

## Simian Foamy Virus Isolated from an Accidentally Infected Human Individual

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**Evidence for natural foamy virus (FV) infections in humans is still lacking. However, accidental infections of humans with simian FV have been demonstrated by serology and PCR, but all previous attempts to recover infectious virus in such cases have failed. Here we describe the isolation of a simian FV from peripheral blood mononuclear cells (PBMC) of a healthy animal caretaker, who acquired the virus 20 years ago from an African green monkey (AGM) bite. Properties of the human isolate such as host range in cell cultures including human PBMC and ability to induce neutralizing antibodies in the primate host proved to be similar to those of FV obtained from AGM. The genomic sequence of the isolate was found to be virtually identical to the proviral sequence present in the host lymphocytes and related to AGM isolates but distinct from those of all FV isolates handled in the laboratory. For successful virus isolation, it was essential to stimulate the host lymphocytes by phytohemagglutinin and interleukin-2 for 2 weeks prior to cocultivation with permissive cells. In contrast to the situation found in FV-infected monkeys, virus isolation from the saliva of the animal caretaker was not possible, and no evidence for FV transmission to family contacts was obtained. We conclude that, in contrast to active infection in monkeys, FV persists in a state of latency following accidental infection of humans.**

Foamy viruses (FV; *Spumavirus* genus of *Retroviridae*) cause persistent infections in a wide range of mammalian species, including nonhuman primates (10). The prevalence of simian FV (SFV) in monkeys and apes kept in primate centers is more than 70% (15). SFV can readily be isolated from infected animals, either from the saliva or from peripheral blood lymphocytes (10, 18). By contrast, evidence for natural FV infections in humans is lacking. Previous reports of FV prevalence in random human populations and association of FV with human diseases could not be confirmed by extended studies in our laboratory (17, 18) and by various other groups (2, 6–8, 16, 20). Most of the previous serological data and recent detection of proviral DNA in human cohorts have most likely resulted from inappropriate testing (2, 18). Some older FV isolates that were claimed to be of human origin (5, 22, 23) have unfortunately been lost. Despite repeated efforts to obtain new isolates from patient samples, only a single FV isolate from a human individual is available. This unique strain, designated human foamy virus (HFV), was obtained from nasopharyngeal carcinoma tissue of an East African patient (1). Based on immunological and sequence data, HFV is closely related to chimpanzee FV, and it was suggested to be a variant strain of chimpanzee FV transmitted by animal contact (4, 9, 13, 19).

Transmission of SFV to humans resulting from professional exposure has been described in few cases (10, 18). However, in these individuals, infection had so far been demonstrated only by serology and PCR. Here, we describe for the first time the isolation of infectious FV from an accidentally infected person, proving the presence of the complete proviral sequence in the host lymphocytes.

**History of the infected human individual.** The male animal caretaker Ka, born in 1932, was exposed to African green monkeys (AGM), rhesus monkeys, and cynomolgus monkeys

from 1974 to 1995. In the 1970s, he was bitten several times by AGM, only once (1976) with deep injury of the left middle finger requiring surgery. He has stayed perfectly well throughout his professional life at the institute and beyond retirement in 1995. His medical history was uneventful. Due to preexisting hyperlipemia with slight elevation of serum aminotransferases, he never donated blood for transfusion or production of plasma products. His serum was tested for FV antibodies by indirect immunofluorescence for the first time in 1980; this testing revealed a titer of 1:160, which has remained unchanged in repeated samples (14, 17, 18). Multiple attempts at virus isolation from throat swabs and native lymphocytes were unsuccessful, and FV DNA could not be demonstrated by Southern blot hybridization of lymphocyte DNA. In 1988, FV DNA was detected by the PCR technique in peripheral blood mononuclear cells (PBMC). Cloning and sequencing of the amplification products revealed a close relationship between proviral DNA present in PBMC of Ka and sequences of FV isolates obtained from the AGM colony attended (19), indicating that Ka is infected with an AGM FV from this colony. The mode of natural primate FV transmission was not directly investigated, but epidemiological observations in monkey colonies as well as shedding of infectious virus in the saliva of infected animals as shown below point to biting as the common route of transmission. Therefore, infection of Ka occurred probably by the bite documented in 1976. Analysis of different lymphocyte fractions of Ka separated by fluorescence-activated cell sorting revealed FV DNA exclusively in the CD8<sup>+</sup> cells (21). FV RNA could not be detected by reverse transcription-PCR (RT-PCR). Ka is aware of his FV infection and showed continued cooperation in donating blood and other samples for characterization of his FV carrier state. Markers of FV infection could not be detected in close family contacts (wife and one daughter) by testing sera and PBMC by indirect immunofluorescence and FV *pol* PCR (19), respectively, at three instances.

**FV isolation from the animal caretaker and from AGM.** Over the last decade, virus isolation has been repeatedly at-

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TABLE 1. Detection of FV by PCR and virus isolation from human and AGM samples<sup>a</sup>

Individuals	Rate of positive PBMC samples by nested <i>pol</i> PCR	Rate of successful virus isolation	
		From saliva	From PBMC
Seropositive AGM	34/38 (22) <sup>b</sup>	50/60 (40) <sup>b</sup>	20/25 (13) <sup>b</sup>
Seronegative AGM	0/8 (5)	0/10 (5)	0/5 (5)
Seropositive human (Ka)	5/5 (1)	0/4 (1)	1/6 (1)

<sup>a</sup> Numbers in parentheses refer to numbers of individuals tested.

<sup>b</sup> Each animal yielded at least one positive result.

tempted from saliva and PBMC of Ka as well as from 40 SFV-seropositive and 5 seronegative AGM. All seropositive individuals tested were also positive by nested FV *pol* PCR (19) on PBMC DNA, whereas PBMC from SFV-seronegative monkeys were always negative by PCR (Table 1).

For virus isolation, PBMC were prepared by Ficoll Paque 400 (Pharmacia, Freiburg, Germany) gradient centrifugation of 10 ml of EDTA-blood. Lymphocytes (10<sup>6</sup>/ml) were kept in RPMI 1640 medium supplemented with 15% fetal calf serum and antibiotics. The cells were stimulated for 2 days with 10 µg of phytohemagglutinin (PHA-P; Difco Laboratories, Detroit, Mich.) per ml and further cultivated with 100 U of interleukin-2 (IL-2; from Sigma, Deisenhofen, Germany, or purified from supernatants of human donor blood mononuclear cell cultures [12]) per ml. Beginning from day 3 of IL-2 stimulation, samples of 10<sup>6</sup> lymphocytes were cocultivated for 15 h with 2 × 10<sup>5</sup> human diploid fibroblasts strain alpha-1 (14). After washing and removal of the lymphocytes, the fibroblasts were kept and subcultivated until a cytopathic effect (CPE) typical of FV was observed. In general, CPE appeared within 2 weeks of cocultivation. Infection of the cultures was demonstrated by indirect immunofluorescence (14) using SFV-positive monkey serum (18). Isolation was considered unsuccessful if no marker of FV infection could be detected in the cultures within 4 weeks. Cocultivation was repeated six times from days 5 to 25 of lymphocyte cultivation. Virus isolation from monkeys was most efficient between days 8 and 12.

For isolation of FV from the oropharynx, saliva was obtained by throat swabs, using cotton-wool sticks, and added directly to the medium of alpha-1 human diploid fibroblasts. FV infection of alpha-1 cells was monitored as described for isolation from PBMC cultures. The results of virus isolation are summarized in Table 1. FV could easily be isolated from the saliva as well as from PBMC from all seropositive monkeys

tested. The constant success of FV isolation from saliva of the monkeys indicates that viral replication occurs over long periods, probably for life, in persistently infected monkeys. By contrast, no virus could be obtained from the saliva of Ka, and only one of six attempts at isolation from his PBMC was successful after 14 days of T-cell stimulation. This isolate was designated SFVka. Probably, the level of infectious virus has been very low in Ka at any time. The fact that viral reactivation required *ex vivo* stimulation and expansion of the peripheral blood lymphocytes from Ka appears to correspond to the latent state *in vivo* of the investigated FV infection. Accordingly, FV has not been transmitted from Ka to his relatives, as his wife and daughter revealed no signs of FV infection.

The success of virus isolation after stimulation of T cells is in good accordance with our previous results on FV tropism for peripheral blood cells. We have shown that CD8<sup>+</sup> T lymphocytes are the major target of FV in the peripheral blood of primates, including Ka and an HFV-infected laboratory worker (21).

**In vitro properties of the human isolate SFVka.** For infection experiments and neutralization assays, virus stocks were obtained by filtering supernatants of infected cells through 450-nm-pore-size Millipore membranes. Virus titers were determined by counting cells stained by FV-specific indirect immunofluorescence 2 days after infection of BHK-21 cultures with appropriate (10-fold) dilutions. The host range in cell culture of SFVka was compared with that of SFV-3 strain LK-3, the prototype of lymphotropic FV derived from the AGM colony attended by Ka (14). Different cell lines (Table 2) were infected at a multiplicity of infection of 0.01 and monitored for CPE, FV mRNA (by RT-PCR detecting spliced FV *env* mRNA as previously described [21]), and virus titers in the supernatant. No significant differences in tropism for cultured cells and development of infection could be detected between the two FV strains; however, SFVka was produced at slightly higher titers than LK-3 (Table 2). To investigate the susceptibility for SFVka of primary human blood cells, Ficoll-purified PBMC obtained from two FV-negative donors were stimulated with phytohemagglutinin and IL-2 and infected with SFVka and LK-3 at a multiplicity of infection of 0.1. Infection was proved 5 days after inoculation by RT-PCR for spliced FV *env* mRNA (data not shown). For neutralization tests, serial dilutions of sera were incubated with equal volumes of SFVka (10<sup>3</sup> infectious particles/ml) for 1 h at 37°C before determining viral infectivity on BHK-21 cells by indirect immunofluorescence as described above. In duplicate experiments, SFVka infectivity was reduced by ≥90% when serum of Ka was used at dilutions up to 1:160. Sera of two FV-negative donors did not neutralize

TABLE 2. Infection of various cell lines with SFVka and SFV-3/LK-3

Cells	Virus	CPE <sup>a</sup>	FV mRNA <sup>b</sup>	Virus yield <sup>c</sup>
Alpha-1 (human diploid foreskin fibroblasts)	SFVka	3	+	10 <sup>5</sup>
	LK-3	3	+	4 × 10 <sup>4</sup>
Molt-4 (human T-cell lymphoma)	SFVka	6	+	10 <sup>3</sup>
	LK-3	6	+	10 <sup>2</sup>
Vero (AGM kidney)	SFVka	6	+	5 × 10 <sup>2</sup>
	LK-3	7	+	10 <sup>2</sup>
Cf2Th (canine thymus)	SFVka	4	+	10 <sup>3</sup>
	LK-3	4	+	10 <sup>2</sup>
BHK-21 (baby hamster kidney)	SFVka	5	+	2 × 10 <sup>4</sup>
	LK-3	5	+	4 × 10 <sup>2</sup>

<sup>a</sup> Day of first appearance of CPE.

<sup>b</sup> RT-PCR on day 3 postinfection.

<sup>c</sup> Titer of supernatant on day 7 postinfection.

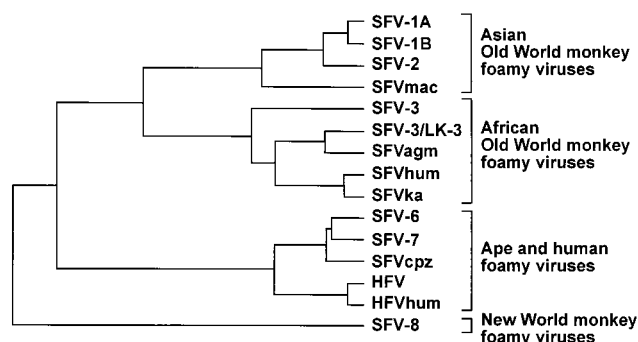


FIG. 1. Phylogenetic tree of primate foamy viruses. SFV-1A SFV-1B, and SFV-2, FV from Taiwan monkeys (*Macaca cyclopsis*); SFVmac, FV from a rhesus monkey (*Macaca mulatta*); SFV-3, FV prototype from AGM (*Cercopithecus aethiops*); SFV-3/LK-3 and SFVagm, FV from AGM of the colony attended by Ka; SFVhum, proviral sequence present in lymphocytes of Ka; SFVka, FV from Ka; SFV-6, SFV-7, and SFVcpz, FV from chimpanzees; HFVhum, proviral sequence present in lymphocytes of an accidentally HFV-infected laboratory worker; SFV-8, FV from a spider monkey (*Ateles* sp.). The EMBL accession numbers for all of the sequences (except SFVka) are given in reference 19.

SFVka. Altogether, cellular tropism, infectivity for cell lines and human PBMC, and the ability to induce neutralizing antibodies were found to be similar in SFVka and LK-3 and in accordance with properties of other SFV isolates previously published (3, 10, 11). Therefore, the difficulties to isolate FV from Ka may be due to host factors rather than attributable to genetic modification of SFVka.

**Sequence analysis of SFVka.** In the past, several reports on FV isolation from humans caused confusion. No sound serological data that could confirm the FV infection of the source individuals have been presented, and none of the isolates has been conserved for analysis by modern methods. In the present case, the authenticity of the new isolate SFVka could be checked by comparing its nucleic acid sequence with the sequence of proviral DNA present in lymphocytes of Ka. For sequencing, the 465-bp amplification product of nested *pol* PCR was cloned by using the TA cloning system (Invitrogen, San Diego, Calif.) and investigated by the dideoxy-chain termination method as described previously (19). The sequence obtained was compared with previously published FV *pol* sequences by using the software CLUSTAL implemented in the PC-Genie program (IntelliGenetics, Mountain View, Calif.) to form phylogenetic trees based on overall similarity. Figure 1 shows a phylogenetic tree of primate FV based on CLUSTAL alignment of the 425-bp-long *pol* region (19), supplemented with the sequence of the new isolate SFVka. FV from Asian or African Old World monkeys, hominoids, or New World monkeys fit into separate clusters, indicating that the phylogenetic branches of the viruses reflect those of their hosts. The sequence of the new isolate SFVka (recovered from a PBMC sample obtained from Ka in December 1995) and the sequence SFVhum (EMBL accession no. X83293), which represents proviral DNA present in a PBMC sample of Ka obtained in 1991 (19), belong to the cluster of African Old World monkey FV, confirming infection of Ka with an AGM FV. SFVka revealed a close relationship to SFVhum. Only three mutations were detected: G at position 284 of SFVhum is replaced by C in SFVka, G at 399 is replaced by C, and G at 403 is replaced by A. Accordingly, two amino acids are exchanged: Asp-Gly at positions 133 and 134 in SFVhum are changed to His-Asp in SFVka. Thus, the sequence homologies between SFVhum and SFVka are 99.3% on the DNA level and 98.6% on the amino acid level. The sequences of FV isolates from monkeys of the

colony attended by Ka were more distantly related: 89.4 to 91.5% on the DNA level and 89.4 to 93.6% on the amino acid level. The virtual identity of SFVka and SFVhum confirms that SFVka originated from Ka, since any FV isolate picked up from the laboratory would have revealed a more different sequence.

Here, we describe the second isolation of an FV from a human 25 years after description of the unique isolate called HFV (1). The history of the host indicates for the first time that FV can persist in an accidentally infected healthy human for two decades. Previously, attempts at virus isolation from accidentally infected humans failed. The difficulties to obtain infectious virus were ascribed to various reasons, such as low-level viral replication, modification of the viral genome, or immune control in seropositive humans. The data presented here prove long-term persistence of complete genomes of SFV in a human. Ka has lived with the FV infection for at least 16, and presumably 20, years without developing any disease that might be associated with the infection. This well-investigated example of missing FV pathogenicity in a long-term-infected human host raises more questions on the mechanisms of persistence of a retrovirus which still reveals unvaried high cytopathogenicity *in vitro*. The nonpathogenicity of FV *in vivo*, not only in naturally infected nonhuman primates but also in accidentally infected human hosts, is an argument in favor of developing FV vectors for human gene therapy.

Previous reports on traces of HFV in normal populations and in certain groups of patients could not be confirmed by appropriate methods (2, 6–8, 16–18, 20). Moreover, the lack of any other FV isolates (besides the unique strain HFV) unequivocally originating from human sources has further reduced the probability of a low-level HFV endemic. Thus, the origin of HFV remains obscure. The high similarity of HFV to chimpanzee FV and the fact that the nasopharyngeal carcinoma patient originally harboring HFV lived in East Africa favor the hypothesis that the virus had been acquired from a chimpanzee by accidental or ritual exposure (4, 9, 13, 19). The isolation of SFVka two decades after accidental transmission, as described in this report, demonstrates that a nonhuman primate FV has been able to persist in an infected human being and indirectly strengthens the probability of the zoonosis hypothesis for the origin of HFV.

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