A Comparative Study of Higher Primate Foamy Viruses, Including a New Virus from a Gorilla

PAUL D. BIENIASZ, AXEL RETHWILM, RICHARD PITMAN, MUTHI A. DANIEL, IAN CHRYSTIE, and MYRA O. McCLURE

Department of Communicable Diseases, The Jenner Institute Research Trust Laboratories, St Mary's Hospital Medical School, Praed Street, London W2 1PG, United Kingdom

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Few foamy (spuma) retroviruses have been investigated in molecular detail, despite their previous isolation from several mammalian species, including ten neutralization serotypes from various primates. Here, we have studied a new gorilla foamy virus (SFV-Gg) and investigated its functional and phylogenetic relationship to the human (HFV) and other primate foamy viruses, including those recently described in orangutans (SFV-11). Nucleotide sequencing of PCR products obtained from the FvUS region of the LTR, gag, and pol genes revealed a close relationship between HFV and three chimpanzee isolates (SFV-6, SFV-7, and SFV-cpz). The SFV-Gg, SFV-11, rhesus macaque (SFV-1), and African green monkey (SFV-3) isolates were more divergent. To explore functional relationships, primate foamy virus transactivation of HFV LTR driven $\beta$-galactosidase expression in a newly constructed cell line, BHLL, was investigated. HFV, SFV-6, and SFV-7 potently transactivated HFV LTR driven lacZ gene expression, SFV-Gg induced expression approximately 10-fold less efficiently, and SFV types 1, 2, 3, and 11 did not significantly transactivate the HFV LTR. It was thus possible to assay serum neutralizing activity in SFV-infected primates against HFV, SFV-6, and SFV-7 by reducing $\beta$-galactosidase activity following infection of the indicator cell line. Sera from infected chimpanzees and gorillas neutralized to varying degrees, each of these three viruses, whereas orangutan sera did not. Our results, based on DNA sequences and functional assays, support the conclusion that HFV is closely related to foamy viruses of chimpanzee origin. © 1986 Academic Press, Inc.

INTRODUCTION

Spumaviridae constitute one of the three subfamilies of the Retroviridae and have been found in several mammalian species, particularly in free and captive primates (Hooks and Gibbs, 1975). Of the known simian foamy viruses (SFV), serotypes 1, 2, and 3 have been isolated from macaques, African green monkeys, and baboons, SFV-4, 8, and 9 from new world monkeys, SFV-5 from prosimians, SFV-6 and 7 from chimpanzees, and SFV-10 from baboons. Of these, SFV-6 has been shown to be antigenically closely related to the prototype human foamy virus (HFV) (Nemo et al., 1978; Brown et al., 1978; Tobal-Tapiello et al., 1990). Recently a foamy virus was isolated from two captive orangutans and designated SFV-11. This virus was distinguished from previously characterized isolates on the basis of serum neutralization, Southern blotting, and sequence analysis of an LTR fragment (McClure et al., 1994). A previously uncharacterized isolate from a captive gorilla (SFV-Gg) is reported here. The phylogeny of primate and human foamy viruses is considered by comparing the sequences of three genomic regions of SFV-6, SFV-7, SFV-Gg, and SFV-11 with the previously published sequences of HFV, SFV-1, SFV-3, and SFV-cpz (Flugel et al., 1987; Maurer et al., 1988; Kupiec et al., 1991; Renne et al., 1992; Herchenroder et al., 1994).

In common with lentiviruses and the human T-cell leukaemia viruses (HTLVs), at least some members of the foamy viruses possess genes encoding transcriptional transactivators (Rethwilm et al., 1991; Keller et al., 1991; Mergia et al., 1990, 1991; Renne et al., 1993), designated Tat proteins (for transactivator of foamy virus). While lentivirus and HTLV gene expression is regulated in part by RNA binding transactivators, Tat proteins have been shown to act solely on the initiation of transcription. Thus, the response elements for SFV-1 Tat, SFV-3 Tat, and HFV Tat (Bel-1) have been mapped within the U3 regions of the respective viral LTRs (Rethwilm et al., 1991; Keller et al., 1991; Venkatesh et al., 1991; Erlien and Rethwilm, 1993; Lee et al., 1993; Mergia et al., 1992; Renne et al., 1993). The activity of Tat proteins is sometimes, but not always, specific for the LTR of the respective virus; for example, SFV-1 Tat transactivates SFV-3 LTR driven gene expression, whereas the converse is not the case (Renne et al., 1993). Tat proteins have also been reported to upregulate gene expression driven by the HIV LTR (Keller et al., 1992; Lee et al., 1992).
Transactivation can be exploited to study various aspects of the retrovirus life cycle; for example, construction of cell lines with an integrated reporter gene under the control of a promoter, including the viral transactivator responsive elements, facilitates quantification of viral infection by counting of the number of cells expressing a reporter gene. In the same way, the potency of transactivation by virus variants or mutants can be assessed by quantification of reporter gene products. This approach is well described for HIV, where cell lines expressing β-galactosidase (Rocancourt et al., 1990) or chloramphenicol acetyl transferase (CAT) (Felber and Pavlakis, 1988) under the control of the HIV LTR have been widely used. Recently the role of accessory genes in HFV replication was investigated using an indicator cell line containing a bacterial lacZ gene under transcriptional control of the HFV U3 promoter (Yu and Linial, 1993).

In this study an indicator cell line containing a HFV U3 regulated lacZ gene was constructed to quantitatively explore crosstransactivation of the HFV LTR by foamy viruses isolated from diverse primates. Furthermore, this cell line has been exploited to assay sera from foamy virus-infected primates for neutralization of the HFV LTR transactivating viruses. These functional properties of the viruses are considered in the light of the phylogenetic relationships deduced by sequence comparison of selected regions of the viral genomes.

MATERIALS AND METHODS

Cells and viruses

BHK-21 clone 13, and its derivative BHLl, cells were cultured in DMEM containing 5% fetal calf serum (FCS), 100 µg/ml streptomycin and 100 U/ml penicillin. Human embryonic lung fibroblasts (HEL) and MRC5s SV2 cells were cultured in the same medium containing 10% FCS. Viruses (HFV and SFV) were propagated on BHK-21 or HEL cells and harvested either from culture supernatants or from twice freeze thawed cells. Virus stocks were filtered (0.45 µm, Nalgene) and stored in aliquots in liquid nitrogen. SFV-11, recently isolated from a captive orangutan (Pongo pygmaeus) with encephalopathy (McClure et al., 1994) and SFV-Gg, isolated from a captive lowland gorilla (Gorilla gorilla) (Hahn et al., 1994), were similarly propagated and harvested. Virus titers were obtained by end point dilution and observation of blue staining of BHLl cells infected with HFV, SFV-6, or SFV-7 or cytopathic effect (CPE) in the case of the other viruses.

Immunoperoxidase staining

Infected cells were fixed for 2 min with 1:1 acetonemethanol cooled to −20°C, washed with phosphate-buffered saline (PBS), and incubated for 45 min at room temperature with PBS/1% FCS diluted human and primordially washed three times with PBS/1% FCS and incubated for a further 45 min with goat anti-human or anti-rabbit IgG conjugated to horseradish peroxidase (1:100 in PBS/1%FCS). The cells were then washed a further four times in PBS and incubated with substrate (3 mg/ml 3,3’-diaminobenzidine, 0.1% H2O2 in PBS). The substrate was removed by washing with PBS and staining of antigen containing cells observed by light microscopy after incubation for 10–15 min at room temperature.

Electron microscopy

SFV-Gg-infected BHK cells were centrifuged for 10 min at 1000 g and the pellet fixed in 2.5% glutaraldehyde in PBS for 60 min. After washing in PBS, the cell pellet was postfixed in 1% osmium tetroxide for 60 min, washed in distilled water, dehydrated in ethanol, and embedded in Agar 100 resin (Agar Aids). Sections were stained with uranyl acetate and lead citrate and examined at 60 kV.

Unintegrated viral DNA extraction

Cells (BHK-21 or HEL) were infected with SFV-6, SFV-7, SFV-Gg, or SFV-11 and DNA harvested 5–10 days postinfection when CPE was evident. Unintegrated DNA was purified by Hirt extraction. Briefly, cells were trypsinized, washed once with PBS, and lysed with 0.6% SDS, 10 mM EDTA for 20 min at room temperature. NaCl was then added to the lysate to a final concentration of 1 M prior to incubation on ice for at least 4 h. Precipitated chromosomal DNA and cell debris was removed by centrifugation (13,000 g, 4 min) and unintegrated viral DNA purified from the supernatant by phenol extraction and ethanol precipitation.

Polymerase chain reaction

Viral DNA (approximately 109 cell equivalents) was amplified in a 50-µl reaction volume containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl2, 0.01% gelatin, 0.2 mM each dNTP, 0.2 µM each oligonucleotide, and 1.25 U Tag polymerase. Thirty thermal cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 60 sec were carried out. Oligonucleotide primer pairs were selected in regions conserved between SFV-1, SFV-3, and HFV. Thus, approximately 300 bp of the LTR regulatory regions were amplified using PBF1 (CAGCTACTCTGCTGGTCAAGGTC, nucleotides nt 786–809 in the published HFV sequence (Maurer et al., 1988) and PBF2 (GGATTTTGTATTTAGTATTCC, nt 1115–1093). Approximately 300 bp of the pol gene were amplified using degenerate oligonucleotides 2360 (GTACACTCCAAGTCACCCCCAAGG, nt 6045–6068) and 2361 (GACACAGAACAGGAGAAGATGAGG, nt 6369–6349) and approximately 630 bp of the gag gene were amplified using M2046 (GGTTATTCAGAATGGAGG, nt 2267–2288) and M2047 (GCAGGCAGGACATCCGCTCCAGTCGTA, nt 2883–2883).
Nucleotide sequence determination and phylogenetic analysis

PCR products were gel purified and subcloned into pCRII (TA cloning kit, Invitrogen). Nucleotide sequences were determined using the dideoxynucleotide chain termination method (Sequenase, USB) and alignments performed using the CLUSTAL program (Intelligenetics). Phylogenetic analysis was performed using PHYLIP (Felsenstein, 1989). Genetic distances were calculated using the Kimura two parameter algorithm of the DNADIST program and used in conjunction with the NEIGHBOR program to construct neighbor joining trees. One hundred bootstrapped data sets were generated with SEQBOOT and used in conjunction with CONSENSE to test the reliability of the generated phylogenetic trees.

Construction of foamy virus indicator (BHLL) cell line

A reporter plasmid containing an Escherichia coli lacZ gene under transcriptional control of the HIV LTR was constructed. The plasmid pL1Bg (A. Rethwilm, unpublished) contains the HIV U3 promoter (−777 to +4 relative to the start of transcription), including sequences previously shown to be critical for HIV Tat-mediated transactivation (Erlwein and Rethwilm, 1993). Termination sequences are also derived from the viral 5¢ LTR (U3/R −374 to +385). These two regions are separated by a unique BglII site into which was inserted a BamHI fragment of pNASS-B (Clonetech), including the complete lacZ coding sequence, to create the reporter plasmid, pD1.

pD1 was linearized outside the U3-lacZ-U3/R region with Apal. This was mixed in a 10-fold molar excess with an Apal fragment of pMAM-neo-lacZ (Clonetech), which included a neomycin resistance gene driven by an SV40 early promoter. The fragments were ligated and the unpurified concatamers transfected into BHK-21 cells by calcium phosphate coprecipitation. Following selection for 10-days in medium containing 1 mg/ml G418, approximately 200 colonies from a 75-cm² flask were pooled and single cell clones obtained by limiting dilution. Sixty-two of these were expanded in 24-well plates and screened by X-gal staining for β-galactosidase expression following infection with 100 TCID of HFV and X-gal staining, as described below. While most clones expressed β-galactosidase following infection, approximately one-third showed constitutive expression. The most sensitive, nonleaky clone, BHLL (for BHK HFV LTR lacZ) was used in subsequent studies.

Infectivity assay by X-gal staining

BHLL cells (2 × 10⁵) in 100 μl of medium were seeded into each well of 96-well plates and on the following day were infected with 100 μl of diluted cell-free virus stock (or virus-infected cells in the case of SFV-Gg). Forty-eight to 60 hr later the supernatant was removed and the cells fixed for 10 min at room temperature with 1% formaldehyde, 0.2% glutaraldehyde in PBS. Fixed cells were washed once with PBS and incubated for 1 hr at 37°C with substrate (0.5 mg/ml X-gal, 4 mM potassium ferrocyanide, 4 mM potassium ferricyanide, and 1 mM MgCl₂ in PBS). Substrate was removed and replaced with PBS and blue foci were counted under light microscopy.

Quantification of infectivity by (p-NPG) reaction

Cells were seeded, infected, and fixed 48–60 hr post-infection as described above for X-gal staining. One hundred microliters of substrate solution (2.5 mg/ml p-NPG in PBS with 1% Nonidet P-40 to permeabilize the cells) were added to each well and the reaction allowed to proceed for 90–120 min (or in some cases up to 16 hr) at room temperature. Expression of β-galactosidase was assayed by transferring the 96-well plate to an automated plate reader (Bio-tek EL-312) and measuring optical density at 405 nm.

Neutralization assays

Neutralizing antisera from primates infected with the homologous foamy virus were serially diluted threefold (from 1:10 to 1:100) in medium. Duplicate samples containing approximately 200 TCID of HFV, SFV-6, or SFV-7 in 50 μl were incubated with 50 μl of diluted serum for 1 hr at 37°C and then cultured with BHLL cells for 48–60 hr. The cells were fixed and viral infection was assayed using the p-NPG reaction. Neutralization titers were recorded as the reciprocal dilution of serum required to inhibit virus-induced β-galactosidase activity by 90%.

RESULTS

Serological and molecular characterization of foamy viruses

Electron microscopy of cells infected with SFV-Gg from the gorilla revealed particles typical of foamy virus (Fig. 1A). In common with other foamy viruses SFV-Gg could be propagated in MRC5 SV2 cells to induce cytopathology characterized by giant multinucleated cell formation. Serum taken from the same animal from which SFV-Gg was isolated reacted strongly in immunoperoxidase staining assays with cells infected with autologous virus (Fig. 1B). Serum samples obtained from three captive gorillas, thirty chimpanzees, and an HFV-infected human were also tested for reactivity with SFV-Gg. Sera from two out of three goril-
FIG. 1—Continued
las, all the chimpanzees, and the single HFV-infected human reacted strongly with SFV-Gg-infected cells in immunoperoxidase staining assays. Conversely, serum from an SFV-11-infected orangutan reacted strongly with autologous virus but only weakly with SFV-Gg, SFV-6, SFV-7, and HFV-infected cells (McClure et al., 1994 and data not shown) and antisera raised in rabbits to recombinant HFV Gag antigens highlighted localization of Gag antigen to the nucleus of SFV-Gg-infected cells (Fig. 1C), as shown for HFV (Schliephake and Rethwilm, 1994).

Amplification of viral DNA was achieved from all higher primate foamy viruses using primers selected in regions of the HFV LTR and pol genes which are well conserved in SFV-1 and SFV-3. However, amplification was not successful in many cases where the HFV primers were selected with no regard for conservation in SFV-1 or SFV-3. To sequence part of the gag gene PCR primers were designed in regions of HFV capsid and nucleocapsid that were also well conserved in SFV-1 and SFV-3. These were successfully used to amplify SFV-6, 7, and 11, but not SFV-Gg and the PCR products subsequently cloned and sequenced (Fig. 2).

Sequence analysis of the LTR PCR products from SFV-6, SFV-7, SFV-Gg, and SFV-11 revealed a closer phylogenetic relationship between HFV, SFV-6, SFV-7, and SFV-cpz (95.7–96.4% nucleotide identity) than with SFV-Gg, SFV-11, SFV-1, or SFV-3 (78.8–81.3% identity) (Figs. 2 and 3). Alignment of the sequences revealed areas of extremely high conservation (for example, including and immediately downstream of a putative polyadenylation signal, (AATAAA) interspersed with regions of considerable sequence and length heterogeneity. The inferred phylogenetic relationships were similar when sequences amplified from the pol gene were analyzed (Figs. 2 and 3). In this case the HFV was 92.5–93.5% identical to each of the three chimpanzee virus sequences at the

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**Fig. 2.** Nucleotide sequences of PCR products amplified from proviral DNA from SFV-6, SFV-7, SFV-Gg, and SFV-11-infected cells aligned with SFV-1, SFV-3, and HFV. LTR RUS sequences (A), gag (B), and pol (C). "." indicates nucleotides conserved with HFV. "-" indicates gaps introduced into the sequences to improve alignments. A conserved putative polyadenylation sequence (AATAAA) in the LTR RUS sequences is underlined.
nucleotide level (94.6–95.7% at the amino acid level) but shared only 81.7–83.9% identical nucleotides (83.9–90.3% amino acids) with the other viruses. Silent mutations in the poL sequences were favored; in the 28 possible pairwise comparisons, the ratio of nonsilent:silent mutations ranged from 0 to 0.5 (mean = 0.296, SD = 0.112). This indicates a strong selection pressure for conservation of amino acid sequence indicating an important function for this region of the genome. The gag region sequenced illustrated further divergence, but the phylogeny remained unchanged from that based on the LTR and poL sequences. HFV was 84.6–85.6% identical to each of the chimp sequences at the nucleotide level, 86.7–88.6% at the amino acid level, while there was only 61.3–63.3% nucleotide homology (51.9–64.3% amino acid homology) with the other viruses. In the gag gene in particular the chimp sequences were more closely related to each other than to HFV.

Rapid quantification of viral infectivity

Transfection of the indicator construct ligated to a neomycin resistance gene and subsequent cloning of the BHLL line has been described under materials and methods. This cell line, in which there was a very low level of constitutive expression with intense X-gal staining in response to infection, was selected for the rapid quantification of viral infectivity.

**β-galactosidase expression** was quantitatively related to the HFV inoculum as measured by the number of X-gal-stained foci of infection or by optical density at 405 nm following incubation of p-NPG with fixed infected cells (Fig. 4A). When BHLL cells were infected with HFV, the number of β-galactosidase positive cells (by X-gal staining) and HFV antigen positive cells (by immunoperoxidase staining) was the same (data not shown). Thus, HFV could be directly titrated by counting β-galactosidase expressing foci. Using p-NPG conversion as a measure of viral infectivity, less than 5 TCID of HFV, SFV-6, or SFV-7 could be detected following overnight incubation of the cells with substrate (Fig. 4).

**Cross-transactivation of HFV LTR by primate foamy viruses**

BHLL cells were infected with foamy viruses isolated from diverse primate species (SFV-1,2, and 3 from macaques, African green monkeys, and baboons; SFV-6 and 7 from chimpanzees, SFV-11 from orangutan and SFV-Gg from gorilla). In some cases, despite obvious CPE, this resulted in little or no expression of β-galactosidase above background (Fig. 4). However, SFV-6 and SFV-7

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**FIG. 2—Continued**
potently transactivated β-galactosidase expression. When BHLL cells were infected with equivalent titers of HFV, SFV-6, and SFV-7, similar levels of β-galactosidase were expressed (Fig. 4B). SFV-Gg could not be produced with sufficient cell-free titer on BHLL cells to carry out comparable experiments. Therefore, increasing numbers of SFV-Gg-infected BHLL cells were used as the inoculum and SFV-Gg antigen positive foci appearing 48 hr postcocultivation were taken as a measure of viral infectivity. SFV-Gg-infected cells were approximately 10-fold less efficient than the equivalent cell-free titers of HFV, SFV-6, and SFV-7 for transactivating HFV-LTR-directed β-galactosidase expression (Fig. 4).

Neutralization of HFV, SFV-6, and SFV-7

Since β-galactosidase expression was quantitatively dependent on infection of BHLL cells by HFV, SFV-6, or SFV-7, inhibition of infection by neutralizing antisera could be conveniently assayed. Each of the three viruses was preincubated with serial dilutions of sera from foamy virus-infected primates (three chimpanzees, three gorillas, and one orangutan) before inoculation of BHLL cells. Forty-eight hours postinfection β-galactosidase expression was assayed by conversion of pNPG. An example of this data is shown in Fig. 6; >90% neutralization of SFV-7 was observed with all three gorilla sera at a dilution of 1/2592. Serum from chimpanzee C neutralized SFV-7 at a dilution of 1/288 while serum from chimpanzee B failed to achieve 90% neutralization even at a 1/32 dilution. Data from this and further neutralization experiments is summarized in Table 1. Although some degree of crossneutralization was observed, SFV-7 was distinguishable from SFV-6 and HFV in terms of neutralizing serotype with this panel of sera. Two out of three chimpanzee sera neutralized HFV and SFV-6 more potently than SFV-7, the third neutralized all three viruses with approximately equivalent titers. The three gorilla sera potently neutralized SFV-7 but only poorly neutralized SFV-6 and HFV, even though both SFV-6 and SFV-7 were originally isolated from chimpanzees. Sera from an SFV-11 infected orangutan showed little neutralizing activity against these viruses, although the orangutan had high titer neutralizing antibodies against SFV-11 (McClure et al., 1994). To confirm the validity of these assays, in some experiments neutralization was also assayed by X-gal staining of infected cells. Where examined, reduction in X-gal stained foci correlated closely with reduction in p-NPG conversion (data not shown).

DISCUSSION

In this study the phylogenetic and functional relationships of HFV with several primate foamy viruses was investigated including a new strain of foamy virus isolated from a gorilla. Nucleotide sequence analysis of three distinct regions of foamy virus genomes revealed a close relationship between HFV (Flugel et al., 1987; Maurer et al., 1988), SFV-cpz (Herchenroder et al., 1994), and two further chimpanzee isolates, SFV-6, and SFV-7. Conversely, isolates from orangutan (SFV-11), gorilla (SFV-Gg), and the previously molecularly characterized isolates from rhesus macaque (SFV-1, Kupiec et al., 1991) and African green monkey (SFV-3, Renne et al., 1992) were genotypically dissimilar. To investigate the functional properties of these viruses, an indicator cell line
for HFV infection was constructed and used to investigate two aspects of primate foamy virus biology, namely Taf transactivation and neutralization. Of the eight viruses tested (HFV, SFV types 1, 2, 3, 6, 7, SFV-11 and SFV-Gg), only HFV-, SFV-6-, SFV-7-, and SFV-Gg-transactivated HFV LTR regulated gene expression, while the other foamy viruses failed to do so. This is not surprising for SFV-1 and SFV-3 given the degree of sequence diversity of their respective U3 regions and taf genes (Flugel et al., 1987; Kupiec et al., 1991; Renne et al., 1992). Earlier experiments using transient transfections and CAT assays have also demonstrated lack of cross transactivation of the HFV LTR by SFV-1 Taf (Mergia et al., 1992) or the SFV-1 LTR by SFV-3 Taf (Renne et al., 1993), although SFV-1 Taf transactivated SFV-3 LTR driven transcription.

Despite the high degree of homology in the env genes

**FIG. 5.** Sample data from neutralization experiments. Neutralization of SFV-7 by sera from two foamy virus infected chimpanzees (○, ▽) and three infected gorillas (○, ▽, ●). SFV-7 (200 TCID) was incubated with the indicated serum dilutions prior to infection of BHL cells. Forty-eight hours later, infection inhibition was measured by pNPG substrate conversion in 90 min as described. Each point represents the mean of duplicate cultures, except the nonneutralized control (●) which is the mean ± SD of quadruplicate cultures.

**FIG. 4.** (A) β-galactosidase expression following HFV infection of BHL cells. Forty-eight hours postinfection with HFV, cells were fixed and incubated with pNPG substrate solution. Optical density (OD) at 405 nm was measured 45 min (○), 90 min (●), or 18 hr (▲) after substrate addition. Each point represents the mean ± SD of quadruplicate cultures. Similar results were obtained using SFV-6 and SFV-7. (B) β-galactosidase expression following infection of BHL cells with HFV (○), SFV-1 (▲), SFV-2 (△), SFV-3 (●), SFV-6 (▲), SFV-7 (△), SFV-Gg (▼), or SFV-11 (●). Forty-eight hours postinfection with the indicated virus titers, cells were fixed and incubated with pNPG substrate solution for 16 hr and OD at 405 nm was measured. For HFV, SFV-6, and SFV-7 the results shown are the mean ± SD of quadruplicate cultures. For SFV-1, 2, 3, 6, 7, and 11, the mean of duplicate cultures is shown.

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* Serum neutralization titer was defined as the reciprocal serum dilution that inhibited HFV-, SFV-6-, or SFV-7-induced β-galactosidase activity by 90%.
* Source animal for SFV-Gg isolation.
* Source animal for SFV-11 isolation.
of foamy viruses, subtypes have been distinguished by serum neutralization. This has been determined in the past by inhibition of CPE in cell lines using end point dilution assays which are cumbersome to execute. We made use of the BHLL cell line to quantify neutralization of HFV LTR-transactivating foamy viruses by sera from various infected primates. Classification of viruses in terms of neutralization only partially correlated with phylogenetic analysis of LTR, gag, and pol sequences. The distinction of SFV-7 from SFV-6/HFV as a separate neutralization serotype (Brown et al., 1978; Nemo et al., 1978) was in part supported by the neutralization results in this study, even though sequences obtained from R/U5, gag, and pol regions were remarkably similar. Interestingly, one of the three SFV-7-neutralizing gorilla sera was obtained from the same animal as SFV-Gg. This suggests that SFV-7 shares neutralization epitopes with SFV-Gg and is, thus, the same neutralization subtype, despite having greater nucleotide sequence homology in the LTR and pol with SFV-6, SFV-cpz, and HFV in the regions examined than with SFV-Gg. Neutralization is likely to be a property of the viral envelope antigens (Weiss, 1993) and env sequences have not been analyzed. It is possible, however, that the gorilla from which SFV-Gg was isolated was dually infected with SFV-Gg and an SFV-7-like virus. In fact, all three gorilla sera potently neutralized SFV-7, a virus originally derived from a chimpanzee. Given the possibility of virus transmission between captive chimpanzees and gorillas housed in close proximity, these observations can be rationalized. Neutralization analysis of SFV-Gg using SFV-7 specific sera (not possible using the assay described here due to the low cell free infectivity of SFV-Gg and the reduced transactivation potency in BHLL cells) and molecular characterization of further gorilla and chimpanzee foamy virus isolates would help to resolve this ambiguity.

Our previous description of SFV-11, isolated from an orangutan with encephalopathy, as a new serotype was supported by these studies. Despite the presence of high neutralization titers against homologous virus (McClure et al., 1994), sera from the source animal of SFV-11 failed to neutralize HFV or SFV-6 and displayed only weak cross-reactivity with SFV-7. Similarly, sera from macaques, infected with SFV types 1, 2, or 3, showed no or only weak neutralizing activity against HFV, SFV-6, or SFV-7. This lack of crossneutralization by macaque (data not shown) and orangutan sera and their divergent nucleotide sequences was consistent with the inability to transactivate the HFV LTR, emphasizing their more distant relationship.

Given the occurrence of foamy viruses in primates native to Africa, Asia, and the Americas, it is likely that these viruses represent the descendants of ancient foamy viruses which were present in primates prior to their geographical separation and/or speciation. An analogous situation exists within the HTLVs/STLVs, in contrast to the immunodeficiency viruses which occur only in African primate species in the wild. Foamy virus phylogeny may therefore reflect the phylogeny of the primates, their geographical distribution, or a combination of both. Predation may also be an influence. The close phylogenetic and functional relationship between HFV and the chimpanzee foamy viruses is thus consistent with the close relationship of their hosts, although we cannot exclude a recent or isolated transmission event between chimpanzees and humans. SFV-Gg, in common with its host, is most closely related to the chimpanzee/human foamy virus cluster based on functional assays of transactivation, neutralization, and DNA sequences. The phylogenetic clustering of SFV-1 and SFV-3 is consistent with their common hosts and the distant relationship of SFV-11 with the other ape foamy viruses may reflect the geographical and phylogenetic separation of its host from other apes.

If foamy virus infections of primates are indeed ancient phenomena, then coevolution of host and virus may account for their apparent lack of pathogenic consequences. Thus, although foamy viruses may have evolved to be apathogenic in primates, the question of whether they have also evolved to be apathogenic in humans remains to be resolved (Weiss, 1988; Aguzzi et al., 1992). Clearly, foamy viruses derived from chimpanzees are phylogenetically and functionally most closely related to HFV and further characterization of these viruses could shed light on the origins of HFV. Furthermore, careful observation of captive chimpanzees where natural infection with foamy viruses appears to be common may yield information as to whether there are pathological consequences of infection.

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