Characterization of blood-borne transmission of simian foamy virus

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BACKGROUND: Simian foamy virus (SFV) is an endemic, nonhuman primate (NHP) retrovirus that is transmitted to individuals who work with or hunt NHPs. The cross-species transmission of simian retroviruses is believed to be the etiology of human immunodeficiency virus and human T-lymphotropic virus infections in humans. Although SFV is not pathogenic in the native host, the shared ancestry with other simian retroviruses has brought into question the potential for acquired pathogenicity after cross-species transmission. This study examines whether SFV also shares the traits of transmissibility through the blood supply.

STUDY DESIGN AND METHODS: Within a controlled environment, blood from an SFV-infected monkey was transfused into an SFV-uninfected monkey. Evidence of infection, pathogenic effects, immune correlates, and viral shedding were followed for 6 months after transfusion.

RESULTS: Molecular evidence of SFV infection manifested 8 weeks after transfusion followed by seroconversion 1 week later. Quantitative analysis demonstrated that the highest level of detectable virus was concomitant with seroconversion followed by establishment of a viral "set-point." Analysis of circulating lymphocytes revealed changes early in infection. Potential routes of transmission of SFV and roles of site-specific immune response are suggested by the late appearance of SFV shedding in the saliva of the transfused animal.

CONCLUSION: The blood supply has historically provided a portal through which novel, occult viruses can become disseminated among humans. The demonstration of transmissibility of SFV through whole-blood transfusion, in an NHP model, contributes to the understanding of potential risks associated with blood donation by SFV-infected humans.

Simian foamy virus (SFV) is an endemic, nonhuman primate (NHP) retrovirus that is transmissible to humans.[1] Studies have demonstrated that 2 to 3 percent of individuals who work directly with NHPs are persistently infected with SFV.[2,3] In addition, a defined seroprevalence of SFV among bush-meat hunters who harvest NHPs for food has recently been reported.[4] Possibly due to targeted on site blood donor clinics, or due to involvement in biomedical research, we found that the SFV-exposed laboratory workers in Canada donate blood at a much higher rate than the general population (J.I. Brooks et al., unpublished observations). With similar factors promoting blood donation at other primate facilities, it is expected that SFV-exposed workers in general would be overrepresented in the blood donor pool.

The issue of transmission of zoonotic retroviruses through the blood supply is significant, given that human

ABBREVIATIONS: CPE = cytopathic effect; LN = lymph node; NHP = nonhuman primate; SFV = simian foamy virus.

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immunodeficiency virus (HIV) and human T-lymphotropic virus (HTLV) infections are both believed to be the result of discrete cross-species transmission events between NHPs and humans.[5] Both of these pathogenic human retroviruses are characterized by prolonged latency before progression to clinical disease. Furthermore, in the case of HTLV, the pathogenic effects of viral infection may only be manifest in a small proportion of infected individuals. Whether SFV shares the pathogenic characteristics of the known human retroviruses after cross-species transmission remains to be determined.

There are relatively few identified human SFV cases, and the period of clinical follow-up remains insufficient for establishing the pathogenic potential of cross-species infection with SFV. Approximately 21 SFV-infected humans have been identified in the published literature with follow-up information available on a few subjects.[1-3,6,7] The available analysis of a subset of infected individuals did not reveal the presence of any medical conditions specifically associated with SFV infection.[1,3] Further clinical information on these infected individuals is being gathered in ongoing follow-up studies. The association of SFV infection with disease is further complicated by an inherent case selection bias in all surveillance programs conducted to date. The recruitment strategy for previous studies captured only SFV-exposed individuals who were relatively healthy and were still working. It is possible that some SFV-infected people, who may have become ill as the result of SFV infection, were no longer working and as a result were not available for studies.

The transmission of the known human retroviruses HIV and HTLV through transfused components of infected blood has resulted in the development of risk management policies specific to populations in whom these infections are prevalent. Since the identification of cross-species transmission of SFV to individuals exposed to NHP, public health agencies in the United States and Canada have attempted to address the risk posed to the blood supply by simian retroviruses.[8,9] A single study reported the lack of SFV transmission to four recipients of blood products from a single SFV-infected donor. The specific circumstances of those transfusions and the small study sample made it difficult to rule out such a risk of SFV transmission.[10] Owing to the absence of SFV transmission events, the potential health consequences from blood-borne transmission of SFV remain unknown. Regulatory authorities have requested more evidence on SFV transmission through blood transfusion to facilitate decision making.

MATERIALS AND METHODS

Cynomolgus macaques

A cohort of three, captive-born, cynomolgus macaque monkeys (*Macaca fascicularis*) was selected from the monkey colony at Health Canada. The cohort consisted of an SFV-infected (SFV(+)) "donor" monkey, a blood group matched, SFV-negative (SFV(–)) blood transfusion "recipient," and another SFV(–) "control" monkey that received a saline transfusion. SFV status was confirmed prior to the start of the experiment based upon repeated seroreactivity or seronegativity on an SFV immunoblot and concordant polymerase chain reaction (PCR) results from isolated peripheral blood mononuclear cells (PBMNCs).[2,11]

Three months prior to transfusion, the SFV(–) animals were segregated from the SFV(–) troop, and housed separately. After transfusion, the recipient animal was initially housed in its own room. In order to mitigate the psychological impact of being isolated for 1 month, an SFV(–) companion animal was subsequently brought into the recipient animal's room. The recipient and companion animal were housed singly and could see each other but had no direct contact nor did they share any toys. The control animal was returned to the SFV(–) monkey troop after the saline transfusion. The protocol was approved by the Health Canada Animal Care Committee at the Animal Resource Division. Animals were cared for in accordance with regulations from the Canadian Council on Animal Care.

ABO blood grouping

Blood group compatibility was determined with reverse typing.[12,13] Serum samples from the donor and eligible recipients were tested for antibodies to A and B antigens with standardized human red blood cells (RBC) reagents (Dominion Biologicals Ltd, Dartmouth, Nova Scotia, Canada). Blood grouping was inferred with Landsteiner's rule from the presence or absence of precipitation to either group A and/or group B RBCs in the presence of serum from the animal in question.

Cell lines

Canine thymocytes, Cf2Th (ATCC Catalog CRL-1430), and baby hamster kidney cells (BHK21 ATCC Catalog CCl-10) were used to culture SFV-1, -3, and -6, respectively. Cell cultures were maintained according to ATCC recommendations.

Preparation of SFV antigens

The SFV immunoblot assay for identifying SFV infection in monkeys and humans with a combination of SFV antigens from chimpanzees, African green monkeys, rhesus macaques, and cynomolgus macaques has been described previously.[2,14] Briefly, BHK21 host cells were inoculated with SFV-6, and Cf2Th host cells were inoculated separately with SFV-1 and -3 and SFVmac-cyn. SFV strains were obtained from ATCC (SFV-1, -3, -6) or from our stock

(SFVmac-cyn). At 50 percent cytopathic effect (CPE), the infected cultures of BHK21 and Cf2Th cells were lysed and assayed for protein content. Control cultures of the same cells were lysed at the same time point.

Preparation of immunoblot

Single-lane NuPage Novex Bis-Tris 4 to 12 percent polyacrylamide IPG gels (Invitrogen, Carlsbad, CA) were loaded with 20 µg of protein from each of the SFV-1, SFV-3, SFV-6, cyn-mac, or 20 µg BHK21 and 60 µg of Cf2Th proteins and run at 200 V for 54 minutes. Three strains of SFV were grown on Cf2Th cells and only one strain on BHK21 cells hence the ratio of proteins loaded on the control gels. Protein was transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Ottawa, Ontario, Canada) with standard techniques.[15] Dilutions of serum (1:100), if available, or plasma were used as the primary antibody. Bound antibody was detected with protein A/G-alkaline phosphatase conjugate (Pierce, Biolynx, Brockville, Ontario, Canada) with chemiluminescent substrate (Immunstar, Bio-Rad, Hercules, CA). Diagnosis of SFV infection was based on seroreactivity to SFV antigens in the 70-kDa range, representing the *gag* doublet, as described previously.[1-3]

Whole-blood transfusion

The SFV(–) recipient was transfused with 20 mL of fresh blood collected in citrated buffer from the SFV(+) donor animal. This volume represented 10 percent of the circulating blood volume of recipient animal approximating a single unit of whole human blood. The control SFV(–) monkey was treated and housed in the same manner except that it was infused with normal saline. After transfusions, the recipient was isolated as described above and the control animal was returned to an SFV-free colony.

Blood samples from the recipient and control monkeys were collected at 1, 2, 4, 6, 8, 12, and 17 weeks after transfusion for SFV testing. Animals were monitored for serologic and molecular evidence of SFV infection at the indicated time points. Blood was also collected from the recipient monkey at Week 9, to confirm SFV infection and at Weeks 21, 25, 29, and 39 for SFV viral load monitoring.

Biochemistry and hematologic testing

Anticoagulated whole blood and serum from the recipient monkey was analyzed with commercial assays at a diagnostic veterinary laboratory (Vita-Tech, Markham, Ontario, Canada). The measured parameters included organ systems reported to have disease association with SFV infection.[16] Measured indices included a complete blood count and differential, electrolytes, blood urea nitrogen, liver enzymes, and thyroid function.

PBMNC isolation

Two milliliters of anticoagulated cynomolgus blood was centrifuged at $1200 \times g$ without brake for 10 minutes. Plasma was carefully removed so as not to disturb the buffy coat. The buffy coat was then removed and layered over 95 percent Ficoll-Paque Plus (Amersham Biosciences, Baie d'Urfe, Quebec, Canada) and centrifuged for 30 minutes, at $1200 \times g$ without brake. PBMNCs were washed twice in RPMI and were cultured immediately or frozen in dimethyl sulfoxide freezing medium (Gibco BRL, Grand Island, NY) and stored in liquid nitrogen.

Coculture

PBMNCs for coculture were stimulated with 30 U per mL interleukin-2 (IL-2; Roche, Laval, Quebec, Canada) and 5 µg per mL phytohemagglutinin-M (Roche) in RPMI 1640 (Sigma Chemical Co., St. Louis, MO) supplemented in the manner described for Cf2Th cultures with the exception of the addition of 20 percent calf serum for the first 24 hours. Stimulation was typically carried out for a total of 72 hours. Stimulated PBMNCs for coculture were treated with 3000 Rads gamma radiation and 1×10^6 to 1.5×10^6 cells were added to a T-25 flask containing 5×10^5 Cf2Th cells. Cultures were maintained for a minimum of 14 days, split three times per week, and monitored daily for CPE. Stimulated and irradiated control PBMNCs were maintained in culture to monitor for cell death. Uninfected Cf2Th cultures were also maintained to ensure that there was no contamination. After the detection of CPE, SFV infection was confirmed with PCR.

PBMNC nucleic acid detection

SFV proviral DNA was detected with nested PCR and quantified with real-time PCR. Briefly, DNA was extracted from Ficoll-separated PBMNCs or 2 mL of whole blood with a commercial kit (DNeasy tissue kit or the QIAamp DNA blood midi kit, Qiagen Canada, Mississauga, Ontario, Canada, respectively). The choice of whole blood or PBMNCs as source material was based on sample availability. Nested PCRs were performed on a thermocycler (ABI 9700, Applied Biosystems Canada, Streetsville, Ontario, Canada) as previously described[11] with the exception of the elimination of the initial two cycles at 38°C. Presence of a 464-bp band on an ethidium bromide–stained agarose gel was deemed a positive. Identity of this band was confirmed, when first appearing in a sample, by DNA sequencing.

Real-time PCR primers were optimized for SFVmac-cyn using computer software (Primer Express, Applied Biosystems) with the target located within the same con-

served area of the *pol* gene. Cycling conditions included denaturation for 15 seconds at 94°C and anneal-extend for 1 minute at 60°C for 50 cycles after following the manufacturer's recommendations for UNG and *Taq* activation (TaqMan universal PCR master mix, Applied Biosystems). Primers and probe were as follows: sense MS5 5′-ACCGGACGAGATGCTACCTTT, antisense MS6 5′-TGA CTTTTACCACATCCTTTCGTAGA, and probe 6-FAM-TGAAAGTCTCTTCCAAGTATTGGTGGCCAA-TAMRA. The standard curve consisted of serial dilutions of a cloned fragment of the *pol* gene SFVmac-cyn in a background of SFV(–) *M. fascicularis* genomic DNA. The assay could reliably detect 10 copies of target DNA in a background of genomic DNA. Real-time PCR procedures were carried out on a sequence detection system (ABI Prism 7000, Applied Biosystems) with 1 µg of target DNA. The effective number of cells added to the quantitative assay was calculated by dividing the amount of DNA added to the reaction by the mass of DNA in a diploid *M. fascicularis* cell.[17] Quantification of SFV viral load is represented as the number of copies per 1000 cells.

Plasma and saliva nucleic acid detection

Total nucleic acid was recovered from 200 µL of plasma or saliva with a virus kit (QIAamp Ultras-Sens, Qiagen) in a volume of 40 µL. Samples were used neat or DNase treated on the column according to the manufacturer's instructions (Qiagen). At Week 39, plasma samples were treated as above with and without passage through a 0.45-µm filter used to eliminate cellular material. For all samples, 10 µL of the extracted material was reverse transcribed and amplified with the a probe reverse transcription (RT)-PCR kit (QuantiTect, Qiagen) according to manufacturer's directions with the MS5 and MS6 primers and Taqman probe with the conditions described above with an initial 30-minute (reverse transcriptaise) incubation at 50°C. Plasmid DNA containing SFV pol sequence, as described above, was used as a positive control.

Analysis of lymph node from recipient monkey

At 17 weeks after transfusion, a left inguinal lymph node (LN) was harvested under general anesthesia from the recipient monkey. The LN was divided in half and real-time PCR was carried out on one portion of the LN after nucleic acid extraction with a tissue kit (DNeasy, Qiagen). The other portion of the LN was teased apart and filtered through a 100-µm cell strainer, and cells were separated with a Ficoll gradient. The purified lymphocytes were then divided into two aliquots. One portion of cells was stimulated as described above, irradiated, and cocultured with Cf2Th until CPE was seen at which point cellular material was analyzed with real-time PCR. The other portion of the purified cells was stimulated as above for 3 days and then cultured for 7 additional days in the presence of IL-2. After 10 days' total culture, these latter cells were harvested and washed. RNA was isolated with an RNA blood mini kit (Qiagen) and treated with the RNase-Free DNase set (Qiagen). Sample was reverse-transcribed and PCR-amplified with a one-step RT-PCR kit (OneStep, Qiagen) according to the manufacturer's directions with primer-3 and primer-4 described previously.[11]

Sequence analysis

Sequencing was performed on a capillary sequencer (ABI 3100, Applied Biosystems), with dye terminator chemistry (Big Dye v.3, Applied Biosystems), according to the manufacturer's instructions with primers 1, 2, 3, or 4.[11]

Flow cytometric analysis

The samples were analyzed with a flow cytometer (FACS-Calibur, BD Biosciences, San Jose, CA) equipped with two lasers: an argon-ion laser (488 nm) and a red diode laser (635 nm). Anti-human monoclonal antibodies CD45, 3, 4, 8, 20, and 16/56 (BD Biosciences, Mississauga, Ontario, Canada) were used for four-color immunophenotyping on fresh ethylenediaminetetraacetate blood.[18] To determine absolute and percentage values with single-platform technology, fluorescent particles (AccuCount Spherotech, Libertyville, IL) were used as internal calibrator beads. Both lymphocyte subset percentage and absolute count enumeration was generated with a CD45-based gating strategy.

RESULTS

Pretransfusion analysis

Initial experiments confirmed that RBC ABO epitopes were not present in sufficient quantity to be detected by forward grouping.[13] Instead, the presence of antibodies to A and B antigens in both the donor and the recipient monkey established a common type O blood group (data not shown).

The donor animal had serologic evidence of SFV infection at all time points tested before transfusion (Fig. 1) and was demonstrated to propagate infectious virus by the production of characteristic CPE after 10 days of coculture of donor monkey PBMNCs with Cf2Th (data not shown). The recipient animal had been identified as SFV(–) 2 years previously and remained seronegative at all points before transfusion (Fig. 1).

Demonstration of seroconversion after transfusion of SFV-positive blood

Consistent with the transfer of passive immunity, 1 week after the transfusion, the recipient monkey showed weak seroreactivity to SFV gag antigens on the immunoblot

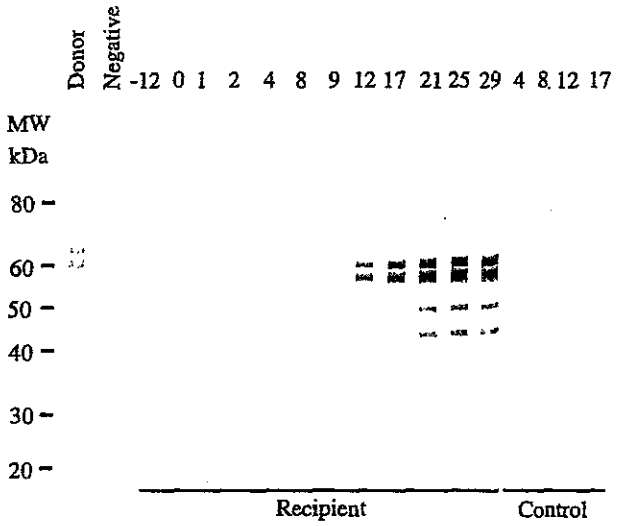

Fig. 1. Seroreactivity of transfused monkey plasma over time (transfusion time points in weeks) toward lysates of SFV-1, SFV-3, SFV-6, and SFV_{Mac} with Western blotting. Negative control serum from cynomolgus macaque.

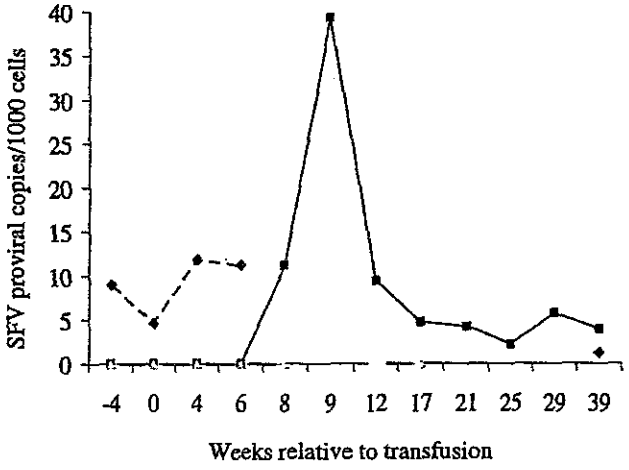

Fig. 2. SFV proviral load. (■) Recipient; (▲) control; (◆) donor.

assay that disappeared by Week 8. On the original immunoblot, a weak antibody response is manifest at 9 weeks after transfusion. At Week 12, however, a robust de novo antibody response is easily appreciated, with increasing antibody titer as demonstrated through the increase in intensity banding at successive sampling times (Fig. 1). The control monkey did not display any serologic evidence of SFV infection through 17 weeks after saline transfusion.

Molecular evidence of transfusion acquired SFV infection

Proviral DNA was detected by nested PCR in DNA isolated from the blood of the transfused monkey 8 weeks after transfusion heralding seroconversion that manifested 1 week later (Fig. 2). Before this, all time points were negative for proviral DNA in the recipient animal. Subsequent analysis by real-time PCR revealed 11 copies of SFV provirus per 1000 cells at this time point (Fig. 2). The highest cellular viral load measured was 39 copies per 1000 cells at Week 9. Subsequent SFV viral load measurement revealed a "set-point" cellular viral load in the recipient monkey of 3 to 4 SFV proviral copies per 1000 cells. These numbers compare with the donor monkey SFV viral load of 13 copies of SFV per 1000 cells. No PCR evidence of SFV infection could be found in the control monkey at any time point (Fig. 2).

Evidence of SFV in plasma

Real-time RT-PCR analysis of total nucleic acid extracted from the recipient animal's plasma showed the presence of SFV at the lower limit of detection. Positive signal was present on replicate testing at the aggregate time point of 8 and 9 weeks and at Week 25 (Table 1). The positive SFV signal detected in the plasma of the recipient monkey disappeared after DNase treatment. In a similar fashion, the donor animal had SFV detected in the plasma at Week 0, but again this signal disappeared after DNase treatment. The control animal had no positive results with this assay.

TABLE 1. Detection of SFV nucleic acid in plasma with real-time RT-PCR*

Sample	Time point (weeks)	Positive results/ replicate number
Donor	0	1/4
Donor	39	0/1
Recipient	0	0/1
Recipient	4	0/4
Recipient	8	2/4
Recipient	9	3/4
Recipient	12	1/4
Recipient	17	0/4
Recipient	21	0/1
Recipient	25	1/2
Recipient	29	0/1
Recipient	39	0/1

* All positive results became negative after DNase treatment. No SFV was detected from the plasma of the control monkey at any time point.

Viral shedding in saliva

The recipient monkey showed molecular evidence of viral shedding in saliva beginning at 29 weeks after infection (Table 2). At 39 weeks after infection, both viral RNA and DNA were detected in saliva of the recipient monkey. The donor monkey's saliva was positive for viral DNA and RNA at all tested time points (Table 2). The quantity of virus detected in the saliva of the chronically infected monkey

was approximately 1000-fold higher than that detected in the plasma.

TABLE 2. Detection of SFV nucleic acid in saliva with real-time RT-PCR*

Sample	Time point (weeks)	Total nucleic acid	After DNase treatment
Donor	6, 29, 39	+	+
Recipient	6-21	–	ND†
Recipient	29	+‡	–
Recipient	39	+	+

* No SFV was detected in the saliva of the control monkey.
† ND = not determined.
‡ Positive in one of two replicates.

LN analysis after transfusion

DNA extracted from inguinal LN tissue, at week 17, contained 4.2 copies SFV proviral DNA/1000 cells by Real-Time PCR analysis (data not shown). Characteristic CPE of SFV infection was seen after 14 days of co-culture with stimulated lymphocytes isolated form the lymph node. Evidence of SFV nucleic acid was present in whole nucleic acid extracts using Real-Time PCR. Nucleic acid extracts of isolated and stimulated lymphocytes demonstrated evidence of SFV by Real Time PCR that persisted after DNase treatment.

Biochemistry, hematologic markers, and clinical changes

Clinical laboratory measures showed little change over the course of the study. White blood cell (WBC) counts differed among animals but remained consistent throughout the experiment. There was no evidence of a hepatitis induced by infection. Thyroid function as measured by the free T4 assay was not affected by infection.

Flow cytometry results

The total lymphocyte count on both the transfusion and the control animals fell after the transfusion consistent with a stress response brought on by housing in isolation (Fig. 3). The CD4+:CD8+ ratio remained fairly constant for the control animal with minor variations over time. At Week 4 after transfusion, however, the recipient animal had a sudden inversion in CD4+:CD8+ ratio from 1.2 to 0.8 (Fig. 4). The ratio of CD4+:CD8+ remained in flux with a value of 1.8 measured at Week 6 appearing to stabilize beyond Week 12.

The change in the CD4+:CD8+ ratio appears due to a precipitous drop in the percentage of CD4+ cells beginning at Week 2 after transfusion, continuing to a nadir at Week 4 with recovery by Week 6 (Fig. 5). With a delay of approximately 2 weeks, the percentage CD8+ cells also showed a precipitous drop contributing to the large rebound in the CD4+:CD8+ ratio. By 12 weeks, the percentages appear to stabilize, but the animal demonstrates a higher relative contribution of CD8+ cells than at baseline.

Sequencing results

Comparison of a 425-bp sequence within the *pol* gene of SFV isolated from the donor and recipient monkeys showed 99.6 percent homology (Fig. 6).

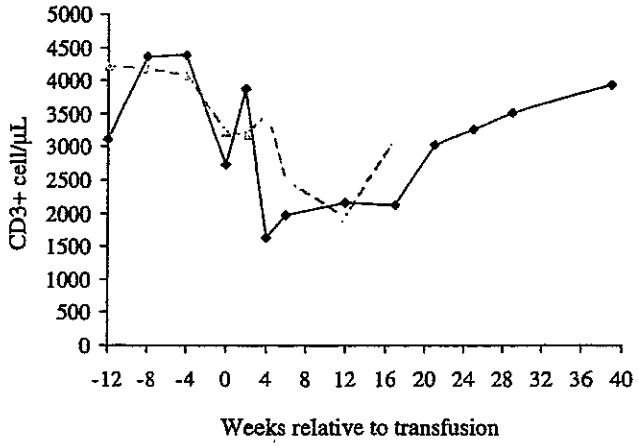

Fig. 3. Total T-lymphocyte count. (◆) Recipient; (▲) control.

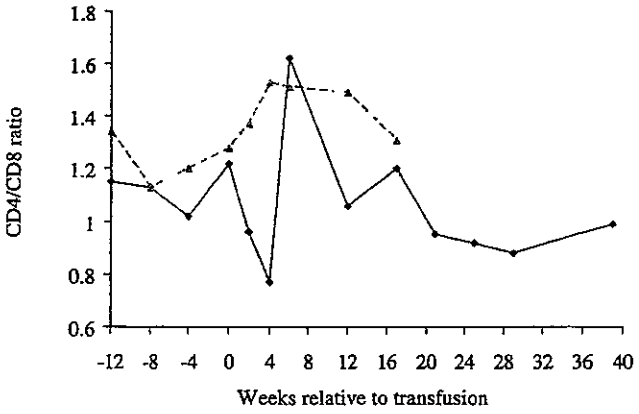

Fig. 4. Changes in CD4/CD8 ratio. (◆) Recipient; (▲) placebo.

DISCUSSION

To our knowledge, this is the first report showing transmission of SFV via blood transfusion in an NHP model. Previous studies demonstrating transmission of SFV have been performed in small animals with techniques such as intradermal inoculation to demonstrate the presence of an infectious agent.[19] By employing stringent experimental conditions to ensure that the recipient animal acquired SFV infection by means of the blood transfusion and not through any other route, we provide compelling evidence

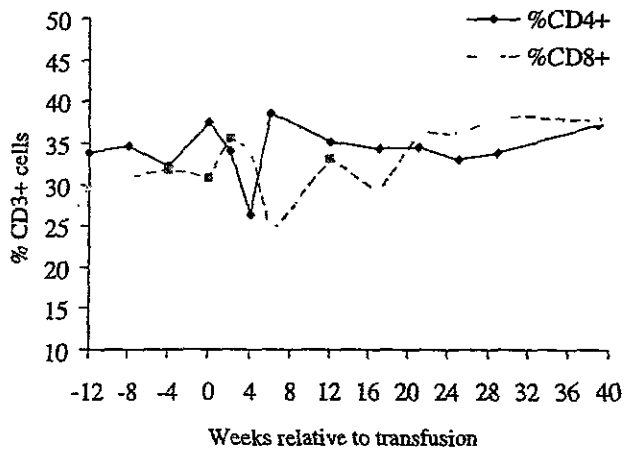

Fig. 5. Relative percentages of lymphocytes in recipient. (◆) Percent CD4+; (■) percent CD8+.

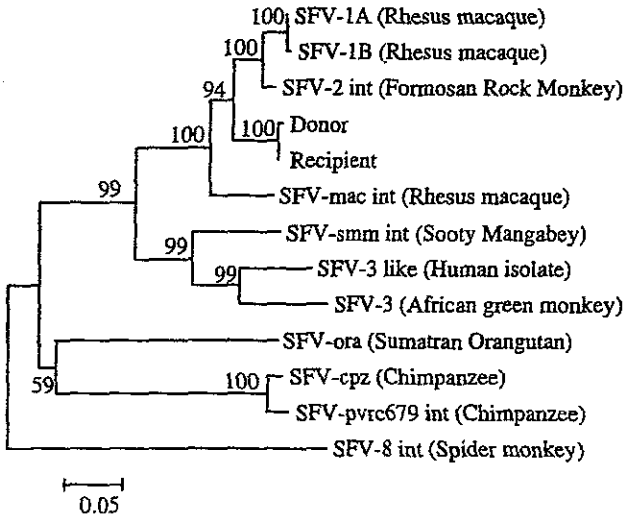

Fig. 6. Phylogenetic relationship of integrase sequences of SFV-infected recipient and donor monkey compared with other primates. The tree was derived by NJ analysis with two Kimura distances. Values on branch nodes represent the percentages of 1000 bootstrap replicates. The scale bar represents evolutionary distance of 0.05 nucleotide per site.

for the blood-borne transmission of SFV with whole blood. This finding suggests that SFV may be transmissible to humans through SFV-contaminated blood. In our previous study,[2] half of 46 animal handlers reported a history of blood donations (J. Brooks, unpublished data). This figure compares with 2 percent of the general population that donate blood on a regular basis,[20] suggesting that animal handlers may be overrepresented in the donor pool. Given this new knowledge, the question on the deferral of potentially SFV-infected persons remains open for debate.

This study also provides the first detailed information on acute SFV infection in the native host. Similar to what has been described for other retroviruses, SFV infection appears to result in a burst of viral replication leading to a spike of circulating virus which then falls to achieve equilibrium of viral set-point after seroconversion. Although the containment of the rapid increase in SFV replication is most likely the consequence of intervention by the immune system, the exact role of humoral and cellular immunity remains to be elucidated. Clearly, there is an increasing titer of specific antibodies against SFV that may be neutralizing as seen in other models.[21] However, the CD4+:CD8+ inversion, in the recipient and not the control monkey, in the 2 to 4 weeks after the transfusion, suggests stimulation of a cell-mediated immune response to the infection. Another possibility is that there may be either viral- or immune-mediated killing of SFV-infected T-cell targets because previous work has shown that lymphocytes are the major reservoir of SFV in the blood of infected animals.[22] Other explanations for the T-cell disturbances could include the nonspecific allogeneic effects of the blood transfusion or cotransmission of an occult pathogen. There was a parallel drop in the total CD3+ in both the transfused and the control monkey in the 4 weeks after the transfusion. This effect is likely due to stress associated with the single housing arrangements.[23]

Analysis of the excised LN indicates that the potentially infectious virus is found in the lymphatics. The LN harvested at 17 weeks had SFV DNA detectable at the time of excision. When stimulated with IL-2 and phytohemagglutinin, these cells then produced a highly transcriptionally active virus indicated by detection of viral RNA from lysed cells.

We also describe the seeding and emergence of active SFV replication in saliva from the recipient animal. SFV DNA is initially detected in saliva followed by the emergence of viral RNA concomitant with the development of SFV-specific immunity and the reduction in measurable virus within the circulating lymphocytes. This finding may suggest a qualitative difference in the capacity of the host immune system to suppress viral replication in the two sites. The presence of SFV in the saliva supports the possibility that the virus may be transmitted through bites.[24] The delay in the appearance of SFV in the saliva suggests that a recently infected animal may not be able to transmit the virus through saliva, until 6 or more months after infection.

The data on isolation of SFV from the oropharynx of infected humans is less clear. In the largest study, SFV was successfully cultured from a throat swab in only one of six SFV-infected individuals but isolation from the saliva was unsuccessful in all cases.[25] Molecular evidence of SFV in the saliva was detected only in that one individual who had the positive throat culture. Another report in the literature describes failure to culture SFV from saliva in a single individual.[7]

The demonstration of viral DNA in the plasma may represent viremia. It could be argued that our results represent contamination from cellular DNA. Our preparative methods, however, are consistent with those used to obtain HIV nucleic acid for viral load testing. Virus in the plasma in association with the peak viral load in cellular DNA is consistent with maximum viral turnover, perhaps at other sites such as the lymphatics, with spillover of free virus into the blood compartment. The detection of viral DNA in the plasma, in association with peak viremia, is not surprising given our understanding that the DNA form is the functionally relevant virus.[26,27] SFV replication is unique among retroviruses as it is reverse-transcribed late in its life cycle,[26] and SFV has been described as a DNA virus with an RNA intermediate.[27] Our detection of RNA forms of SFV from the cell culture may represent the cell-associated intermediate forms of SFV associated with the viral life cycle.

Suggestive molecular evidence of SFV in the plasma provides new information relevant to the risk of SFV transmission through blood products. Measures to protect the blood supply include leukoreduction, which reduces the total number of WBCs by approximately 3 logs.[28] Although this effort has been shown to reduce infectivity of cell-associated agents,[29] risk of infection is not eliminated. In addition to blood products containing cells, the possibility now exists that plasma may be a source of transmissible SFV. Although most current plasma products undergo a variety of inactivation processes, the presence of antibodies to infectious retroviruses in source plasma and plasma pools could be considered grounds for donor exclusion.[29]

The risk of SFV becoming endemic in the human population depends on the prevalence of SFV infection, the transmissibility of the virus, and the opportunities for human-to-human transmission. There is a measurable prevalence of infection and ongoing transmission of SFV to animal workers and people who hunt NHPs. Studies have shown that 2 to 3 percent of people who work with NHPs are infected with SFV.[1,2] The majority of captive NHPs are SFV-infected.[30] Previously, we have demonstrated human SFV infection resulting from cross-species transmission of the virus from macaques, the most common NHP used in the laboratory setting.[2] This study establishes that SFV is transmissible through blood transfusion with a homologous virus challenge in the native host. Taken together, these findings indicate that there may be a risk of SFV transmission through the blood supply from individuals occupationally exposed to NHPs.

The true risk of human-to-human transmission of SFV remains unknown due to limited data. The only published study on blood donation by an SFV-infected human failed to demonstrate transmission to four recipients of blood products.[10] The largest collection of data examining the risk of transmission of SFV between humans is limited to six spouses of SFV-infected men where transmission was not observed.[3] The absence of transmission from a small number of sexual partners of SFV-infected men, however, does not rule out transmissibility of SFV between humans via other risk behaviors. Furthermore, in the context of the blood supply, retroviral transmission risk may be 3 orders of magnitude higher than through unprotected sexual contact.[31,32]

In principle, dissemination of a novel retrovirus into the human population should be prevented. The blood supply has historically provided a portal through which novel occult viruses can become disseminated among humans. Data provided by this study may guide decisions regarding blood donor deferral of people with ongoing exposure to NHPs, to minimize the risk of transmission of SFV through blood transfusions. These decisions will have to be weighed against negative health effects, including blood availability.[33]

NOTE

Subsequent to submission of this work, similar findings were published by Khan et al. in Transfusion 2006;46:1352-9.

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