strains currently circulating in North America. This region of the protein has been assigned to antigenic sites (*17*) and has been associated with adaptation to growth in eggs (*18*). Phylogenetic analysis showed that each of the 8 viral genes of A/Canada/1158/2006 clustered with A/swine/Ontario/33853/2005 (H3N2) and other swine/turkey Canadian isolates from 2005. Although the HA gene of these isolates were shown to be closely related to American viruses that were first isolated from pigs in 1999, they represent a new distinct cluster (*2*). The NA genes are phylogenetically distinct from the US swine isolates and are represented by human influenza (H3N2) isolates from Asuncion, Paraguay (2001), and New York (2003) (*2*).

A recent review described 50 cases of symptomatic human infection with SIV, documented in the literature through April 2006; 46 cases were infected with subtype H1N1 and 4 were infected with subtype H3N2 (*19*). The spectrum of pathogenicity of SIV infection ranges from asymptomatic infection (*6*) to death; 7 of these 50 patients died (*5,20–24*). Laboratory-confirmed swine influenza in humans may be "the tip of the iceberg." Diagnosis of the current case was serendipitous because typing was performed only because the case occurred outside of influenza season.

The mode of spread of SIV in humans is not established. Because of his young age, the index patient was not likely to have had unrecognized direct contact with swine. That aerosolization of influenza virus occurs is increasingly recognized (*25*), but the child was reportedly never in the barns that housed the swine. However, other members of the farm reported that infants were sometimes taken for walks through the barn. The child also may have acquired the virus from person-to-person spread or from fomites. All 13 patients in the Fort Dix outbreak and 15 of 37 previously reported civilian case-patients also had no swine contact (*19,20*).

The Fort Dix outbreak of SIV in humans lasted only 21 days and never spread outside the military base. The calculated basic reproductive rate ($R_0$) was only 1.1 to 1.2. This suggests that person-to-person spread of the implicated H1N1 strain was not efficient enough to produce a major epidemic (*26*). However, future strains of SIV could have a higher $R_0$, and documentation of a case of swine influenza (H3N2) in a child with unrecognized transmission within the community adds another possible mechanism by which major epidemics of influenza could arise. Swine influenza infection in humans most commonly results in either no symptoms or a self-limited illness (*6*). However, routine surveillance for cases among swine workers may enable early detection of a strain with the potential for person-to-person transmission, prompting institution of infection control measures and vaccine development.

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別紙様式第2

別紙(3)-2

# 医薬品　研究報告　調査報告書

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|---|---|---|---|---|---|---|
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| 一般的名称 | | | 研究報告の公表状況 | Clinical and virological characterization of persistent human infection with simian foamy viruses. AIDS research and human retroviruses, Nov 2007, 23 | 公表国<br>米国 | |
| 販売名（企業名） | タココンプ（CSL ベーリング株式会社） | | | | | |

**研究報告の概要91**

問題点（SFV 感染者は輸血や移植）

HIV と HTLV の病原性のレトロウイルスは、非ヒト霊長類から SIV と STLV のヒトへの感染が定着し、人から人への感染が拡大した。simian foamy viruses ( SFV ) 感染は、捕獲された非ヒト霊長類間でかなり伝播している。職業上非ヒト霊長類やその体液と接触したり、咬まれたり、引掻かれた人達に SFV 感染が確認されてきた。遺伝子配列を解析すると、人への SFV 感染はチンパンジー、ヒヒ、アフリカサバンナモンキーなど多様な非ヒト霊長類を起源としていることが分かっている。

筆者らは、SFV 感染した人 13 名の中で 7 名を長期追跡調査した。遺伝子配列を解析すると、SFV は参加者 2 名がヒヒ由来であり、他の 5 名はチンパンジー由来であった。

参加者 7 名は、非ヒト霊長類やその唾液、尿などの体液に接する機会があり、体液が参加者の皮膚と粘膜と暴露したり、皮膚損傷があると報告している。

全ての参加者の抹消血単核球（PBMC）から SFV DNA が検出され、口腔の検体、尿、精液からも検出された。

自己申告による健康状態を検討したが、SFV 感染と関連がある共通した臨床的な症候群は示唆できなかった。

臨床検査を調べ最も興味ある事は、参加者 3 名で好酸球減少、血小板減少の軽度の異常があったことである。しかし臨床上重要ではない。

参加者 3 名の妻を SAV 感染者との接触者として、ウエスタンブロット法と PCR 法で調べたが陰性であった。少人数での限られた追跡調査期間での観察のため、SFV 感染に関連する病状や人の二次的 SFV 感染は特定できなかった。

筆者らは、人における感染は医学的に重要性が解明されていないので、SFV 感染者は輸血や移植を控えるべきであると警告している。

使用上の注意記載状況・その他参考事項等

| 報告企業の意見 | 今後の対応 |
|---|---|
| SFV 感染の人での病態や二次感染について明確になっていない。SFV はレトロウイルスであることから、本剤の製造工程で不活化できると考えられる。 | 今後とも情報収集に努める所存である。 |

(4)

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# Clinical and Virological Characterization of Persistent Human Infection with Simian Foamy Viruses

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## ABSTRACT

**Persons occupationally exposed to nonhuman primates (NHPs) can be persistently infected with simian foamy virus (SFV). The clinical significance and person-to-person transmissibility of zoonotic SFV infection is unclear. Seven SFV-infected men responded to annual structured interviews and provided whole blood, oral, and urogenital specimens for study. Wives were tested for SFV infection. Proviral DNA was consistently detected by PCR in PBMCs of infected men and inconsistently in oral or urogenital samples. SFV was infrequently cultured from their PBMCs and throat swabs. Despite this and a long period of intimate exposure (median 20 years), wives were SFV negative. Most participants reported nonspecific symptoms and diseases common to aging. However, one of two persons with mild thrombocytopenia had clinically asymptomatic nonprogressive, monoclonal natural killer cell lymphocytosis of unclear relationship to SFV. All participants worked with NHPs before 1988 using mucocutaneous protection inconsistently; 57% described percutaneous injuries involving the infecting NHP species. SFV likely transmits to humans through both percutaneous and mucocutaneous exposures to NHP body fluids. Limited follow-up has not identified SFV-associated illness and secondary transmission among humans.**

## INTRODUCTION

TWO PATHOGENIC RETROVIRUSES, human immunodeficiency virus (HIV) and human T-lymphotropic virus (HTLV), established endemicity in human populations following infection of individual humans with simian immunodeficiency viruses (SIV) and simian T cell lymphotropic viruses (STLV) from nonhuman primate (NHP) reservoirs, respectively, and viral adaptation facilitating human-to-human spread.[1] Continued direct contact between human and NHPs in occupational and other settings provides an ongoing opportunity for introduction of additional simian retroviruses across species into human populations.

Foamy viruses (FVs), retroviruses in the *Spumavirus* genus,[2,3] establish persistent infections endemic to many mammalian species. Simian FV (SFV) infection is highly prevalent among captive NHPs.[4–6] Despite SFV coevolving with primates for at least 30 million years,[7] endemically infected human populations have not been identified.[6] A prototype FV (PFV), previously termed "human" FV (HFV) because it was isolated from a nasopharyngeal carcinoma (NPC) from a Kenyan man in 1971,[8] is now known to be of chimpanzee origin.[9,10] SFV infections have been identified in persons exposed directly to NHPs and their body fluids occupationally or through hunting, butchering, or habitat sharing.[10–16] Sequence analysis suggests

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that these human SFV infections originated from multiple NHP species, including chimpanzee, baboon, African green monkey, macaque, De Brazza's guenon, mandrill, and gorilla.[4,6,10] Putative associations between FV infection and various human diseases, including NPC, have not been supported in well-designed studies.[6,17,18]

Unlike HIV and HTLV, the clinical significance and secondary transmissibility of human infections with SFV are unknown. SFV is strongly cytopathic in human and NHP cells *in vitro*,[6] but is not recognized to be associated with disease in any natural host, though this has not been systematically evaluated. SFVs are easily transmitted between NHPs, mostly by contact with oral secretions during grooming or biting.[5,6]

Surveillance for simian retrovirus infection in persons occupationally exposed to NHPs at research centers and zoos in North America identified 14 persons with serological evidence of SFV infection.[10,12] Using archived sera, most workers were shown to have long-standing zoonotic infection with unclear exposure risks or public health significance [10,12] We report preliminary results from the first prospective cohort study of persons persistently infected with SFV. We characterize the potential for secondary transmission by determining the presence of virus in various body fluids and by longitudinal testing of intimate contacts, evaluate the health status of humans persistently infected with SFV, and assess possible occupational risk exposures leading to infection.

## MATERIALS AND METHODS

### *Study design and enrollment*

Persons with documented SFV infection identified through surveillance of occupationally exposed workers were eligible to provide informed consent and enroll as primary participants in a prospective cohort study approved by the Centers for Disease Control and Prevention (CDC) Institutional Review Board.[10,12] Documented SFV infection was defined as seroreactivity to SFV antigens by Western blot (WB) combined with evidence of proviral DNA sequences in peripheral blood mononuclear cells (PBMCs) by polymerase chain reaction (PCR) and/or isolation of SFV from PBMCs.[10,12]

Spouses/partners and children of SFV-infected humans or other persons living in the same household were also eligible to enroll as contact participants. Informed consent was obtained from all participants before enrollment. All enrollees were offered follow-up for a minimum of 5 years.

### *Participant interviews*

Participants were interviewed by telephone at enrollment and annually for 5 years using a standard questionnaire. Information was collected on demographics and health status at the time of enrollment. Participants were asked about their general health status including personal health observations and conditions diagnosed by physicians and about symptoms known to be associated with retroviral infections such as malignancies or lymphoproliferative, inflammatory, and neurological diseases.[3] To evaluate exposure opportunity, information was collected on the duration of occupational exposure to specific NHP species, work activities and practices including use of protective equipment, past injuries, and specific exposures to NHP blood or body fluids. To identify opportunities for secondary transmission, participants were questioned about sexual contacts, practices, and other activities that may result in intimate exchange of body fluids, including donation of blood or other living biological material. Participants were counseled regarding current knowledge about human SFV infection and provided the opportunity to ask questions.

### *Specimen collection and preparation*

Whole blood, parotid saliva, swabs of saliva and the posterior oropharynx ("throat swabs"), urine, and semen (all primary participants were male) were requested from participants annually for clinical, virological and immunological testing. Parotid saliva was collected in intraoral (Schaefer) cups and immediately transferred with a pipette to cryovials.[19] Throat and saliva swabs were collected with viral culturettes (Becton/Dickinson). Nonblood specimens were shipped to the CDC immediately after collection on wet ice; whole blood was shipped at room temperature.

Upon arrival at the CDC parotid saliva was centrifuged for 2 min at $1000 \times g$ and cell pellets and supernatant were aliquoted and frozen at $-80°C$ until tested. Throat and saliva swabs were placed in 2 ml phosphate-buffered saline (PBS), vortexed, and then centrifuged for 5 min at $1000 \times g$ to pellet any cells present. The cell pellet was washed twice with PBS, then divided equally for PCR testing and for tissue culture for some participants. Urine and, when available, semen samples were centrifuged for 10 min at $800 \times g$ to pellet any cells present and washed twice with PBS and stored at $-80°C$. Urine and semen supernatants were aliquoted and stored at $-80°C$ for mucosal immunity studies.[19]

### *Clinical laboratory testing*

Clinical testing was performed by a commercial diagnostic laboratory. Clinical testing included complete blood counts (CBC) with differential analysis of white blood cells, testing of serum for electrolytes, glucose, creatinine, blood urea nitrogen, uric acid, total protein, albumin and globulin, total bilirubin, adenosine phosphatase, lactate dehydrogenase (LDH), serum aspartate aminotransferase (AST), and serum alanine aminotransferase (ALT).

### *Virological and immunological analysis*

DNA lysates were prepared from PBMCs and from pelleted cells from parotid saliva, mixed saliva, throat swabs, and urine and tested for SFV polymerase (*pol*) proviral sequences using nested PCR.[10,12] DNA quality was confirmed by $\beta$-actin PCR as previously described.[10,12] Virus isolation was attempted from selected participant's PBMCs, throat, and saliva samples. Specimens were cultured on canine thymocytes and/or *Mus dunni* fibroblasts and monitored biweekly for up to 40 days for cytopathic effect, reverse transcriptase (RT) activity, and proviral *pol* sequences.[10,12]

Plasma was tested for SFV antibodies using a WB assay that can detect both monkey and ape SFV as described in detail elsewhere.[4,10,19,20] Saliva and urine were tested for the presence of anti-SFV IgG and IgA by WB analysis.[19]

To perform mononuclear cell phenotyping, 20 μl of well-mixed MultiTEST four-color reagent (CD3/CD8/CD45/CD4) (Becton Dickinson Biosciences, San Jose, CA) and 50-μl aliquots of EDTA-anticoagulated whole blood were added to a TruCOUNT tube (Becton Dickinson Biosciences, San Jose, CA) containing a known concentration of beads. The mixture was incubated for 20 min at room temperature in the dark before 450 μl of FACS lysing solution (Becton Dickinson Biosciences, San Jose, CA) was added. After 15 min of incubation, the lyse/no-wash-stained samples were analyzed with the FACSort flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). Data were analyzed using MultiSET software (Becton Dickinson Immunocytometry Systems, San Jose, CA).

### *Contact enrollments*

Contacts of SFV-infected participants were evaluated annually for evidence of SFV infection by WB testing of serum or plasma and PCR testing of PBMC DNA, but are not interviewed.

## RESULTS

### *Primary participants*

Seven of 13 persons eligible to participate enrolled for long-term follow-up. We refer to these participants as cases 2, 3, 6, 7, 9, 10, and 12 to be consistent with previous reports and the chronological order in which they were identified as SFV-infected.[10,12] Sequence analysis indicates that the virus infecting cases 2 and 3 originated from baboons while virus infecting the remaining five participants all originated from chimpanzees.[10,12] Case 11 dropped out of the initial surveillance study and was not available for further study.[10]

Table 1 summarizes demographic and exposure data of SFV-infected persons by enrollment status. Participants are not entirely representative of the eligible population, limiting interpretation of results. Male gender, higher level job status, and longer duration of both occupational exposure and SFV seropositivity are overrepresented among study participants.

All participants are male compared to four/six (67%) eligible persons who did not enroll ("nonparticipants"). Two of five (40%) animal caretakers, both animal care supervisors, the sole research associate, and two/four (50%) veterinarians participated. At the time SFV infection was confirmed, the median age of participants (median 56, range 41–62 years) was similar to that of three nonparticipants for whom age was available (median 57, range 49–58). Participants enrolled a median of 2 years (range 1–5) after infection was confirmed. Participants were exposed to NHPs longer prior to confirmation of infection than were five nonparticipants for whom adequate data were available (median 26, range 8–37 years versus median 19, range 10–29 years, respectively).

The availability of stored serum allowed determination of a minimal duration of seropositivity for six/seven participants and four/six nonparticipants. Prior to documentation of SFV infection,

TABLE 1. DEMOGRAPHIC AND EXPOSURE CHARACTERISTICS OF ELIGIBLE AND ENROLLED PARTICIPANTS

| *Case* | *Sex*[a] | *SFV species origin* | *Occupation* | *Age when SFV infection documented (years)* | *Year SFV infection documented* | *Year first positive sera archived* | *Minimal duration (years) SFV infection when identified* | *Duration of NHP exposure (years) when SFV infection identified* |
|---|---|---|---|---|---|---|---|---|
| | | | | *Enrolled participants* | | | | |
| 2 | M | Baboon | Research associate | 56 | 1996 | 1978 | 18 | 29 |
| 3 | M | Baboon | Animal care supervisor | 57 | 1996 | 1988 | 8 | 37 |
| 6 | M | Chimpanzee | Veterinarian | 60 | 1998 | 1981 | 17 | 19 |
| 7 | M | Chimpanzee | Veterinarian | 41 | 1999 | 1990 | 9 | 23 |
| 9 | M | Chimpanzee | Animal caretaker | 41 | 1999 | 1980 | 19 | 8 |
| 10 | M | Chimpanzee | Animal care supervisor | 50 | 1999 | 1976 | 23 | 26 |
| 12 | M | Chimpanzee | Animal caretaker | 62 | 2001 | NA[b] | NA | 32 |
| | | | | *Eligible nonparticipants* | | | | |
| 1 | M | AGM[c] | Animal caretaker | 57 | 1996 | 1995 | 1 | 19 |
| 4 | M | Baboon | Veterinarian | 49 | 1997 | 1994 | 3 | 20 |
| 5 | M | Baboon | Veterinarian | 58 | 1998 | 1979 | 19 | 29 |
| 8 | M | Chimpanzee | Animal caretaker | NA | 1999 | 1985 | 14 | >14 |
| 13 | F[d] | Chimpanzee | Veterinary technician | NA | 2002 | NA | NA | 11 |
| 14 | F | Chimpanzee | Animal caretaker | NA | 2002 | NA | NA | 10 |
| | | | | *Ineligible nonparticipants* | | | | |
| 11 | M | Chimpanzee-like[d] | Research technician | NA | 1999 | NA | NA | 10 |

[a]M, male; F, female.
[b]NA, not available.
[c]AGM, African green monkey.
[d]Based on SFV-type-specific WB.

participants with available stored sera were seropositive a median 9 years longer than were nonparticipants (median 17.5, range 8–23 years versus median 8.5, range 1–19 years, respectively). Initial clinical testing of participants under this protocol occurred after a median of 19 years (range 2–24) of documented infection.

### *Exposure history and use of protective equipment*

All seven participants reported direct, frequent, and intimate opportunity for exposure to NHPs and their biological fluids including animal saliva, urine, feces, and blood. All seven workers reported histories of both mucocutaneous exposures to NHP body fluids and skin-penetrating injuries. Five of seven participants (71%) described NHP bite wounds, five/seven (71%) described scratch wounds, and six/seven (86%) described percutaneous exposure to NHP body fluids via skin penetrating sharp injuries. However, only four/seven (57%) participants (cases 3, 6, 7, and 9) described a percutaneous injury that was associated with the NHP species from which sequence analysis suggests their infecting virus arose.

All participants reported currently wearing leather or latex gloves when handling NHPs. However, these seven participants worked with NHPs for a median of 16 years (range 5–29) prior to 1988 when universal precaution guidelines were established. Five workers consistently and two inconsistently wore gloves prior to 1988. All reported historically inconsistent use of face shields and goggles transitioning to more regular use in recent years. Two participants denied use of goggles or face shields for mucocutaneous protection; four reported wearing them 25–75% of the time for specific tasks such as working with chemicals or infected animals or cleaning cages. One wore face shields consistently "when required." The use of protective equipment was rare prior to 1988, and workers noted that current use of face shields did not always protect them from mucocutaneous exposure to NHP saliva and other body fluids.

### *Body distribution of SFV*

The distribution of SFV in human body fluids is summarized in Table 2. SFV DNA sequences were found in PBMCs from all seven persons and in all of their 19 serial samples tested. Virus culture was attempted at least once on PBMCs from six/seven participants. SFV was isolated from only one/two PBMC samples from each of cases 6 and 10, representing 33% of persons and 20% of specimens from which virus culture was attempted.

SFV DNA was detected in oral cavity specimens from three/seven (43%) participants; overall, 6/16 (38%) throat swab samples from these seven participants were SFV DNA positive. Saliva specimens from two/seven (29%) persons were positive for SFV DNA; 6/23 (26%) serial samples were positive. Interestingly, SFV was isolated from throat swab samples from case 6, for whom six/seven (86%) oral cavity specimens were positive for SFV DNA. SFV was not isolated from saliva available from the other five participants (seven samples).

Although all subjects provided urine specimens, most (13/18, 72%) samples had insufficient cellular DNA present to support SFV PCR testing. Of five samples from four persons with sufficient cellular DNA, two persons (cases 3 and 6) were positive for SFV DNA. Two participants (cases 2 and 12) each provided two semen samples; a third (case 6) provided a single specimen. Specimens from case 2 (who had received a vasectomy more than 20 years ago) and case 12 tested negative for B-actin sequences, indicating the absence of cellular DNA or the presence of PCR inhibitors. Thus these semen specimens were not suitable for further PCR testing. The semen specimen from case 6, who had benign hemospermia, was positive for SFV DNA. Insufficient material was available to attempt virus isolation from semen or urine specimens.

SFV-specific immunoglobulin G (IgG) antibodies against structural (Gag) and accessory (Bet) proteins were detected by WB in plasma, saliva, and urine from participants. Immunoglobulin A antibodies were not detected in any specimens, as previously reported.[19]

### *Clinical status*

Self-reported medical histories identified chronic conditions, nonspecific symptoms, and diseases of aging common in the U.S. population. In addition, case 9 reported congenital heart disease and mild thrombocytopenia since 1997 and case 3 underwent aortic valve replacement during this observational pe-

TABLE 2. PRESENCE OF SFV IN BODY COMPARTMENTS

| | *Number of specimens positive/number of specimens tested (%)* | | | | | |
|---|---|---|---|---|---|---|
| | *Peripheral blood mononuclear cells* | | *Oral cavity*[a] | | | |
| *Enrollee* | *SFV DNA (%)* | *SFV isolation (%)* | *SFV DNA (%)* | *SFV isolation (%)* | *Urine*[b] *SFV DNA (%)* | *Semen*[b] *SFV DNA (%)* |
| Case 2 | 2/2 (100) | 0/1 (0) | 0/6 (0) | 0/2 (0) | 0/1 (0) | NT[c] |
| Case 3 | 4/4 (100) | 0/2 (0) | 0/4 (0) | 0/4 (0) | 1/1 (100) | NT |
| Case 6 | 4/4 (100) | 1/2 (50) | 6/7 (86) | 1/4 (25) | 1/2 (50) | 1/1 (100) |
| Case 7 | 4/4 (100) | 0/2 (0) | 0/9 (0) | 0/4 (0) | 0/1 (0) | NT |
| Case 9 | 1/1 (100) | 0/1 (0) | 1/2 (50) | 0/2 (0) | NT[c] | NT |
| Case 10 | 3/3 (100) | 1/2 (50) | 0/6 (0) | 0/2 (0) | NT | NT |
| Case 12 | 1/1 (100) | NT | 3/5 (60) | NT | NT | NT |
| Total | 19/19 (100) | 2/10 (20) | 10/39 (26) | 1/18 (6) | 2/5 (40) | 1/1 (100) |

[a]Includes throat and saliva swabs and parotid saliva.
[b]Specimen quantity insufficient for SFV isolation.
[c]NT, not tested.

riod. No symptom or diagnostic patterns suggested a common clinical syndrome associated with SFV infection.

Results of clinical laboratory testing for each participant are summarized in Table 3. Repeated clinical laboratory testing was within normal limits for cases 2, 10, and 12. Testing identified unremarkable patterns of mildly abnormal glucose and renal function tests compatible with a three decade history of diabetes (case 3) and of fluctuating mild liver transaminase elevations (case 7).

Clinical laboratory testing identified hematological abnormalities for three participants. Three times on annual testing case 6 had eosinophil counts at the lower limit of or below normal range; his eosinophil count was within normal limits on subsequent testing in May 2002. Mild thrombocytopenia without other blood count abnormalities was confirmed in case 9. In addition to mild laboratory abnormalities expected to accompany long standing diabetes, case 3 had the unexpected findings of intermittent mild thrombocytopenia accompanied by natural killer (NK) cell lymphocytosis (NKCL). NK cells [$CD3^-CD16^+56^+$ cells] at three time points were 44% (780/$\mu$l), 39% (854/$\mu$l), and 38% (840/$\mu$l), respectively (upper normal limit 25% or 480/$\mu$l). Additional hematological tests performed by a specialized laboratory on specimens collected 9 months apart (personal communication, W.G. Morice, II and A. Tefferi, Mayo Clinic) repeatedly showed that 40% of lymphocytes were CD16-positive, CD3-negative NK cells, expressing CD56 and CD57, and CD161 with partial loss of CD94. These findings along with other tested markers indicated monoclonal NKCL consistent with a chronic NK-large granular lymphocytosis (indolent large granular lymphocytosis) of unclear clinical significance. The patient has no related clinical symptoms.

### *Secondary transmission*

Wives of cases 2, 3, and 9 were tested for SFV infections as contact participants. All remained WB and PCR negative despite a collective minimum of 57 person-years of intimate exposure to an SFV-infected partner (median 20, range 13–24 years). The wives of three additional nonparticipants also tested SFV negative.[10,12] All participants reported intimate marriages that provided frequent opportunity for exposure to saliva and other body fluids. All denied the routine use of barrier precautions or other practices documented to minimize sexual transmission of infections.

Case 2 ceased regular blood donation in 1969. A serum collected in 1967 was WB negative; the next available specimen collected in 1978 was WB seroreactive. Cases 6, 7, and 10 ceased regular blood donation when notified of SFV infection; retrospective testing of archived sera confirmed earlier seropositivity. As previously reported elsewhere, four recipients of leuko-reduced blood products from one SFVcpz-infected donor (case 6) showed no evidence of SFV infection.[20]

## DISCUSSION

This is the first longitudinal description of the clinical, immunological, and virological status of humans infected with SFV. After a minimum median of 26 years (range 6–31) of documented infection as of 2007, participants continue to demonstrate strong antibody response by WB testing. Viral DNA can be consistently detected in all subjects' PBMCs but inconsistently from other body sites. SFV was successfully cultured from the throat swab and PBMCs of one participant and from the PBMCs of a second. SFV DNA was also detected in the urogenital tract of two persons. Despite this, no secondary transmission among humans via intimate exposure or blood product transfusion from one SFV-infected donor was identified.[20] Our data support a persistent infection in humans consistent with the demonstrated nature of endemic infections in NHPs, but suggest that the presence of detectable viral DNA in human body fluids does not correlate with transmissibility.

Repeated exposure to SFV DNA in body fluids may be insufficient for human-to-human transmission due to a limited viral load, the persistent presence of virus in a latent noninfectious state, or other mechanisms. SFV isolations were most frequent from case 6, the only participant whose specimens were received and processed within 8 h of collection. This may suggest that time elapsed from collection to processing influenced virus recovery, or may reflect higher viral loads in case 6 than other participants. Quantitative PCR testing for viral DNA and RNA levels in blood and other body compartments might provide further insight on the relationship between variations in viral load and inconsistencies between detection of SFV DNA in, and isolation of SFV from, body fluids. Fluctuations in viral load may also explain the variable detection of SFV in some body fluids.

Six of seven retrovirus genera are associated with hematological, neurological, dermatological, arthritic, or oncogenic diseases affecting nearly all vertebrate species, typically after long incubation periods.[3] Despite being highly cytopathic in cell culture, FVs have not been associated with any *in vivo* disease.[6] Our preliminary observations on humans with prolonged infection, while limited, are reassuring. Most clinical laboratory results were within normal limits or explained by the presence of conditions common in human populations of comparable age.

The most intriguing clinical observations were mild hematological abnormalities in three participants. The fluctuating, inconsistent and mild eosinopenia of case 6 appears to be clinically insignificant. The mild thrombocytopenia in two/seven participants deserves attention, but also does not appear to be clinically significant.

Although the total lymphocyte counts in all participants were normal, the NKCL in one participant with minimal thrombocytopenia is a notable finding. NKCL is a rare condition of unknown etiology, accompanied by thrombocytopenia in 12% of subjects in one series.[21] Persistent viral infection (e.g., Epstein–Barr, hepatitis B or C viruses)[22] or persistent immunological stimulation[23] has been hypothesized to play an etiological role. With the caveat that we do not know whether case 3 has other persistent infections, we may speculate that persistent SFV infection and, possibly, the presence of long-term diabetes mellitus[24] might contribute to case 3's NKCL. Future studies quantifying SFV integration in specific blood cells or cell lines may cast light on whether SFV plays a role in the observed hematological abnormalities.

SFV transmits naturally among NHPs via casual exposure to oral and respiratory secretions, and has been experimentally

TABLE 3. ABNORMAL CLINICAL LABORATORY TEST RESULTS OVER THE PERIOD OF FOLLOW-UP

| *Participant* | *Years of age, Year 1* | *Known duration of infection (years), Year 1* | *Year 1* | *Year 2* | *Year 3* | *Year 4* | *Year 5* |
|---|---|---|---|---|---|---|---|
| Case 2 | 62 | 23 | WNL[a] | WNL | WNL | NA[b] | NA |
| Case 3 | 62 | 13 | Glucose 111 mg/dl (65–109)[c]; creatinine 1.7 mg/dl (0.5–1.4); BUN[d] 33 mg/dl (7–25); NK[e] cells 780/μl, 44% (68–482, 4–25%) | NK cells 854/μl, 39%; uric acid 8.6 (1.7–8.2); platelets 138,000 (140,000–400,000) | Glucose 131 mg/dl; creatinine 1.4; ALT[f] 116 U/liter (5–35); NK cells 38% | NA | NA |
| Case 6 | 62 | 19 | Eosinophils 34/mcl (50–500) | Eosinophils 17/mcl | Eosinophils 56; platelets 122,000 | Eosinophils 33 (15–500) | WNL |
| Case 7 | 42 | 10 | AST[g] 48 IU (0–42); ALT 70 IU (0–48) | ALT 67 | WNL | NA | WNL |
| Case 9 | 41 | 20 | Platelets 107,000 | NA | NA | NA | NA |
| Case 10 | 50 | 24 | WNL | WNL | WNL | NA | NA |
| Case 12 | 62 | 2 | WNL | NA | NA | NA | NA |

[a]WNL, within normal limits.
[b]NA, not available.
[c]Limits of normal are shown in parentheses.
[d]BUN, blood urea nitrogen.
[e]NK, natural killer.
[f]ALT, alanine aminotransferase.
[g]AST, aspartate aminotransferase.