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Simian Foamy Virus Infections in a Baboon Breeding Colony

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The prevalence, transmission, and variation of simian foamy viruses (SFVs) in baboons was investigated. Over 95% of adult baboons in the breeding colony as well as recently imported adult animals had high titers of anti-SFV serum IgG. Maternal antibody was detectable in infants' serum up to 6 months of age. Approximately 30% of infants in breeding harems experienced SFV infections by 1 year of age. Shedding of SFV in oral secretions was common, with 13% of samples from normal adult animals and 35% from immunosuppressed animals containing infectious SFV. SFV was isolated from three baboon subspecies (olive, yellow, and chacma baboons) and sequences from both the *pol* and the LTR regions of the provirus were amplified by PCR and sequenced. Phylogenetic analysis indicated that all baboon isolates formed a single lineage distinct from SFVs of other African monkey species. Within the baboon SFV lineage, two distinct clades were apparent, which consisted of isolates from yellow and olive baboons and isolates from chacma baboons. Competition ELISAs indicated that, while SFV isolates of these two groups were very closely related, antigenic differences do exist between them. SFV isolates from a drill and a mandrill were distinct from baboon SFV isolates, both genetically and antigenically.

INTRODUCTION

Foamy viruses are members of the *Spumavirus* genus of the family *Retroviridae* (Aguzzi, 1993; Linial, 1999; Neumann-Haefelin *et al.,* 1993). Serological surveys indicate that these viruses are widespread and that many nonhuman primate species, in particular, show evidence of infection with foamy viruses (Lecellier and Saïb, 2000). Despite the high prevalence of antibodies and the isolation of foamy viruses from many species of primates, no disease or pathology has been firmly associated with infection by these viruses. Similarly, humans accidentally infected with simian foamy viruses (SFVs) have not been noted to have any clinical disease as a result of infection (Neumann-Haefelin and Schweizer, 1997; Schweizer *et al.,* 1995). Foamy viruses, however, have been shown to encode a transcriptional transactivator, which, although usually specific for the LTR and an internal promoter of the homologous virus, may in some cases also transactivate transcription of other viruses like HIV, leading to questions regarding their role in diseases associated with the nonfoamy viruses (Mergia and Luciw, 1991; Neumann-Haefelin *et al.,* 1993).

SFVs have been isolated from a variety of primate species and were originally classified into different serotypes, based on cross-neutralization assays. Single SFV serotypes have been isolated from multiple simian species, but it is not clear whether this is the result of cohousing of different monkey species and horizontal transfer of SFV between species or whether SFV isolates of different simian species actually do group together by neutralization tests. Most of the different SFV serotypes have been isolated from peripheral blood lymphocytes and appear to infect multiple host species. For example, SFV serotypes 1, 2, 3, and 10 have been isolated from baboons (*Papio spp.*), while serotypes 1, 2, and 3 have also been isolated from rhesus macaques (*Macaca mulatta*) and African green monkeys (*Cercopithecus aethiops*). The genomic arrangement of different SFV serotypes is very similar (Kupiec *et al.,* 1991; Mauer *et al.,* 1988; Renne *et al.,* 1992). With the isolation of the same serotype SFV from multiple primate species and the ease of molecular characterization of such isolates, SFVs are now commonly designated by their host species of origin rather than by serotype (Schweizer and Neumann-Haefelin, 1995; Broussard *et al.,* 1997). Phylogenetic analyses based on sequences derived from conserved regions of the LTR and *pol* gene suggest that, despite the finding of the same SFV serotypes in multiple species, these viruses still exhibit a pattern of genetic relatedness that broadly reflects that of the host species' phylogeny, implying that SFVs have coevolved with their hosts (Schweizer and Neumann-Haefelin, 1995; Broussard *et al.,* 1997).

In this study, we describe various characteristics of SFV infections in a captive breeding colony of baboons.

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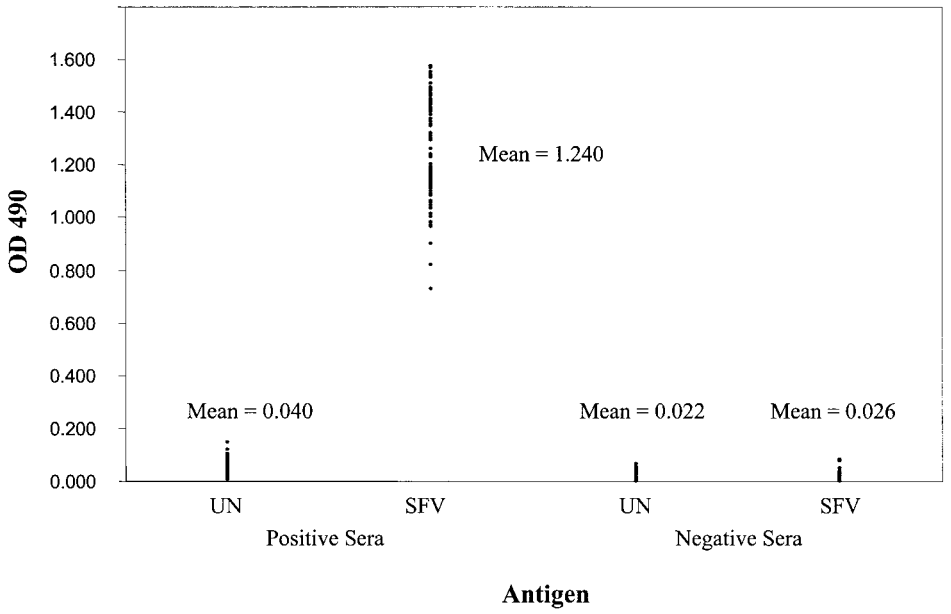

FIG. 1. Detection of anti-SFV IgG in baboon serum by ELISA. Uninfected (UN) or SFV (OCOM1-5) infected (SFV) human diploid cell detergent extracts were used as antigen to coat microtiter plates. A total of 254 baboon sera were tested (adults and juveniles > 1 year old). The mean OD values for positive (n = 150) and negative (n = 104) sera on each antigen are shown.

We examined the prevalence of anti-SFV antibody in the colony, shedding of infectious virus in oral secretions of adult animals, the decay of maternal antibody in infants, and the acquisition of SFV infection by infant and juvenile animals prior to reaching sexual maturity. We also describe the isolation of multiple SFV isolates from colony animals and their relationship both to one another and to other existing SFV isolates, based on nucleotide sequence data. The results are similar to those recently reported by Schweizer *et al.* (1999) for green monkey SFV isolates, in that some variation was detected among baboon SFV isolates. However, all baboon SFV isolates constituted a single phylogenetic lineage distinct from other primate SFVs. SFV isolates obtained from a drill and a mandrill were markedly different from the baboon isolates and form a separate phylogenetic clade, concurring with the classification of *Mandrillus spp.* as distinct from *Papio spp.*

RESULTS

Prevalence of SFV in adult baboons

An ELISA was developed to screen serum samples for anti-SFV IgG. As shown in Fig. 1, there was a clear separation of positive and negative serum samples based on the OD values using this ELISA. Positive animals had a mean OD value of 1.240, whereas negative animals had a mean OD of 0.040 on SFV antigen. Mean values of positive and negative sera on uninfected control antigens were 0.022 and 0.026, respectively. A number of ELISA-positive (n = 35) and ELISA-negative (n = 8) baboon sera were also screened by IFA and by Western blot against purified SFV antigen for reactivity with the p70/p74 proteins. As shown in Fig. 2, all baboon sera that were positive for anti-SFV antibody by ELISA recognized the p70/p74 proteins of purified SFV-3 (agm) in Western blots, while ELISA-negative sera did not. Most

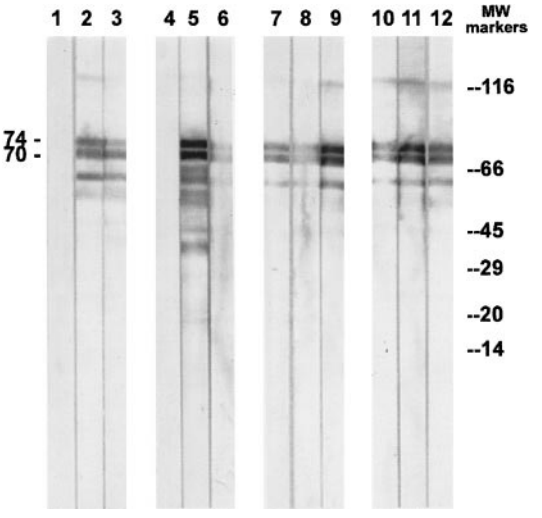

FIG. 2. Western blot analysis of several baboon sera. Purified SFV-3 (agm) proteins were separated on 4–12% polyacrylamide gels and transferred to membranes for use as antigen. Sera that tested as positive or negative by ELISA were used at a dilution of 1:100. Reactivity with the p70/p74 proteins was considered the minimal reaction necessary to classify sera as SFV-positive by Western blot. Most positive sera also recognized the bet protein (60 kDa). Sera used were as follows: lanes 1–3, wild-caught *P. anubis*; lanes 4–6, colony-reared *P. anubis*; lanes 7–9, wild-caught *P. ursinus*; lanes 10–12, colony-reared *P. cynocephalus.* Sera in lanes 1 and 4 were negative by ELISA; all other sera were positive by ELISA.

TABLE 1

Isolation of SFV from Baboon Oral Secretions

	Immunosuppressed	No. positive/no. tested[a] Number of animals	Number of samples
P. anubis (Group 1)	−	1/4	1/12
P. anubis (Group 2)	−	10/58	11/98
P. cynocephalus	−	10/54	13/81
Total		21/116 (18.1%)	25/191 (13.1%)
P. anubis	+	2/2	3/24
P. ursinus	+	9/10	27/60
Total		11/12 (91.9%)	30/84 (35.7%)

[a] Multiple samples were collected from individual animals.

ELISA-positive sera also recognized the 60-kDa bet protein. For all 43 sera tested, there was complete agreement between the ELISA, IFA, and Western blot results in determination of sera as positive or negative for anti-SFV IgG.

Sera from all adult animals comprising the Oklahoma University Health Sciences Center (OUHSC) baboon colony were screened at a dilution of 1:100 using this ELISA. The overall incidence of anti-SFV antibody in adult baboons was 88.0% (132/150). Among long-time OUHSC colony residents (*P. anubis*) maintained in small breeding harems of 6–10 adults, the incidence was 100% (76/76). Yellow baboons (*P. cynocephalus*), transferred to the OUHSC colony from another breeding colony, where they had been maintained in an open breeding corral, also had a high prevalence of anti-SFV antibody (30/44, 68.2%). Two groups of baboons that had been recently captured from the wild in Africa and imported into the OUHSC colony also showed a high incidence of SFV infection: *P. ursinus,* 100% (10/10) and *P. anubis,* 80% (16/20). These groups represented two geographically separated subspecies of baboons (chacma and olive, respectively). Based on these results, SFV appears to be a common infection in free-ranging baboon populations as well as in captive colony animals.

Although most reported SFV isolates were obtained from peripheral blood lymphocytes, shedding of SFV in saliva was previously reported to be a common occurrence (Schweizer *et al.,* 1997). To gain an estimate of the frequency of shedding of infectious SFV in the oral cavity, throat swabs and saliva samples were collected from adult baboons during semiannual TB testing, and virus isolation was performed by inoculation onto subconfluent human diploid fibroblast cell cultures. As shown in Table 1, SFV was isolated from a number of normal adult animals on different occasions. Of a total of 191 samples tested, 25 were positive, giving an estimated frequency of shedding of SFV of approximately 13.1%. When animals on immunosuppressive research protocols were similarly tested, the frequency of shedding of infectious SFV rose significantly to 35.7% (30/84 samples positive).

SFV in infant baboons

The exact mode and timing of SFV transmission in baboons is unknown, although both sexual and oral transmission are suspected (Broussard *et al.,* 1997). To determine whether SFV is commonly acquired at an early age, we tested serial bleeds from a number of infant baboons born in the OUHSC colony between 1996 and 1998. As shown in Fig. 3, all infants had high levels of maternal antibody at and shortly after birth, levels that were comparable to that of the dam. Decay in levels of infants' serum anti-SFV IgG began at about 2 months, dropping off to background levels by 150–200 days (5–7 months) of age.

Several infants developed a high titer of anti-SFV IgG which persisted, indicating an active infection by SFV. Of 39 animals for which sera were tested up to 2 years of age, 16 seroconverted to SFV. Based on paired serum samples, 11 of these infants seroconverted to SFV-positive status between 0 and 1 year of age, one between 1 and 2 years of age, and one between 2 and 3 years of age; because of a lack of appropriate sequential serum samples, the other three SFV-positive infants could only be determined to have seroconverted before 2 years of age. Once infants seroconverted, serum anti-SFV IgG levels remained high at levels comparable to those observed in adult animals. As can be seen in Fig. 3, in all cases in which spacing of sequential serum samples made it possible to determine, maternal antibodies had waned to a substantial degree or disappeared altogether before infants became infected. It should be noted that, although the majority of infants (~60%) remained seronegative for SFV past 2 years of age, infants were routinely removed from the breeding harems and placed into small peer group housing (2–3 juveniles/cage), which restricted their interactions with other animals, thus greatly lessening the opportunity for contact with SFV-positive animals. These results demonstrate that SFV is readily transmitted to/among young group-housed animals, indicating that nonsexual mode of transmission is a primary means of SFV transmission.

Genetic characterization of baboon SFV isolates

To investigate the extent of variation among strains of baboon SFV, we analyzed nucleic acid sequences of SFV isolates obtained from baboons with different colony histories and SFV isolates from different baboon subspecies. Using PCR primers and conditions described by Broussard *et al.* (1997), regions of the *pol* gene and LTR were amplified from infected cell proviral DNA and directly sequenced.

In the *pol* gene, sequence was very conserved among

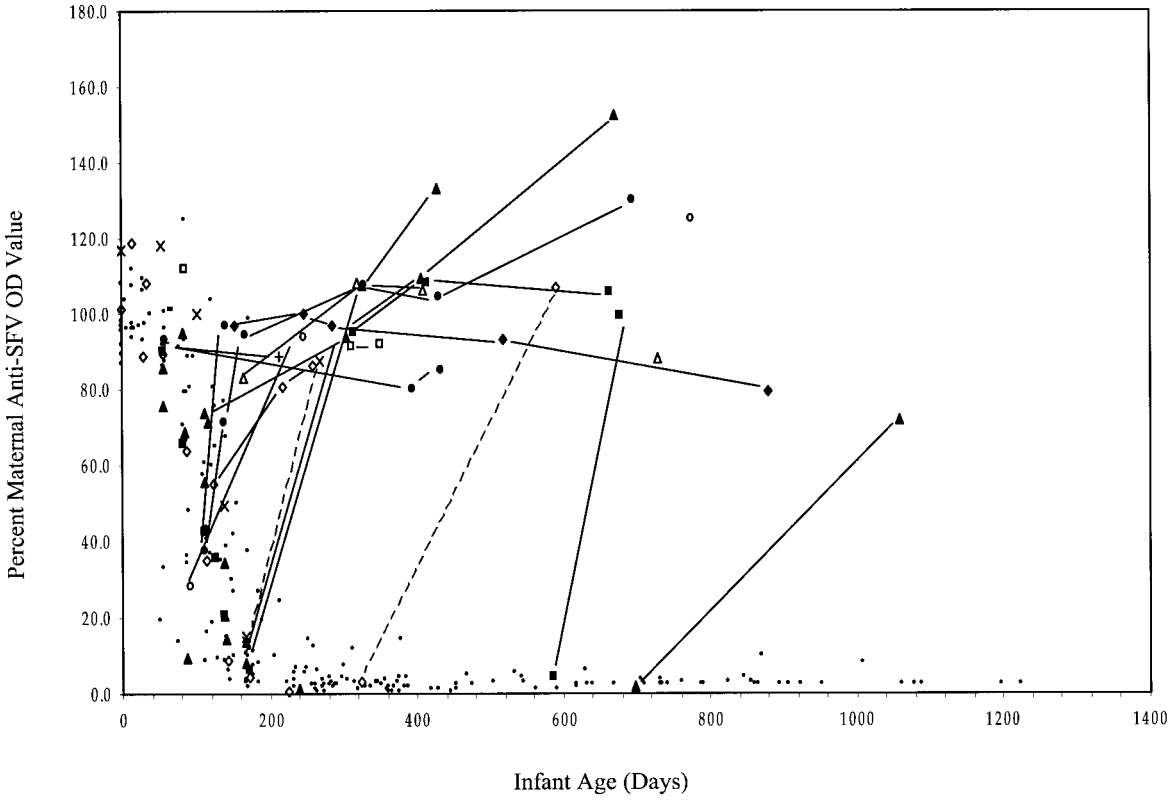

FIG. 3. Decay of maternal anti-SFV IgG in serum of infant baboons. Anti-SFV IgG in infant baboon sera was measured by ELISA and expressed as the percentage of maternal anti-SFV levels. All infants which did not seroconvert to SFV are represented as anonymous data points (•). Individual infants that seroconverted to SFV are indicated by other symbols, connected by lines beginning at the last point at which anti-SFV levels were still declining.

all baboon SFV isolates, with no gaps/insertions necessary to align the sequences (Fig. 4). In the 279 aligned base pairs (93 codons), there were 53 positions (19%) which varied among baboon SFV isolates. The codon position of the variant nucleotides were as follows: 11 in the first, 2 in the second, and 40 in the third position. However, of the 53 variant positions, only 11 produced nonsynonymous amino acid substitutions, and 8 of these 11 were conservative substitutions (S/A/T, V/I/L, or E/D).

Of the 53 variant positions in the *pol* gene sequence, 15 varied characteristically between chacma and yellow/olive baboons isolates, but only 2 of these 15 substitutions resulted in an amino acid change (both S/T substitutions). Thirteen of the 51 alignment positions containing nucleotide substitutions were present in a single virus isolate, and these were limited to three particular virus isolates (two yellow and one chacma isolate), with the chacma baboon isolate (OCOM4-47) accounting for 11 of these 13 substitutions. These results reflect the overall highly conserved nature of the pol protein sequence.

As previously noted by Broussard *et al.* (1997), there was more variation in sequences from the R/U5 region of the LTR than in the *pol* gene. Even so, within baboon SFV isolates there was considerable conservation of sequence (Fig. 5). Although insertion of a number of gaps was necessary to produce an optimal alignment with nonbaboon SFV sequences (not shown), only four gaps were necessary to align baboon LTR sequences, if the Bab1 sequence of Broussard *et al.* (1997) was excluded (this isolate varied considerably from all other baboon isolates and was therefore not used as a reference strain in alignments). Of these four gaps, one (at position 26) was characteristic of chacma SFV isolates and a second (at position 76) was present in all isolates except Bab1. A third gap, 5′ of the polyadenylation signal (at position 119–134), was 0–1 nucleotide long in yellow and olive SFV isolates but 12–16 nucleotides long in chacma isolates. The fourth region of sequence variability was located about 25 nucleotides 3′ of the polyadenylation signal (position 207–210). This was interesting, in that the most strictly conserved region of the aligned LTR sequence was located between these two latter gaps and spanned the polyadenylation signal. Such noncoding regions of viral genomes are frequently more variable than coding regions. The absolute sequence conservation in this region (even in nonbaboon SFV isolates) suggests that this region serves a critical function.

Phylogenetic analysis of the *pol* sequences placed all baboon SFV isolates in a single clade separate from SFV

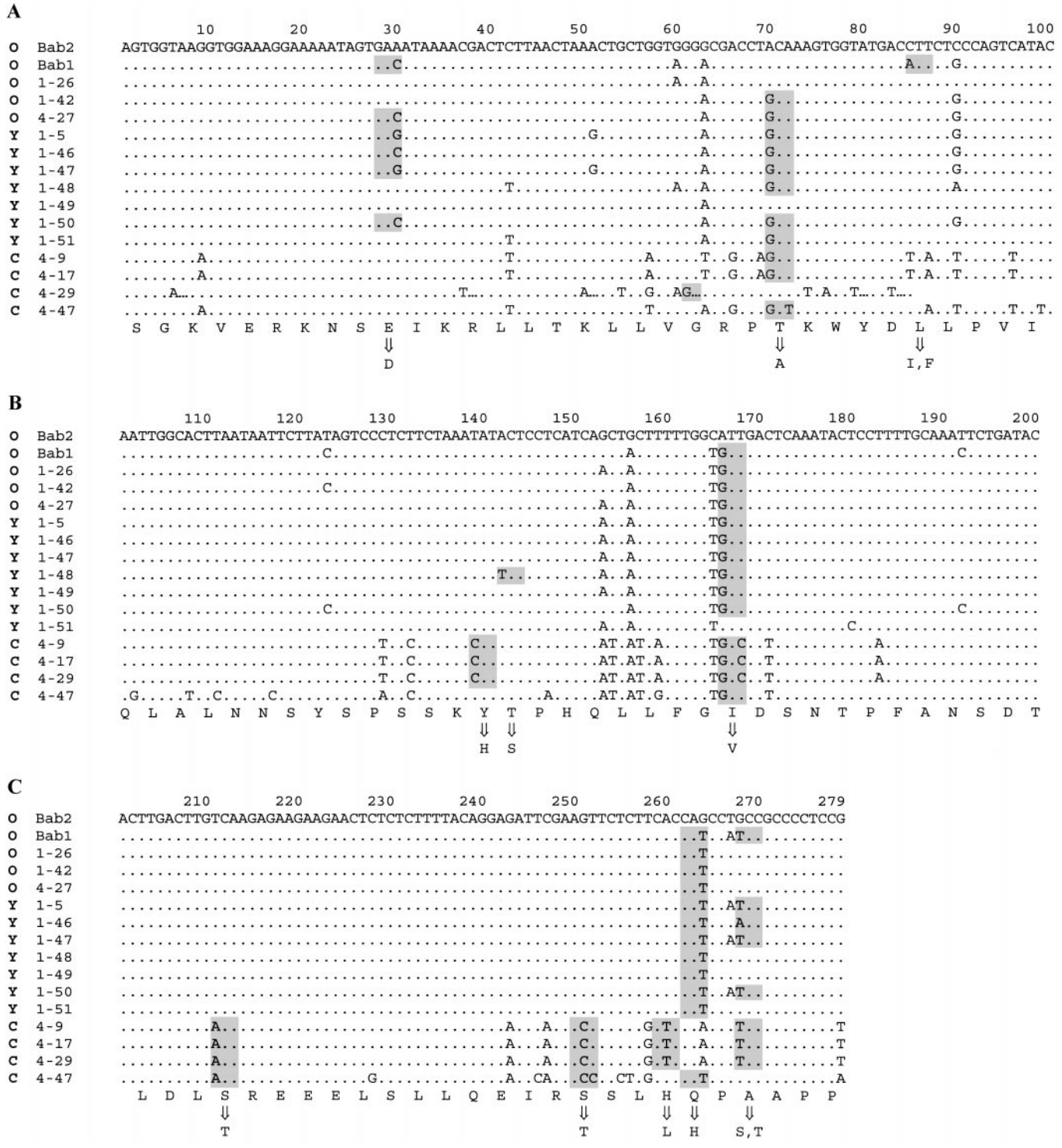

FIG. 4. Alignment of DNA sequence from the *pol* gene of baboon SFV isolates. Infected cell DNA was amplified by PCR and products were sequenced directly. The species of baboon from which the viruses were isolated is indicated at left (O, olive; Y, yellow; C, chacma). The alignment was made using Bab2 as the reference sequence, with identical residues in other sequences indicated by dots. The translated amino acid sequence of Bab2 is shown below the alignment, with residues that vary among the various isolates indicated below, and the corresponding variant codons are indicated by shading. SFV isolates from OUHSC were as follows: yellow baboons, 1-5, 1-46, 1-47, 1-48, 1-49, 1-50, and 1-51; olive baboons, 1-26, 1-42, and 4-27; chacma baboons, 4-9, 4-17, 4-29, and 4-47. Sequences for all other viruses were taken from GenBank and were previously described in Broussard *et al.* (1997).

isolates from macaque, drill, mandrill, and other African monkeys (Fig. 6). Within the baboon SFV lineage, chacma baboon isolates formed a clade that was separate from olive and yellow baboon isolates. SFV isolates from yellow and olive baboons did not form distinct lineages related to these two baboon subspecies. Sim-

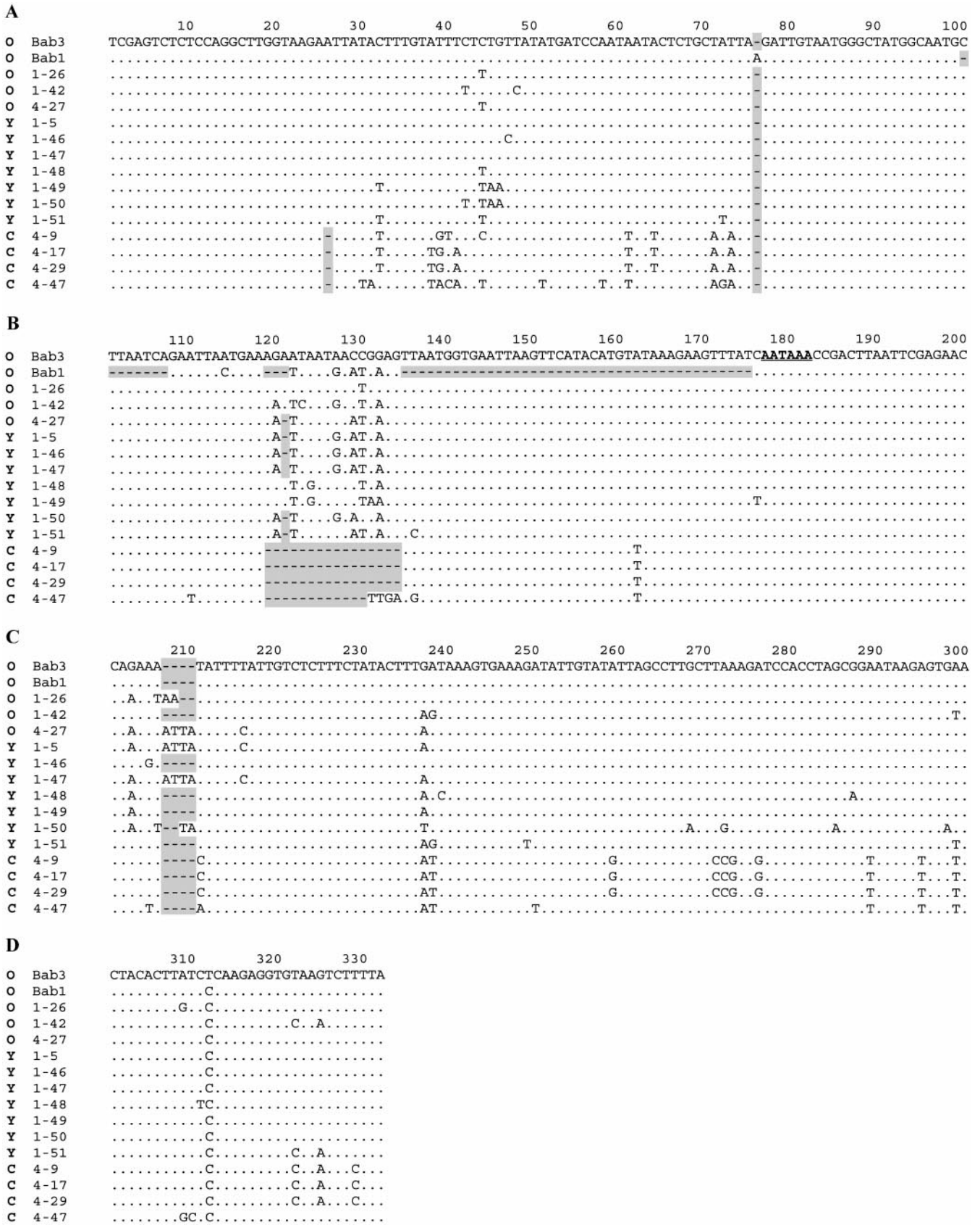

FIG. 5. Alignment of the LTR region of baboon SFV isolates. LTR nucleotide sequences were amplified from infected cells by PCR and the products were directly sequenced. The species of baboon from which the viruses were isolated is indicated at left (O, olive; Y, yellow; C, chacma). Using the Bab3 sequence of Broussard *et al.* (1997) as a reference, identical nucleotides in other sequences are indicated by dots and gaps by shaded dashes. The polyadenylation consensus signal is highlighted in underlined boldface at positions 177–182.

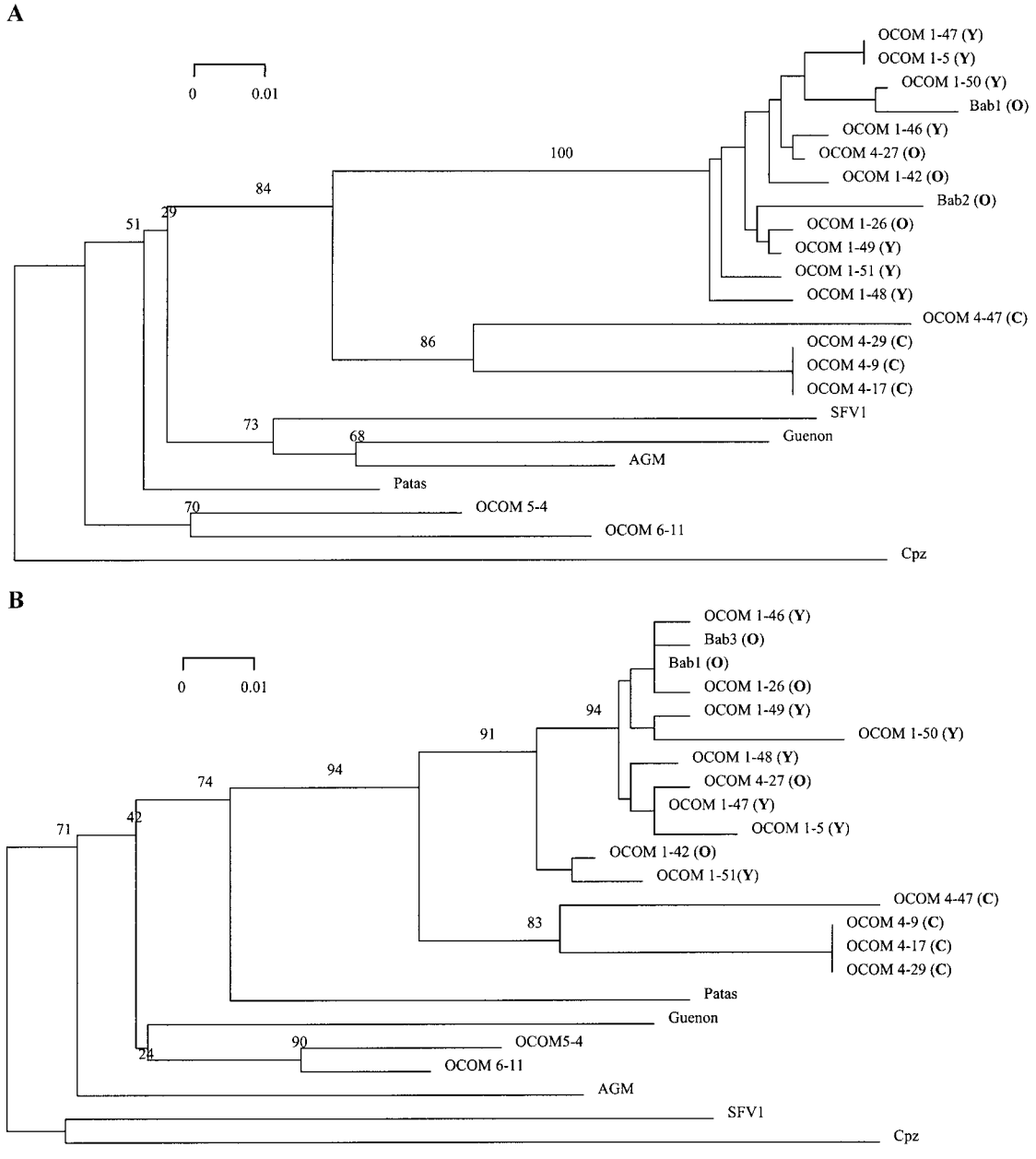

FIG. 6. Phylogenetic relationships of primate foamy viruses. Phylogenetic trees were generated for both the *pol* (A) and LTR (B) sequences. The species of baboon from which the viruses were isolated is indicated (O, olive; Y, yellow; C, chacma). The Tamura–Nei method was used to calculate distances and trees were constructed using the neighbor-joining method (Kumar *et al.,* 1993).

ilar results were obtained when LTR sequence data were analyzed, with all baboon SFV isolates forming a single lineage separate from other primate SFV isolates. Within this LTR-based baboon SFV clade, isolates from chacma baboons again formed a subgroup distinct from the yellow and olive baboon SFV isolates. One of the chacma isolates, OCOM4-47, was somewhat distant from the other three chacma isolates. The chacma baboons from which the other three viruses were isolated were imported as a group, whereas the animal from which SFV OCOM4-47 was isolated had been imported as part of a separate group about 5 years earlier. SFV isolates from yellow and olive baboons were intermixed, although specific groupings of isolates within this subgroup were different from those obtained based on *pol* sequences. Although minor variations among the yellow and olive baboon isolates occurred, this same overall tree topology was consistently obtained using different distance estimate methods and tree construction algorithms.

The mandrill and drill SFV isolates were distinct from all baboon isolates. Phylogenetic analyses based on both *pol* and LTR sequences placed them in a separate lineage from that of baboon isolates. Although their position relative to other primate SFVs varied within both

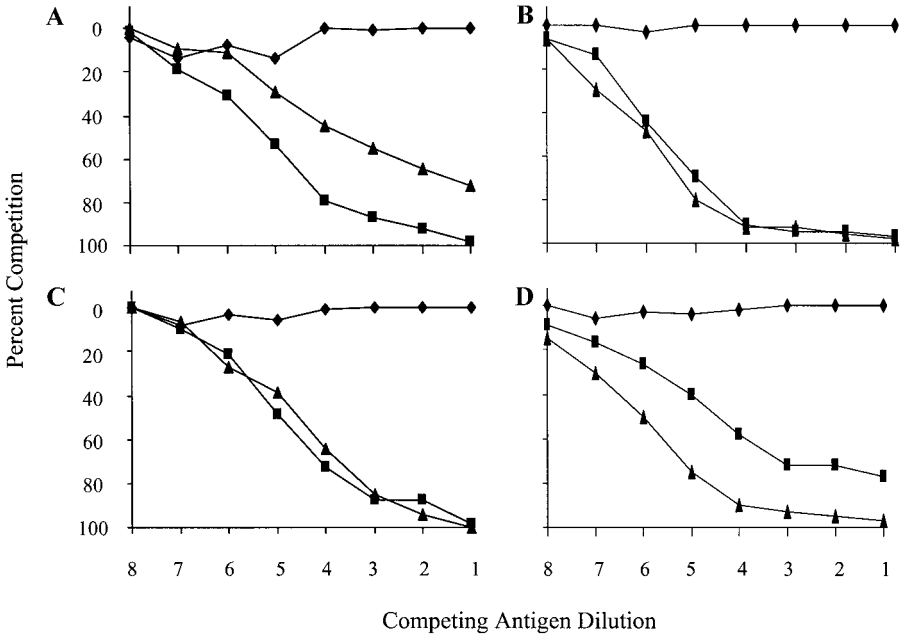

FIG. 7. Detection of sub-species-specific antigenic determinants of chacma and yellow/olive baboon SFV isolates. Competitive ELISAs were performed by reacting SFV-positive sera with SFV antigen and competing these antibody–antigen reactions with different soluble antigens. The antibody–antigen reactions are: (A) yellow baboon serum–yellow baboon SFV antigen; (B) chacma baboon serum–yellow baboon SFV antigen; (C) yellow baboon serum–chacma baboon SFV antigen; and (D) chacma baboon serum–chacma baboon SFV antigen. These antibody–antigen reactions were competed using serial twofold dilutions of soluble uninfected cell (◆), yellow baboon SFV (■), or chacma baboon SFV antigens (▲).

pol- and LTR-sequence-based trees, these two SFV isolates appeared to be more closely related to SFV isolates from cercopithecine monkeys than to baboon SFV isolates.

Antigenic variation among baboon SFV isolates

Given the consistent phylogenetic appearance of two subgroups of baboon SFV isolates (chacma and olive/yellow SFVs), the possibility of antigenic variation between isolates of these two groups was examined. Standard ELISAs were performed with various primate sera using SFV isolates from both baboon subgroups as well as the mandrill SFV isolate as antigens. Sera from yellow and olive baboons appeared to react slightly better (gave a higher OD) with yellow or olive baboon SFV antigens than with chacma SFV antigens. Similarly, chacma baboon sera reacted slightly better with chacma SFV antigens than with yellow or olive baboon SFV antigens. All baboon sera reacted much better with any baboon SFV antigen than with mandrill SFV antigen and, conversely, mandrill serum reacted far better with mandrill SFV antigen than with baboon SFV antigens. Sera from all other primate species tested exhibited approximately equivalent reactivity with baboon and mandrill SFV antigens, indicating that these sera were recognizing only antigenic determinants common to baboon and mandrill SFV isolates.

Although these ELISAs indicated the presence of baboon SFV-specific antigenic determinants and suggested the existence of baboon SFV subgroup-specific determinants, they could not be considered definitive. Therefore, more sensitive competition ELISAs were performed. As shown in Fig. 7, antigenic differences were readily apparent between chacma and yellow/olive baboon isolates of SFV. Competition of the reactivity of yellow baboon sera with yellow baboon SFV antigen is shown in Fig. 7A. Soluble yellow baboon SFV antigen competed this antibody–antigen reaction more completely than did soluble chacma baboon SFV antigen (100% vs 75% maximal competition, respectively). Similar results were obtained when competition of reaction of chacma baboon serum with chacma baboon SFV antigen was examined (Fig. 7D). Soluble chacma baboon SFV antigen competed this reaction better than did soluble yellow SFV antigen (100% vs 80% maximum competition). Consistent with these results, the reactivity of chacma sera with yellow SFV antigen and yellow baboon sera with chacma SFV antigen were both competed equally well by soluble chacma and yellow SFV antigens (Figs. 7B and 7C, respectively). These results clearly demonstrate that some antigenic differences do exist between the chacma and olive/yellow baboon SFV isolates.

DISCUSSION

In examining the presence of SFV infections in the OUHSC baboon colony, we made a number of observations that are relevant to the biology of SFV in baboons.

SFV infections are obviously very widespread in adult baboons. Over 85% of adult animals tested had high levels of serum IgG to SFV. This was true for both wild baboons recently caught and shipped into the United States and for long-time breeding-colony resident animals. The ready isolation of infectious SFV from saliva suggests a likely source and mode for transmission of the virus infection to nonadult baboons.

Infants acquired high titers of antibodies directed against SFV from their dams, and these IgG titers dropped to undetectable levels by 6–7 months of age. By 1 year of age, approximately 20% of infant baboons seroconverted to SFV. During years 2 and 3, only about 10–15% of all infants seroconverted to SFV. Since over 60% of young baboons remained seronegative up to three years of age, it appears unlikely that SFV is commonly passed from the dam to the infant as either a prenatal or neonatal infection. Rather, SFV appears to be acquired at a later point in life, as indicated by the seroconversion of several infants. In almost all cases this appears to occur after maternal antibodies have waned to low or background levels in the serum of infants. However, the low incidence of seroconversion of juvenile animals 1–3 years of age needs to be viewed in the context of these animals' removal from the breeding harems at 6–9 months of age and the consequent reduction in opportunity for interaction with SFV-positive animals likely to be shedding infectious virus. The finding of Broussard *et al.* (1997) that very few infants or juvenile baboons (<15 months old) are PCR-positive for SFV is consistent with these conclusions based on serological results. While we expect that infant and juvenile baboons probably acquire SFV by oral infection, the fact that many juveniles do remain SFV antibody-free up to 3 years of age does allow for the possible sexual transmission of SFV as well.

Our genetic analyses of multiple SFV isolates obtained from baboons demonstrated the existence of two major subgroups of SFV, representing isolates from chacma baboons and isolates from yellow and olive baboons. These two subgroups of baboon SFV were distinguishable both by sequence analysis of regions of the LTR and *pol* genes and by antigenic differences using sensitive competition ELISAs. The baboon SFV isolates were very closely related to each other, but were distinct from SFVs isolated from other primate species, including isolates obtained from both a drill and a mandrill. Thus, the baboon SFVs appear to represent a well-defined subgroup of the simian foamy viruses.

With few exceptions, most SFV isolates from different monkey species that have been examined in any detail are represented by a single isolate. Because they are RNA viruses, and thus subject to a higher rate of error in replication of the viral genome than DNA viruses, the extent of genetic variability within SFV serotypes and the influence such variation might have on genetic stability of the viral genome is unknown. Recently, Schweizer *et al.* (1999) addressed this problem by analyzing multiple isolates obtained over a number of years from a group of 19 cohoused African green monkeys. Based on comparison of the predicted amino acid sequence of an *env* surface domain and nucleotide sequence homologies, these investigators identified four distinct subtypes of SFV serotype 3 circulating in the green monkey colony. Consistent with these observations, a number of sequence differences were evident among individual SFV isolates from both baboon SFV types. Although the baboon population from which these SFV isolates were obtained is nowhere nearly as defined as the AGM population examined by Schweizer *et al.* (1999), it is evident that there is variation among individual SFV isolates, not only in the LTR but in the conserved *pol* gene as well. However, as phylogenetic subgroupings of yellow/olive SFV isolates shifted between LTR vs *pol* sequence data and also with use of different distance and tree construction algorithms, we did not find firm evidence for the existence of specific subtypes of baboon SFV isolates beyond the yellow/olive–chacma split.

It has been proposed that the primate foamy viruses coevolved with their host species (Schweizer and Neumann-Haefelin, 1995; Broussard *et al.,* 1997). Although we detected only two distinct subgroups of SFV from three different baboon species, these results are not necessarily inconsistent with the proposed coevolution of these viruses and their hosts. Chacma baboons inhabit a geographically distinct area from that occupied by yellow and olive baboons (southern Africa vs sub-Saharan Africa, respectively). Thus, it is not surprising that SFV isolates from chacma vs yellow/olive baboons should be different, if these viruses have coevolved with their hosts. In contrast, SFV isolates from yellow and olive baboons did not separate into host species-specific lineages, and the position of the various SFV isolates in the yellow/olive baboon SFV clade shifted relative to one another with use of different distance algorithms and/or tree drawing programs, indicating that these isolates were clearly very closely related to one another. Since the range of yellow and olive baboons in Africa overlaps, this is not unexpected. It is also notable that, although these various yellow and olive baboon SFV isolates were obtained from both newly imported and long-time captive baboons, and from captive baboons that until approximately 6 months before virus isolation were housed in different primate centers, there was relatively little sequence variation among them. While it is possible that animals newly added to the OUHSC colony may have acquired SFV infections from existing colony animals, we consider this unlikely, as all baboons added to the OUHSC colony were seropositive for SFV when they arrived at the OUHSC facility.

Finally, past use of ELISA for detection of anti-SFV antibody in sera gave variable results, at least for testing

of human sera (Mahnke *et al.,* 1990; Schweizer *et al.,* 1995). The ELISA described in this study for assay of serum IgG to SFV correlated 100% with results obtained by both IFA and Western blot analysis using purified SFV antigen. In addition, the difference in OD values observed between positive and negative sera (means of 1.240 vs 0.040, respectively) provides a clear demarcation between positive and negative sera, resulting in very few, if any, equivocal results (none, to date, in the 262 adult baboon sera that we tested). Although this standard ELISA format could not reliably differentiate between baboons infected with each of the two baboon SFV subgroup viruses, this lack of specificity made the assay very robust for detection of SFV serum antibody in infected animals; baboons infected with either SFV subgroup reacted strongly, regardless of the subgroup SFV isolate used as antigen. The few sera from other nonhuman primates that we tested in this assay suggests that this basic assay would perform similarly for detection of anti-SFV IgG in these monkeys as well. Thus, this ELISA appears to be a reliable, sensitive, and rapid test for identification of SFV-infected monkeys.

MATERIALS AND METHODS

Animals

All baboons investigated in this study were housed in the AAALAC-approved University of Oklahoma Health Sciences Center laboratory animal facility. Most animals were long-time residents of the colony (5 to >15 years). Two groups of animals, one of olive baboons (*P. anubis*) and another of chacma baboons (*P. ursinus*), were newly imported from Kenya and South Africa, respectively (Eberle *et al.,* 1997). Unless experimental protocols required otherwise, baboons were group-housed in breeding harems consisting of one adult male, 6–10 adult females, and their infants. At approximately 6–9 months of age, young baboons were removed from the harems and placed in small peer groups of 2–3 animals or, if required for experimental reasons, into single-cage housing. Mandrills (*Mandrillus sphinx*) and drills (*M. leucophaeus*) were resident in two U.S. zoos.

Several groups of baboons tested for virus shedding were on immunosuppressive protocols as part of another study. These animals received myelosuppression by administration of cytoxan (750 mg/m^2) plus adriamycin (75 mg/m^2). Myelosuppression was defined as a platelet count of <20,000/μl and an absolute neutrophil count of <500/μl. Drugs were administered iv three times/week over a period of 28 days.

Virus isolation

All SFV isolates were obtained from throat swabs or saliva samples. Samples from baboons were placed in Dulbecco's minimal essential medium after collection and inoculated onto subconfluent human diploid fibroblast cell monolayers within 8 h of collection. Cultures showing syncytial CPE typical of SFV were harvested for further studies. Drill and mandrill samples were not inoculated onto cell cultures until 36–48 h after collection. Isolates were confirmed as SFV by PCR and DNA sequence analysis as described below.

PCR and sequence analysis

Cell monolayers in 100-mm petri dishes were infected with SFV isolates. When CPE involved >70% of the monolayer (which sometimes required passaging of infected cell cultures), cells were lysed in 10 mM Tris/0.5 mM EDTA containing 0.2% SDS and 200 μg/ml proteinase K, and incubated at 37°C overnight. Infected cell DNA was then isolated by phenol–chloroform extraction. PCR was performed using the primers and conditions described by Broussard *et al.* (1997), to amplify sequences from the LTR and *pol* gene. All PCR products were sequenced directly in both directions without cloning, to avoid any potential bias by selection of a single PCR product sequence. Sequence data were initially aligned using the Clustal algorithm and alignments then adjusted manually. Phylogenetic analyses were performed using the MEGA program package as described (Kumar *et al.,* 1993; Smith *et al.,* 1998). All sequences were deposited in the GenBank database and are available under sequential accession numbers AF291379–AF291410.

Indirect fluorescence assay (IFA)

SFV-1-infected and uninfected Cf2th cells were grown to confluency and acetone-fixed on 16-well chamber slides. To detect SFV specific antibody, control and test sera, previously diluted (1:8) and preabsorbed with uninfected Cf2th cells overnight at 4°C, were each incubated with both an infected and uninfected cell well. Bound antibodies were labeled by incubation with fluorescein-conjugated goat anti-human IgG for 30 min at 37°C. Finally, the slides were examined for specific nuclear fluorescence in infected but not uninfected cells, to signify the presence of SFV-specific antibody.

Western immunoblotting

Western immunoblots were prepared from pelleted SFV-3 (agm) viral lysate, which was electrophoretically separated through a 4–12% SDS–polyacrylamide gel and transblotted onto polyvinyl membranes. The membranes were cut into strips that were incubated overnight at room temperature with either control or test sera that was previously diluted (1:100) and preabsorbed with uninfected Cf2th cells overnight at 4°C. The presence of bound antibody was detected by subsequent incubation with horseradish peroxidase-conjugated goat anti-monkey IgG for 1 h at room temperature followed by 4-chloro-

1-naphthol substrate for 20 min at room temperature. The presence of SFV characteristic bands at 70 and 74 kDa was required for a positive interpretation.

ELISA

Sera were screened for anti-SFV IgG antibody using an ELISA. SFV antigen was prepared from human diploid cell monolayers infected with SFV isolate OCOM1-5. When cell monolayers exhibited >70% CPE, infected cells were collected and resuspended in 0.5% TX-100 at approximately 2×10^7 cells/ml, and the lysates were clarified by centrifugation at 14,000 rpm in a microfuge for 15 s. The supernatant was used as antigen to coat microtiter plate wells. The ELISA procedure was exactly as described for detection of anti-herpesvirus IgG in primate serum (Ohsawa *et al.,* 1999). Sera giving OD values against SFV antigen that exceeded three times the OD obtained on uninfected cell antigen were considered positive.

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