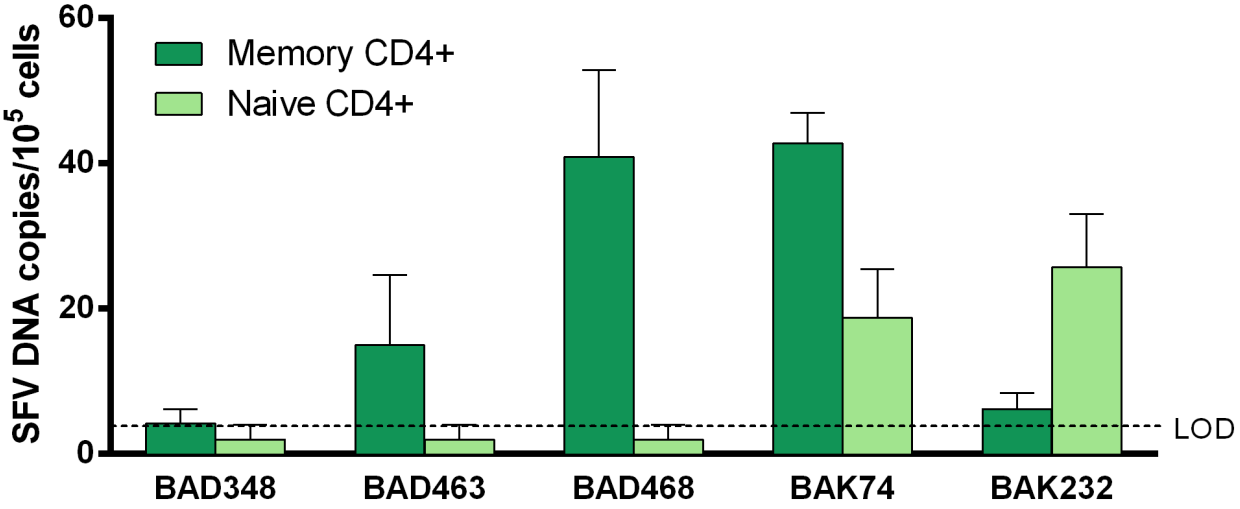


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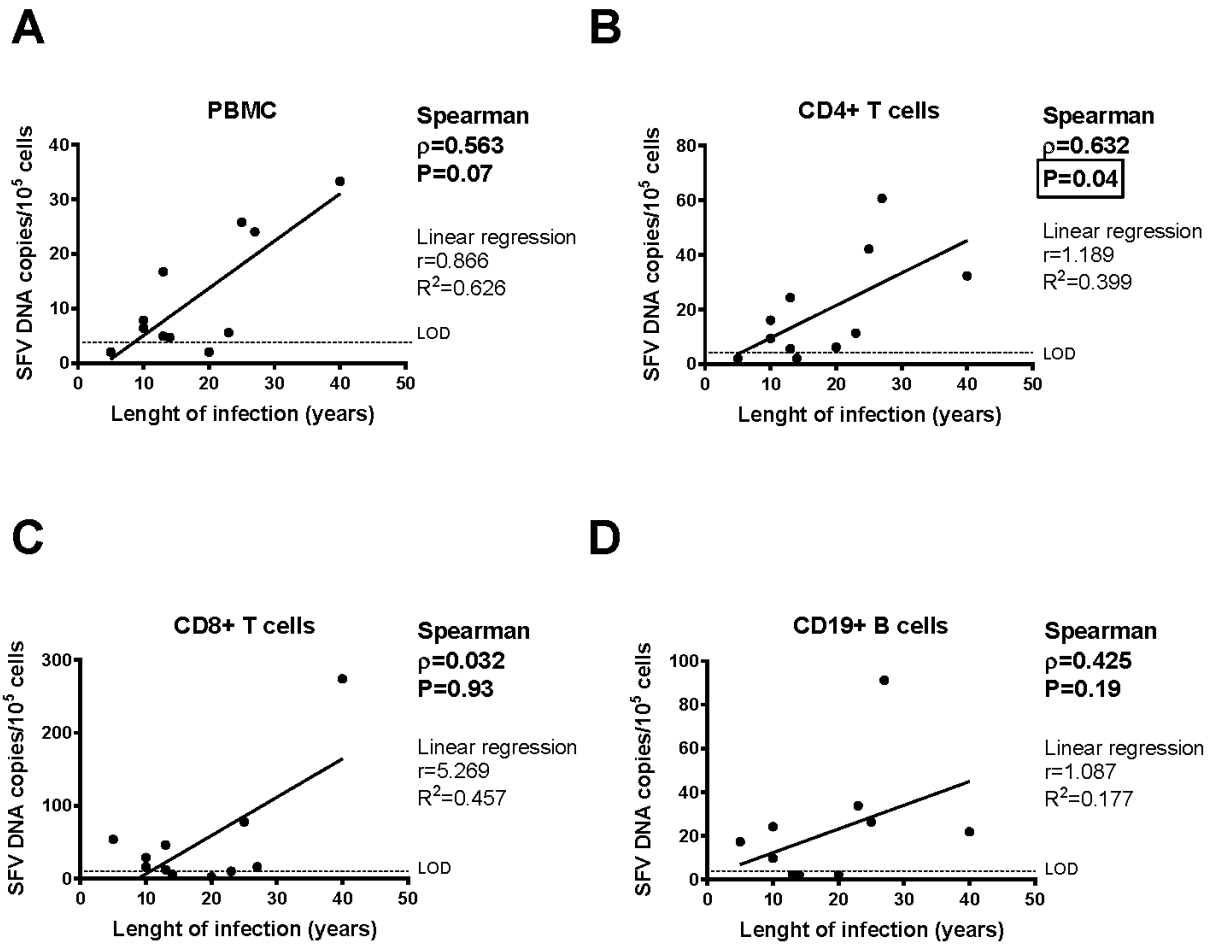


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