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SHORT COMMUNICATION

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Simian and human foamy virus (FV) DNA can be readily detected in peripheral blood leukocytes. However, it is unknown which leukocyte populations harbor the virus *in vivo.* We, therefore, analyzed blood samples from nine African green monkeys, four chimpanzees, and two humans for the presence of foamy virus proviral DNA in different FACS-purified leukocyte populations, using a highly sensitive nested polymerase chain reaction (PCR). The $CD8^+$ lymphocytes were PCR positive in all 15 samples and the average viral burden was highest in this population. FV DNA was detected in 10 of 15 cell samples enriched for B lymphocytes, and 4 of 9 $CD4^+$ lymphocyte, 3 of 13 $CD14^+$ monocyte, and 4 of 13 polymorphonuclear leukocyte samples. A highly sensitive reverse transcriptase PCR was performed to detect viral transcripts in peripheral blood leukocytes. All samples were negative. In conclusion, lymphocytes, and especially $CD8^+$ T lymphocytes, were found to be a major target for foamy virus in the peripheral blood, but viral gene expression was not detected.

Simian foamy viruses (SFVs) are complex retroviruses that have been isolated from a wide range of nonhuman primates (*1*). SFVs can be classified into serogroups by neutralization assays. A similar classification of foamy viruses (FVs) is obtained by analyzing sequence homologies in the pol region (*2*). Human foamy virus (HFV) was isolated from a single patient with nasopharyngeal carcinoma (*3, 4*). FVs are generally considered to be apathogenic (*5, 6*). They have a broad cell tropism *in vitro* including many mammalian and even avian cell lines (*7*). Virus can be isolated from a wide variety of tissues and from peripheral blood leukocytes of infected animals (*8, 9*). However, the exact cell types which harbor virus *in vivo* have not been defined. In this study, several leukocyte populations were analyzed for the presence of FV proviral DNA by polymerase chain reaction (PCR).

Blood samples from nine African green monkeys (AGM), four chimpanzees, and two humans (one was infected while handling HFV in the laboratory, the other was bitten several times by SFV-infected AGM) were analyzed. The animals were held at the Biomedical Primate Research Centre in Rijswijk, at the Behring primate facilities in Marburg, or at the Department of Virology in Freiburg (Table 1). All AGM in Freiburg were known to be infected with SFV-3 (*10*). To classify the FV type for the cases from outside of Freiburg, a 425-bp fragment from the pol region was amplified by PCR and sequenced as described previously (*2*). The sequences were aligned to the known prototype sequences. The homologies to the prototype viruses are given in Table 1. All AGM were infected with SFV-3; all chimpanzees with a FV closely related to SFV-6, SFV-7, and SFVcpz; one human with SFV-3; and the other with HFV.

Leukocyte populations were purified from peripheral blood for PCR analysis as follows. Peripheral blood leukocytes (PBL) were separated using Ficoll–Paque gradient centrifugation (Pharmacia density medium; Uppsala, Sweden). Peripheral blood mononuclear cells (PBMC) from the interphase were then stained with the monoclonal antibodies anti-CD4 (Leu3a; Becton–Dickinson [BD] San Jose, CA), anti-CD8 (Leu2a; BD), and anti-CD14 (detects monocytes, IOM2; Immunotech, Marseilles, France). Human PBMC were also stained with anti-CD19 (detects B lymphocytes, OKB19a; Ortho Diagnostic Systems, Raritan, NJ). The cells in the pellet of the Ficoll gradient (polymorphonuclear leukocytes [PMNL] and erythrocytes) were resuspended in erythrocyte lysing buffer (150 m*M* NH_4Cl, 10 m*M* $KHCO_3$, 0.1 m*M* EDTA, pH 7.4), incubated for 8 min at room temperature, and then washed three times with phosphate-buffered saline. The remaining cells, which were highly enriched for PMNL, were stained with anti-CD16, which detects PMNL, and anti-CD14, which detects monocytes (ION16, IOM2; Immunotech).

Several leukocyte fractions were purified on a FACStar^{plus} cell sorter (BD). The $CD4^+$ T helper cells and $CD8^+$ cyto-

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TABLE 1

List of Species and Viruses Analyzed

	Species	Infecting virus	Homology to prototype[a]	Animal colony
A1	AGM[b]	SFV-3	ND	Freiburg
A3	AGM	SFV-3	ND	Freiburg
A4	AGM	SFV-3	ND	Freiburg
A5	AGM	SFV-3	ND	Freibrug
A6	AGM	SFV-3	ND	Freiburg
A7	AGM	SFV-3	ND	Freiburg
CB617	AGM	SFV-3	89%	Marburg
CB695	AGM	SFV-3	91%	Marburg
CB699	AGM	SFV-3	88%	Marburg
Wb	Man (laboratory worker)	HFV	>99%	—
Ka	Man (animal caretaker)	SFV-3	89%	—
D	Chimpanzee	SFV-6	92%	Rijswijk
Ke	Chimpanzee	SFV-6	89%	Rijswijk
I	Chimpanzee	SFV-6	96%	Rijswijk
Wi	Chimpanzee	SFV-6	95%	Rijswijk

[a] The homologies were determined based on a 425-bp pol sequence as described previously (*2*).

[b] AGM, African green monkey (*Cercopithecus aethiops*); ND, not done.

toxic T lymphocytes as well as $CD4^-CD8^-$ non-T-lymphocytes were sorted. The latter population consists mainly of B lymphocytes and natural killer cells. In six of nine AGM less than 1% of the cells in the lymphocyte gate were CD4-positive. The reason why these AGM had such a low number of $CD4^+$ T lymphocytes was unclear. A possible explanation could be that the animals were all older than 15 years. For these animals, only the $CD8^+$ T lymphocytes and the total $CD8^-CD4^-$ non-T-lymphocytes were sorted. For human blood samples, markers for B lymphocytes are available and, therefore, the $CD19^+$ B lymphocytes could be sorted instead of the $CD4^-CD8^-$ lymphocytes. $CD14^+$ monocytes were sorted from all samples. The $CD14^-$ PMNL with high forward and sideward light scatter were sorted from the cell pellet of the Ficoll gradient. For human blood samples, PMNL were additionally selected by their staining with anti-CD16. Cells (10^5) were sorted for each cell population. Sorted cells had a purity of >98%.

The FACS-purified cells (10-fold cell dilutions, starting with 10^5 cells) were resuspended in lysis buffer (10 m*M* Tris at pH 8.3, 2.5 m*M* $MgCl_2$, 50 m*M* KCl, 0.5% Tween 20, 0.5% NP-40) with 0.2 mg/ml proteinase K. After an incubation period of 2 hr at 60°, the proteinase K was inactivated at 95° (10 min). The crude DNA extract was used for nested PCR amplification of a 465-bp segment from the pol region as described previously (*2*). This nested PCR was highly sensitive and <5 copies of viral DNA were regularly detected (*5*). To confirm the high sensitivity of this PCR, cells of the latently infected cell line Vero-L (containing one copy of proviral SFV-3 DNA per cell [*11*]) were diluted in non-infected Vero cells and a crude DNA extract was prepared as described above. Using the nested FV pol PCR, a single Vero-L cell was detected in 10^5 uninfected Vero cells (Fig. 1). To prove the suitability of each DNA sample for PCR, an aliquot equivalent to 10 cells was tested by amplification of the cellular single-copy gene c-myc as described previously (*5, 6*).

The results in Table 2 are given in the minimal number of cells which were needed for a positive PCR result. This number ranged from 10^3 to 10^5 cells, which corresponds to <1% infected cells. The $CD8^+$ lymphocytes were positive in all samples and the average viral load in $CD8^+$ cells was higher than in the other infected leukocyte populations. $CD4^+$ lymphocytes were positive in only 4 of 9 samples, but in 2 of 4 chimpanzees, the highest viral load was found in the $CD4^+$ lymphocytes. Ten of 15 samples enriched for B lymphocytes ($CD8^-CD4^-$ lymphocytes or $CD19^+$ B lymphocytes) were PCR positive. The estimated viral load in this population was always low (<0.01%). The $CD14^+$ monocytes were positive in 3 of 13 samples and PMNL in 4 of 13 samples. The viral load in these two populations was also low (<0.01%). These data clearly show that lymphocytes, and especially $CD8^+$ T lymphocytes, represent the major reservoir for FV in peripheral blood.

To detect spliced FV transcripts in PBMC, a reverse transcription PCR (RT-PCR) was performed. Total mRNA from 2×10^6 PBMC was extracted using the PolyAtract System 1000 mRNA isolation kit (Promega, Madison, WI) according to the manufacturer's recommendations. After precipitation, mRNA was resuspended in 25 μl RNase-free water. Reverse transcription was performed using random primers and a first-strand cDNA synthesis kit (Pharmacia). Viral cDNA was amplified by nested PCR under standard conditions. The primers are located in the leader and in the env region (Table 3). This RT-PCR

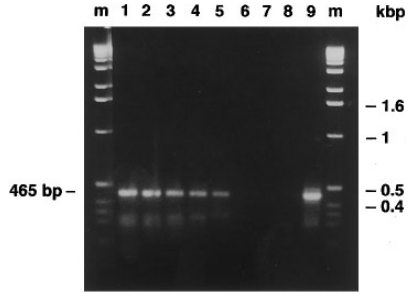

FIG. 1. Sensitivity of nested foamy virus pol PCR. Amplification products of the second round were separated by electrophoresis through a 2% agarose gel. Source of the amplified DNA: lanes 1–8, DNA from latently infected Vero-L cells (each containing one copy of integrated SFV-3 DNA) diluted in uninfected Vero cells (total number of cells 10^5). Lane 1, 10^4 Vero-L cells. Lane 2, 10^3 Vero-L cells. Lane 3, 10^2 Vero-L cells. Lane 4, 10^1 Vero-L cells. Lane 5, 10^0 Vero-L cells. Lane 6, 10^{-1} Vero-L cells. Lane 7, 10^{-2} Vero-L cells. Lane 8, no DNA (negative control). Lane 9, 1 ng DNA from lytically infected Molt-4 cells (positive control). m, molecular weight marker (1 kb DNA ladder; Gibco-BRL, Eggenstein, Germany).

TABLE 2

Detection of Foamy Virus DNA in FACS-Purified Leukocyte Populations

(A) African green monkey (*Cercopithecus aethiops*)

	$CD8^+$ T lymphocytes	$CD4^+$ T lymphocytes	Non-T-lymphocytes ($CD4^-CD8^-$)	$CD14^+$ monocytes	PMNL
A1	10^3	ND	(10^5)	Neg	Neg
A3	10^4	ND	10^5	Neg	Neg
A4	10^5	ND	10^5	Neg	Neg
A5	10^4	ND	10^5	Neg	Neg
A6	10^4	ND	10^5	10^5	Neg
A7	10^3	ND	(10^5)	(10^5)	(10^5)
CB617	10^5	Neg	Neg	Neg	Neg
CB695	10^5	Neg	Neg	ND	ND
CB699	10^4	10^4	10^5	ND	ND
Sum	9/9	1/3	7/9 (5/9)	2/7 (1/7)	1/7 (0/7)

(B) Humans

	$CD8^+$ T lymphocytes	$CD4^+$ T lymphocytes	$CD19^+$ B lymphocytes	$CD14^+$ monocytes	$CD16^+$ PMNL
Wb	10^5	Neg	Neg	Neg	Neg
Ka	10^4	Neg	Neg	Neg	Neg
Sum	2/2	0/2	0/2	0/2	0/2

(C) Chimpanzees

	$CD8^+$ T lymphoyctes	$CD4^+$ T lymphocytes	Non-T-lymphocytes ($CD4^-CD8^-$)	$CD14^+$ monocytes	PMNL
D	10^5	Neg	Neg	Neg	Neg
Ke	10^5	10^4	10^5	Neg	10^5
I	10^5	10^4	10^5	Neg	10^5
Wi	10^5	10^5	10^5	10^5	10^5
Sum	4/4	3/4	3/4	1/4	3/4

Note. For each cell population 10-fold dilutions of 10^5 cells were analyzed and the minimal number of cells needed for a positive PCR (mnpc) is given. If the viral load in a cell population was 100-fold lower than in others, the mnpc was set into parentheses. Here, it cannot be excluded that the PCR signal results from contamination of the sample with cells of the population with the higher viral load, since the purity of cells ranged between 98 and 99.5%. Neg, no foamy virus DNA was detected by polymerase chain reaction in 10^5 FACS-purified cells.

was found to detect the cDNA prepared from less than 10 infected dog thymus cells (Cf2TH; American Type Culture Collection). No viral transcripts were detected in the PBL of the 13 animals and the two humans.

As the $CD8^+$ samples of most individuals were shown to carry the highest amount of FV DNA, $CD8^+$ samples of three AGM (CB-617, -695, and -699), all four chimpanzees, and both humans were analyzed by RT-PCR as

TABLE 3

Primers Used for Amplification of Viral cDNA

Primer	Outer/inner	Location (nucleotides)[a]	Region	Virus detected	Sequence
P1	5′ outer	1337–1358 of SFV-3	LTR/leader	SFV, HFV	5′-ctcaccactgctcgctgcgtcg-3′
P2	5′ inner	1359–1380 of SFV-3	LTR/leader	SFV, HFV	5′-agagtgttcgagtctctccagg-3′
P3	3′ outer	7410–7389 of SFV-3	env	SFV-3	5′-cattccaatcaatgactggacc-3′
P4	3′ inner	7377–7356 of SFV-3	env	SFV-3	5′-tatccctattccattgaatcct-3′
P5	3′ outer	1577–1558 of HFV	env	HFV, SFV-6, -7	5′-acctccacatagtttttggaac-3′
P6	3′ inner	1531–1511 of HFV	env	HFV, SFV-6, -7	5′-caatccttctagtctgtaagg-3′

[a] The EMBL Accession Nos. are M74895 for SFV-3 and M54978 (3′ region only) for HFV.

described. Also in these samples, enriched for FV-positive cells, viral transcripts were not detected. Since the $CD4^+$ cell numbers of AGM 1–7 were very low and the majority of the PBMC were $CD8^+$ cells, $CD8^+$ samples of these monkeys were not analyzed separately.

This study clearly shows that lymphocytes, and especially $CD8^+$ lymphocytes, represent a major target for FV *in vivo.* This finding furthers several previous studies, in which FV was isolated from unfractionated leukocytes or from enriched leukocyte samples (*9*). In one study, FV was isolated from nonadherent peripheral blood mononuclear cells, which had been stimulated with PHA for 4 days prior to cocultivation with cells susceptible to FV. This protocol is expected to enrich T lymphocytes, and these results indicated that T cells may be a reservoir for FV *in vivo* (*12*). In another study, FV was isolated from an EBV-immortalized B cell line, which indicated that B lymphocytes also may be a viral reservoir (*13*). In both studies, total mononuclear cells were cultured prior to selection of the lymphocytic cells and isolation of FV. Virus could have been passed on from one cell type to another during culture. However, this study, in which we analyzed leukocytes directly after isolation, confirms the previous findings that B lymphocytes as well as T lymphocytes can harbor FV *in vivo.*

It is unclear why lymphocytes are the major FV reservoir in the blood. One likely explanation is that lymphocytes have a much longer half-life than other leukocyte populations and are highly mobile. The probability of encountering virus-infected cells or free infectious virus during a life span is expected to be higher for lymphocytes than for PMNL or monocytes. It is more difficult to explain why $CD8^+$ lymphocytes have a higher viral burden than the other lymphocyte populations. $CD8^+$ lymphocytes could be more susceptible than other lymphocytes, due to a higher expression of virus receptor. $CD8^+$ cytotoxic T lymphocytes could also be preferentially exposed to the highly cell-associated FV while killing FV-infected cells. Lastly, FV could be less cytopathic for $CD8^+$ lymphocytes and establish latency in these cells more readily than in other leukocyte populations. Further *in vitro* studies on the tropism of FV for lymphocyte subsets will be necessary to clarify this issue.

The fact that FV is only rarely detected in cells of the myelomonocytic lineage (PMNL and monocytes) shows that the myeloic progenitor cells in the bone marrow are most likely not a major reservoir for FV. We cannot completely rule out that progenitor cells harboring FV lose the capacity to differentiate into mature PMNL and monocytes. However, preliminary data from our laboratory indicate that infection of human $CD34^+$ hematopoietic progenitor cells with FV does not inhibit hematopoiesis in clonal assays *in vitro.*

Viral transcripts could not be detected in PBL by a method as sensitive as RT-PCR. The FV LTR promoter thus functions inefficiently in leukocytes in comparison to other promoters, such as the human immunodeficiency virus or cytomegalovirus promoters. For these viruses, we could regularly detect transcripts in PBL by the RT-PCR technique described here (*14*) (for HIV unpublished data). However, the RT-PCR used was not designed to detect mRNA transcribed from the internal FV promoter (*15, 16*). We can, therefore, not exclude that the internal promoter is functioning normally and possibly even producing a factor that inhibits transcription from the LTR, such as bet. This mechanism has recently been described for HFV mutants, which lack the functional transactivator bel1 but harbor an intronless *bet* gene (*17*). This defective mutant behaves like classical defective interfering particles. It suppresses gene expression of wild-type virus and thereby induces chronic infection. Similar defective FVs were indeed found for SFV in PBL of AGM using a PCR which spans the deletion downstream of the internal promoter (R. Renne, personal communication). Further possible explanations for the lack of detectable viral transcription could be methylation of the DNA, the lack of essential cellular transcription factors in leukocytes, or mutations in the LTR (*18*). Further studies are necessary to clarify this issue.

In conclusion, lymphocytes are the major reservoir for FV in peripheral blood, whereas virus is rarely detectable in cells of the myelomonocytic lineage. However, FV transcripts were not detected in PBL. These findings are especially important in the light of recent attempts to develop FV-based viral vectors for use in human gene therapy (*19, 20*).

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