別紙様式第２－１

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	日刊薬業, 第 12105 号, 平成 18 年 9 月 6 日	公表国 日本	
販売名（企業名）	－					

研究報告の概要

エイズウイルス(HIV)のうち、世界的にも感染例が少ない HIV-2 に日本人男性が初めて感染していたことがわかった。厚生労働省は、感染例の多い HIV-1 に比べ HIV-2 は感染力が弱いため、診断や治療などの体制は従来通りとする一方、HIV-2 の抗体検査の実施を徹底するよう都道府県に通知した。
HIV-2 は主に西アフリカで流行しており、感染してから発病までの期間が長いという。国内では 1993 年に韓国籍の 2 人の感染が確認されたが、今まで日本人の感染例は無かった。
男性は気管支喘息を患い、国内の医療機関に入院し、検査の結果、HIV-2 に感染していることが判明した。過去に西アフリカに渡航し、現地で輸血した経験があるため、これが感染経路と見られている。男性は既に症状が改善し退院している。
入院先の医療機関から依頼を受けた厚生労働省研究班が検査を行い、遺伝子検査の結果、HIV-2 であると確定、8 月 11 日に厚生労働省に報告した。
HIV 抗体検査で陽性だった場合には、抗体の種類を判別する確認検査を行うが、国内感染者のほとんどが HIV-1 のため、HIV-1 の検査だけをして HIV-2 の検査を行わない医療機関もあり得るため、厚生労働省では 8 月 11 日付で各都道府県に対し、HIV-2 の検査も確実に行い、検査漏れがないよう通知した。

報告企業の意見	今後の対応
世界的にも感染例の少ない HIV-2 に感染した初の日本人男性に関する報告である。当社血漿分画製剤は原料血漿の段階で抗 HIV-2 抗体陰性を確認している。また、HIV に対するウイルスクリアランス指数が 9 以上であることを確認しているので、安全性について特に問題ないと考えられる。	今後とも HIV-2 に関する安全性情報等に留意していく。

使用上の注意記載状況・その他参考事項等

「重要な基本的注意」に原材料となる血液について抗 HIV-2 抗体陰性を確認している旨を記載。

厚労省

日本人初のＨＩＶ２型感染で検査徹底を通知

エイズウイルス（ＨＩＶ）のうち、世界的にも感染例が少ないＨＩＶ２型に日本人男性が初めて感染していたことが４日分かった。厚生労働省は、感染例の多いＨＩＶ１型に比べ２型は感染力が弱いため、診断や治療などの体制は従来通りとする一方、２型の抗体検査の実施を徹底するよう都道府県などに求めている。

２型は主に西アフリカで流行しており、１型に比べ感染力が弱く感染してから発病までの期間が長いという。国内では１９９３年12月と２００２年１月に韓国籍の２人の感染が確認されたが、今まで日本人の感染例はなかった。

男性は気管支喘息を患い、国内の医療機関に入院し、検査の結果、ＨＩＶ２型に感染していることが判明した。過去に西アフリカに渡航し、現地で輸血をした経験があるため、これが感染経路とみられている。男性はすでに症状が改善し退院している。

入院先の医療機関から依頼を受けた厚労省研究班が検査を行い、遺伝子検査の結果、２型であると確定、先月11日に厚労省に報告した。ＨＩＶ抗体検査で陽性だった場合には、抗体の種類を判別する確認検査を行うが、国内感染者のほとんどが１型のため、１型の検査だけをして２型の検査を行わない医療機関もあり得る。このため厚労省では先月11日付で各都道府県に対し、２型の検査も確実に行い、検査漏れがないよう通知した。

先端医療振興財団

助成事業の概要を公表

神戸市の先端医療振興財団は５日、２００７年度から実施する、「がん情報普及・啓発に関する講演会等への助成事業」の公募に関する概要を公表した。財団傘下の神戸臨床研究情報センター（神戸ＴＲＩ）の活動の一環で、１件当たり50万円、年間総額２５０万円を計上した。助成対象は、07年度に行われる市民・患者を対象にした、がん情報の普及啓発に関する講演会やシンポジウム。応募対象団体は日本がん患者団体協議会（ＪＣＰＣ）に加盟し、推薦を受けたＮＰＯ（民間非営利団体）などの民間団体。同一団体からの複数応募はできない。助成先は財団の審査委員会で決める。公募申し込み受付期間は９月15日～11月15日。詳細は、財団ホームページ（http://www.ibri-kobe.org/）で。

テムリック

第１種医薬品製造販売業許可を取得

ＣＲＯ（医薬品開発業務受託機関）のテムリックは４日、東京都から「第１種医薬品製造販売業」の業許可を８月24日付で取得したと発表した。ＣＲＯ業務とは別に行っていた、自社開発品の承認申請のために取得した。テムリックはがん領域に特化したＣＲＯとして２００２年に事業を開始したが、04年に「ＴＭ－４１１」（多発性骨髄腫）の開発権・販売権を導入し、創薬事業を始めた。同剤は現在治験実施中で、終了は来年以降になる見通し。今年４月にはすでに２品目としてシエーリングからがん治療薬の導入契約を締結し、７月末に「ＴＭ－５１１」として治験届を提出した。

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 8. 22	該当なし	

一般的名称	洗浄人赤血球浮遊液	研究報告の公表状況		公表国
販売名(企業名)	洗浄赤血球「日赤」(日本赤十字社) 照射洗浄赤血球「日赤」(日本赤十字社)		H Cordel, I Quatresous, C Paquet, E Couturier. Eurosurveillance weekly release: 2006,volume 11, 8, 2006 Aug 10.	インド

研究報告の概要

○インドにおけるチクングンヤの再興:高まる脅威

チクングンヤウイルス(CHIKV)の感染がインドで拡大している。2005年12月以降最も被害の大きい5つの州から896,500人以上のチクングンヤ疑い症例が報告されている。北部の州からは1例も報告されていない。2004年末以降、チクングンヤはインド洋の南西部の島々で流行し、その後マダガスカルとインドでも報告された。インドにおけるチクングンヤは、1963年にコルカタで最初に検出された。1973年のインド西部での流行以降はサーベイランスは実施されておらず、インドから消滅したと考えられてきた。最近の研究では発症患者の約50%がRT-PCRでRNA陽性だったが、実際の発生率ははるかに高いと考えられる。病院に行かない患者が多く、受診してもRNA陽性となるのは1日目から4日目のみで臨床検査は難しい。症状は、38.5～40℃の高熱、筋肉痛、頭痛、関節の腫れと激痛、発症5日以降のかゆみを伴う斑点状丘疹で、多くは自己限定的で1～10日持続した。関節痛は症例の約10%で3週間以上持続し、数ヶ月～数年間続くこともある。温暖湿潤な気候と貯水池は媒介蚊の繁殖に適した環境で、貧しい人々はより感染しやすくなっている。インド洋のCHIKV分離株の遺伝子構造はウイルスが急速に変異することを示唆している。疾患は自己限定的であるが、流行地域への渡航者の感染リスクは引き続き存在する。輸入症例が欧州の多くの国から報告されており、フランスでは2006年3月に血液暴露によると考えられる国内感染例が発生している。媒介蚊の一つであるヒトスジシマカは欧州でも見られるため、ウイルス流入と地域内/持続感染のリスクについてさらに調査が必要である。

抗ウイルス剤とワクチンの開発が急務である。さらなる感染拡大を抑えるための対策の強化が求められる。臨床管理を改善する方法、特に早期検出、患者の栄養補給、その他の予防手段によって症状を大きく緩和できる。

報告企業の意見	今後の対応
チクングンヤウイルスの感染がインドで拡大しており、ヨーロッパへのウイルス流入と地域内感染が危惧されるとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。今後も引き続き、新たなウイルス等による感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

洗浄赤血球「日赤」
照射洗浄赤血球「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Resurgence of chikungunya virus in India: an emerging threat

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Since December 2005, an outbreak of chikungunya virus (CHIKV) infection has been ongoing in various states of India (Karnataka, Maharashtra, Andhra Pradesh, Tamil Nadu, Madhya Pradesh, Gujarat, Orissa and Kerala) with potential spread to neighbouring states [1,2]. Cases were first recognised and reported in December 2005. In July 2006, India's National Vector Borne Disease Control Programme (NVBDCP) reported a reduction in the number of cases in the affected districts while other districts are now becoming affected for the first time. The spread is of unprecedented magnitude and over 896 500 suspected chikungunya cases have been reported since December 2005 from the five worst affected states (Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu and Madhya Pradesh) [3]. No chikungunya cases have been reported from the northern states.

Recent large-scale outbreaks of fever caused by CHIKV infection in India have confirmed the reemergence of chikungunya in this part of Indian subcontinent. Since the end of 2004, chikungunya has emerged in the islands of the southwestern Indian Ocean (Comoros, Mauritius, Seychelles and Reunion), where several hundred thousand cases have been reported. Chikungunya was later also reported in Madagascar and in India [4,5]. Chikungunya is not new to the Indian subcontinent. Since it was first detected in Calcutta in 1963 [6], there have been reports of CHIKV infection in different parts of India [7,8,9]. Previously, the most recent Indian chikungunya outbreak was reported in 1973 in western India, in Barsi, Sholapur district, Maharashtra state [10]. Subsequently, there has been no active or passive surveillance carried out in India and it was believed that chikungunya had disappeared from the Indian subcontinent [11,12].

A recent study looked at samples taken from over 140 symptomatic patients with clinical picture of chikungunya who were presented to the Nizam's Institute of Medical Sciences hospital in Hyderabad (the capital of Andhra Pradesh) in March and April 2006. About 50% were found positive for the presence of CHIKV specific RNA (through demonstration of the virus-specific 500 bp amplicon) by reverse transcription-polymerase chain reaction (RT-PCR) [V Lakshmi et al, unpublished data]. However, the true incidence is thought to be much higher, because due to the self-limiting nature of the illness a large proportion of patients did not go to hospital, and even for those who did, laboratory diagnosis proved difficult as RT-PCR was positive for the virus in samples collected between the first and fourth day only, indicating the viraemic phase of the infection. Most patients with acute CHIKV infection presented with high fever (ranging from 38.5°- 40°C), muscle pain, headache and swelling and severe pain in the joints with polyarthralgia (pain in several joints) followed by an itching maculopapular rash five days after onset. Symptoms were generally self-limiting and lasted 1-10 days. Almost 10% of cases reported had prolonged joint pain for more than three weeks. However, joint pain may persist for several months or years. Females were more affected than males, a feature probably associated with the daytime and indoor feeding habits of the mosquito vector in India, *Aedes aegyptii*. All age groups were evenly represented.

Warm, humid climates and water reservoirs serve as an excellent breeding ground for the vector of the virus, *Aedes* mosquitoes. With an increase in temperature, susceptibility of mosquitoes to CHIKV increases [13]. High population density, lack of adequate resources for vector control and hygiene added to the vulnerability of poor people to chikungunya infection. The unique molecular features of the recently analysed Indian Ocean isolates of CHIKV [4] suggest that the virus can evolve rapidly. Studies are in progress to confirm genomic structure and virulence of the recent CHIKV from India.

Although the disease is self-limiting, the risk to non-immune travellers from other parts of the world to areas with a chikungunya epidemic, including India, continues to exist and should be included in the differential diagnosis of travellers returning home with fever. The magnitude of this risk cannot be precisely determined at this time. There is a risk of importing the virus to Europe from affected parts of the world, including Africa and South East Asia, where the virus is endemic. Imported cases have been reported from a number of European countries, including an autochthonous case from France in March 2006, probably contaminated through a blood exposure incident [14]. Considering the extent of the current chikungunya outbreak, the risk of introduction and autochthonous/sustained transmission of the virus in Europe needs further investigation, because one vector, the tiger mosquito *A. albopictus*, is also present in Europe and could increase the likelihood of its future autochthonous transmission in these countries. Various recommendations have been suggested by European experts to ensure the measures to prevent the emergence of imported viral diseases are strengthened in

Europe [5,15]. Pregnant women, families with young children, older people, and those with significant comorbidity should be advised to consult their physician before travelling to the Indian subcontinent, and travellers should be informed about the magnitude of the risk of contracting the disease and decide according to their own judgment. There are no specific preventive medications or vaccines for chikungunya fever, but there are steps travellers can take to reduce risk of being bitten by infected mosquitoes [15]. Despite infecting millions of people worldwide, chikungunya infection has been neglected since its discovery. Worldwide, there are a number of other infections with mosquito-transmitted viruses (arboviruses) with similar symptoms which may be confused with chikungunya, such as Sindbis, Ross River and dengue, and these, together with a detailed travel history, should be considered in the differential diagnosis in returning travellers.

Considering high number of cases, and lack of specific antiviral therapy, it is imperative that specific antiviral agents and vaccine be developed. Although the disease is self-limiting, sustained and intensified control measures (such as regular fogging with pesticides, awareness of the disease and vector, detection and elimination of vector breeding sources, proper facilities for health care and community awareness about the prophylactic measures) are required to control the further spread of the disease. The government of India has taken up necessary steps, in accordance with the NVBDCP guidelines on reducing mosquito breeding sources, use of temephos larvicide in recommended doses, the release of larva-eating fish (*Gambusia*) into the wells and the water bodies to control the mosquito menace and deployment of mobile teams (three teams per district in the affected districts, consisting of epidemiologists, public health specialists, microbiologists and entomologists for assessment of the situation and providing technical assistance and guidelines) and mobilisation of health workers and volunteers [16,17]. Finally, measures to improve clinical management, especially early detection, nutritional support to the affected patients, and other preventive measures may largely mitigate the disease. The wider issues of ecology, current agricultural practices, water management systems, and human behaviour patterns will need to be reviewed. This requires a combination of strategies and we need to proceed with a sense of urgency in this matter.

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医薬品

医薬品　研究報告　調査報告書

化粧品

別紙 3-13

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 8 月 8 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Simian foamy virus infection by whole-blood transfer in rhesus macaques: potential for transfusion transmission in humans Khan, A.S. and Kumar, D. Transfusion, 46, 1352-1359 (2006).	公表国 米国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
アカゲザルでの輸血によるサル免疫不全ウイルス(SFV)の伝播が実験的に示されている。2 頭の自然感染したサルをドナー(D1 及び D2 と命名)として用い，各ドナーあたり 2 頭のレトロウイルス陰性サルに全血を輸血したところ，D1 から輸血された 2 頭のサルだけが感染した(追跡期間は輸血後 1 年間)。感染は以下の方法で証明された。 i) 輸血されたサルにおける特定 SFV 抗体の発現 ii) 感染ドナーサルの末梢血単核細胞(PBMNCs)からの SFV 特有配列の PCR 増幅 iii) 末梢血単核細胞(PBMNCs)からの感染及び複製 SFV の分離 興味深いことに，ドナーのサルはそれぞれ異なる複製動態を持つ SFV 菌株に感染していた（D1 の SFV は D2 の SFV よりも複製速度が早く，D2 は D1 より中和抗体価が顕著に高かった)。また，その他の要因，例えばウイルス接種量なども感染症の伝播において重要であると示唆された。	BYL-2006-0240 Detection and molecular characteri-zation of foamy viruses in Central African chimpanzees of the Pan troglo- dytes troglodytes and Pan troglodytes vellerosus subspecies. Calattini, S. et al, J. Med. Primatol. 35, 59-66 (2006).

報告企業の意見	今後の対応
これまで,非ヒト霊長類では SFV 感染による病変の報告は無かった。また，ヒトの感染例もほとんどなかった。しかし，SFVs は，in vitro で高い細胞変性を示し，ヌクレオチド配列は宿主ゲノム内で安定して取り込まれる。現時点では，ヒト間で SFV の伝播が起こるかは明らかではない。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

Simian foamy virus infection by whole-blood transfer in rhesus macaques: potential for transfusion transmission in humans

Arifa S. Khan and Dhanya Kumar

BACKGROUND: Cross-species infection of humans with simian foamy virus (SFV) has been reported in European and North American nonhuman primate (NHP) handlers, primarily due to wound injuries involving infected animals in research centers and zoos. Additionally, African hunters have been found to be infected with SFV by exposure to body fluids, blood, or tissues of infected NHPs in the wild. The persistence of infectious virus in peripheral blood mononuclear cells (PBMNC) and the recent identification of some infected blood donors has raised safety concerns regarding potential virus transmission by blood transfusion.

STUDY DESIGN AND METHODS: SFV infection by blood transfusion was evaluated by whole-blood transfer from two naturally-infected rhesus macaques (designated as D1 and D2) to retrovirus-free monkeys. Blood from D1 was transfused to two recipient monkeys R1 and R2 and from D2 to monkeys R3 and R4. Virus transmission was evaluated by immunoassays, polymerase chain reaction assays, and coculture of PBMNC for SFV isolation.

RESULTS: SFV infection was seen in R1 and R2 based on development of virus-specific antibodies, identification of SFV sequences in monkey PBMNC, and isolation of infectious virus from PBMNC. Furthermore, both R1 and R2 remained SFV-positive at about 1 year after transfusion, which was the last time tested. No evidence of SFV infection was seen in R3 and R4.

CONCLUSION: SFV transmission in macaques occurred by transfusion of blood from one of two infected donor animals. These results indicate the potential of SFV transfusion transmission in humans, which may depend on virus-specific or donor-related factors.

Cross-species transmission of retroviruses to humans is an important public health concern as exemplified by the origin of human immunodeficiency virus (HIV) from simian immunodeficiency virus (SIV).[1] The extensive use of nonhuman primates (NHPs) in biomedical research and broad exposure to infected animals in the wild has facilitated cross-infection of humans with simian foamy virus (SFV), which is highly prevalent in all NHP species and possesses a broad host range and cell tropism.[2-4] The first human transmission was reported in 1971 due to injury by an infected chimpanzee.[5] Reports of cross-species human infection with SFV have increased since the mid-1990s[6-9] and the use of more sensitive detection assays have further indicated additional NHP handlers infected with SFV due to injury incurred by infected animals[10-12] as well as identification of people infected in Africa due to exposure to body fluids and meat while hunting and butchering of NHPs.[13]

It is noteworthy that although infectious virus has been demonstrated to persist long-term in human cells, in vivo and in vitro[6,14,15] there is, thus far, no report of disease associated with SFV and no evidence of SFV transmission between humans.[6]

The persistence of stably integrated, infectious retrovirus sequences in human peripheral blood cells raises

ABBREVIATIONS: CPE = cytopathic effect; IUPM = infectious units per million total PBMNC; NHP(s) = nonhuman primate(s); PBST = phosphate-buffered saline with 0.05 percent Tween; PBST+5 percent = PBST plus 5 percent milk; SFV = simian foamy virus; SIV = simian immunodeficiency virus; RT = reverse transcriptase.

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concerns, however, regarding the safety of blood transfusion from SFV-infected blood donors. In fact, testing of archived sera identified six SFV-seropositive blood donors.[10] A retrospective study of four recipients of blood components (red cells [RBCs], filtered RBCs, and platelets [PLTs]) from one infected blood donor failed to demonstrate SFV infection; however, it was noted that additional studies are warranted to further evaluate the potential risk of SFV transmission by blood transfusion.[16] This is especially important since transmission by transfusion has been demonstrated as an important mode of acquisition of infections in humans with other retroviruses.[17,18] In this article, we have examined SFV transmission by whole-blood transfusion in a monkey model. Blood from SFV-infected donor animals was transfused into retrovirus-free monkeys, which were analyzed for SFV infection and persistence. This study evaluates the potential human risk of SFV infection by infected blood donors.

MATERIALS AND METHODS

Monkeys and blood transfusion

SFV-negative blood recipients were juvenile, rhesus macaques (*Macaca mulatta*) that were obtained from a group of animals in a domestic breeding colony (LABS of Virginia, Morgan Island, SC), which were free of SIV, simian T-lymphotropic virus, and simian retrovirus. Animals were identified as SFV-negative with a dot blot antibody assay[19] (Simian Diagnostic Laboratory, San Antonio, TX) and shipped in individual cages to the FDA animal facility (National Institutes of Health, Bethesda, MD). All animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* [20] under an approved protocol by the Institute Animal Care and Use Committee. The animals were housed in single cages and in a separate room from the SFV-infected blood donor monkeys. Only animals that were confirmed SFV-negative by serology and by polymerase chain reaction (PCR) analysis of peripheral blood mononuclear cell (PBMNC) DNA at the time of study initiation were used in the study. A control animal was housed in the same room as the blood recipient animals to demonstrate absence of cross-contamination due to housing and handling of the animals.

Donor animals, RhK3T and RhA2V (designated as D1 and D2, respectively, in this article) were adult rhesus macaques, naturally-infected with SFV that were maintained in single housing and in a separate room from SFV-negative animals. Donor animals were well characterized: SFV from D1 and D2 (designated as SFV-D1 and SFV-D2, respectively) were previously isolated from monkey PBMNCs and characterized in replication studies to evaluate virus fitness and nucleotide sequences were determined in the long terminal repeat region. The status of SFV infection in D1 and D2 was confirmed by serology and virus isolation from samples stored on day of blood transfer.

Blood was collected under sedation with ketamine hydrochloride (10 mg/kg). Before transfusion, blood was collected in anticoagulant (heparin or ethylenediaminetetraacetate [EDTA]) from the donor and recipient animals for preparation of PBMNC, plasma, and serum. At the time of transfusion, blood was collected in EDTA for additional PBMNC and plasma preparation and in separate tubes for blood chemistry and hematology. For blood transfer, blood (20 mL) was collected in heparin (1000 U, 1 mL, Elkins-Sinn Inc., Cherry Hill, NJ) from D1 for transfusion (10 mL each) with a butterfly catheter into the right saphenous vein of two recipient monkeys, RhCK2T and RhCK3H (designated as R1 and R2, respectively, in this article). Each animal was separately handled, and mats were changed in between each animal. Similarly, blood from D2 was transferred to RhCJ3K and RhCJ52 (designated as R3 and R4, respectively, in this article). After the blood transfer, 10 mL of saline was injected into a "housing control" animal RhOVG. Monkeys were monitored for healthy recovery after the blood transfusion based on temperature, heartbeat, and respiratory rate. After transfusion, blood was collected at various times in EDTA for PBMNC and plasma preparation for analysis of virus infection. Additionally, at each time of blood collection, serum chemistry and hematology were performed (Antech, Lake Success, NY).

Detection assays for SFV antibodies

SFV-specific antibody was detected by dot blot immunoassay[19] performed by the Simian Diagnostic Laboratory. The samples from each animal were collected and stored for concurrent analysis in the same assay.

SFV-seropositive animals were confirmed by Western blot analysis. Cell lysates were prepared from uninfected and SFV-2-infected *Mus dunni* cells (wild mouse fibroblasts; ATCC, Manassas, VA) as previously described.[21] Protein concentration was determined with a protein assay dye (Bio-Rad, Hercules, CA). Sixty micrograms of protein was heat-denatured and analyzed on an 8 percent Tris-glycine gel (Novex, San Diego, CA), run 1.5 hours at 125 V (Novex X-cell II system, Novex, San Diego, CA) in 1× Tris-glycine running buffer (24.8 mmol/L Tris, 192 mmol/L glycine, 0.1 percent sodium dodecyl sulfate). Proteins on the gel were transferred to nitrocellulose membrane (Invitrogen, Carlsbad, CA; 0.45 µm) at 30 V for 1 hour in 24.8 mmol per L Tris, 192 mmol per L glycine, 20 percent methanol. The membrane was cut into strips so that each strip contained 5 µg of protein. The strips were placed, protein side up, in individual wells of a plastic tray; rinsed at room temperature for 5 minutes each with Ultrapure water, phosphate-buffered saline (PBS) without Ca^{2+}-Mg^{2+}, PBS (pH 7.3)-0.05 percent Tween (designated as PBST); and blocked overnight at room temperature in PBST containing 5 percent nonfat dried milk (designated as

PBST+5%). The strips were then incubated with 1:100 dilution in PBST+5 percent of plasma or serum, except in case of monkey D1 where 1:500 dilution of plasma was used. The membrane strips were initially incubated for 2 hours at room temperature and then overnight at 4°C on a rocker. The strips were brought to room temperature and washed three times for 5 minutes each in PBST+5 percent and then incubated for 2 hours at room temperature with a 1:500 dilution in PBST+5 percent of horseradish peroxidase (HRP)-conjugated goat anti-monkey IgG (Cappel-ICN Pharmaceuticals Inc., Aurora, OH). The strips were then washed five times for 5 minutes each in PBST, and the protein bands were visualized by chemiluminescence with a substrate system (Supersignal CL-HRP substrate system, Pierce, Rockford, IL). The substrate was added to the membrane strips for 2 minutes, the strips then blotted with paper (Whatman 3 MM, Maidstone, Kent, England) to remove excess substrate and exposed for various times ranging from 5 seconds to 2 minutes with film (BioMax MR, Kodak, Rochester, NY).

Neutralizing antibody endpoint titers were determined in assays with homologous virus (SFV-D1 with plasma from D1 and SFV-D2 with plasma from D2). MRC-5 cells (ATCC CCL-171; human lung fibroblast) were planted in a 24-well plate with 30,000 cells per well (Passage 25) in Eagle's minimum essential medium (modified) with Earle's salt without L-glutamine (Cellgro, Mediatech, Herndon, VA) containing 10 percent heat-inactivated fetal bovine serum (FBS; Hyclone, Logan, UT), 2 mmol per L glutamine, 250 U of penicillin per mL, 250 µg of streptomycin per mL, 1× nonessential amino acids (MEM-NEAA 100×, Quality Biological, Inc., Gaithersburg, MD) 1 mmol/L sodium pyruvate in a total volume of 2 mL. Cells were incubated overnight at 37°C, and 0.2 mL was removed to replace with test sample. Monkey plasma (heat-inactivated) was diluted twofold (ranging initially from 1:50 to 1:1600) in PBS, pH 7.4, without calcium and magnesium (Quality Biological, Inc.), initially ranging from 1:50 to 1:1600. Plasma samples were incubated for 1 hour at room temperature with equal volume of SFV (100 $TCID_{50}$ per 0.1 mL), after which 0.2 mL was removed and added to each well, in triplicate. The tray was incubated at 37°C and cells observed for cytopathic effect (CPE) up to Day 13, when the final results were recorded. The antibody endpoint was the highest dilution of plasma that inhibited CPE in all replicate wells.

DNA preparation and PCR analysis

Cryopreserved PBMNCs were recovered in RPMI and washed with cold PBS (without Ca^{2+} and Mg^{2+}), and DNA was prepared with a DNA blood mini kit according to the manufacturer's protocol (QIAamp, Qiagen, Valencia CA) except that all spins were at 15,800 × *g* at room temperature and the DNA elution time was increased to 5 minutes at room temperature. DNA was aliquoted and stored at –80°C.

SFV sequences in PBMNC DNA were amplified by PCR with previously described conditions with set B outer primer pair and inner primer pair (3+5 and 6+7, respectively[21]). The sensitivity of the outer primer set was shown to be 10 viral copies in 10^5 cell equivalents of cellular DNA. The identity of the SFV sequences was confirmed by nucleotide sequence analysis of gel-purified DNA fragments (gel DNA recovery kit, Zymoclean, Orange, CA), obtained with primers 6 and 7. PCR primers, which amplified an 838-bp fragment of the human β-actin gene (Clontech, Palo Alto, CA), were used as a control for the presence of DNA in the sample. The PCR mixture without DNA was used as the negative control.

Nucleotide sequence analysis

Nucleotide sequence reactions were set up with primers 6 and 7, according to the protocol with a cycle sequencing kit according the manufacturer's protocol with 5X sequencing buffer (BigDye Terminator Version 3.1 cycle sequencing kit, Applied Biosystems, Foster City, CA). The sequence reactions were purified with spin columns (CentriSep, Princeton Separations, Adelphia, NJ), and sequences were determined with a DNA sequencing system (ABI Prism 377, Perkin-Elmer Applied Biosystems, Foster City, CA).

Blood processing and virus isolation

PBMNC and plasma were prepared from blood containing EDTA as preservative (SeraCare Bioservices, previously BBI Biotech Research Laboratories, Inc., Gaithersburg, MD). Plasma was aliquoted and stored at –80°C. PBMNC were prepared by the Ficoll-Hypaque method, aliquoted, and cryopreserved. For virus isolation, PBMNC were stimulated in a 24-well plate with 5 µg per mL phytohemagglutin (PHA; Murex Biotech Ltd, Dartford, Kent, England) for 72 hours in RPMI containing 10 percent (1000 U) human interleukin-2 (Roche, Indianapolis, IN), 10 percent FBS (heat-inactivated 56°C for 30 min; Hyclone), 2 mmol per L glutamine, 250 U of penicillin per mL, 250 µg of streptomycin per mL. PHA-stimulated PBMNC were added to *M. dunni* cells (1.3×10^6-1.9×10^6) in a 75-cm^2 flask for coculture in Dulbecco's minimum essential medium containing 10 percent FBS, 2 mmol per L glutamine, 250 U of penicillin per mL, 250 µg of streptomycin per mL in a total volume of 20 mL. Cultures were passaged every 3 or 4 days when the cells reached confluency and maintained until culture termination due to extensive CPE or at least 30 days. PBMNC were added back to the cultures for three passages after the initial coculture. Filtered supernatants were collected and stored at various times during the culture period for Mn^{2+}-dependent reverse transcriptase (RT)

assay.[21] SFV identity was confirmed at culture termination by PCR amplification and nucleotide sequence analysis.

PBMNC viral load determination

MRC-5 cells were planted overnight as described above for the neutralization assay. One-milliliter of medium was removed and replaced with 1 mL containing fivefold serially diluted monkey PBMNC ranging from 1×10^6 cells per mL to 320 cells per mL per well. Each dilution was tested in at least four replicates. The plate was incubated at 37°C for 14 days. Filtered supernatant was collected and analyzed for SFV by a PCR-enhanced RT assay (STF-PERT[22]). The $TCID_{50}$ was calculated by the Kärber method[23] and infectious units per million total PBMNC (IUPM) expressed as the reciprocal of the $TCID_{50}$.

RESULTS

SFV infection occurred in two recipient monkeys (R1 and R2) that were transfused with blood from donor animal D1, but not in the two animals (R3 and R4) that received blood from donor animal D2 or in a saline-injected control animal.

Detection of SFV-specific antibodies in transfused monkeys

Plasma from study animals was analyzed for SFV-specific antibodies at various times after transfusion. The results of dot blot assays are shown in Table 1. The earliest time at which SFV antibody was detected in R1 and R2 was 22 and 16 weeks, respectively, after which time both animals remained positive. The control animal was negative at all tested times.

The antibody status of the animals was further evaluated by Western blot analysis. The results in Fig. 1 indicate the presence of SFV antibodies as early as Week 1, which decreased over time, representing passive transfer of donor antibodies. The resurgence of antibodies was seen at Week 22 in R1 and at Week 16 in R2 indicating the development of antibodies in response to virus infection after transfusion. Antibodies to SFV proteins persisted at Week 48, the last time point tested: the 65K and 70K proteins most likely correspond to the diagnostic Gag doublet seen in all infected species (p68/71[24]). Passive antibody transfer also occurred in R3 and R4 after blood transfusion from D2; however, there was no evidence of new antibody development due to virus infection (data not shown). No SFV-specific antibodies were seen in the control animal.

Detection of SFV sequences in monkey PBMNC

The kinetics of SFV infection by blood transfer were evaluated by PCR analysis of monkey PBMNC DNA. SFV-specific primers amplified a 349-bp fragment from R1 and R2 from PBMNC at Week 8 after transfusion and thereafter (Fig. 2). The expected size β-actin fragment was seen in all the samples, indicating the presence of intact DNA in the samples. The identity of the PCR-amplified fragment from

TABLE 1. Development of SFV-specific antibodies by blood transfusion*

Weeks after transfusion	Monkeys		
	R1	R2	Control
0	–	–	–
1	–	–	–
2	–	–	–
4	–	–	–
8	–	–	–
11	–	–	–
16	–	+/–	–
22	+	+	–
30	+	+	–

* All samples were run in the same assay, and each sample was analyzed in two independent assays. Differences in the results in the two assays are indicated. Negative is less than 1:5.

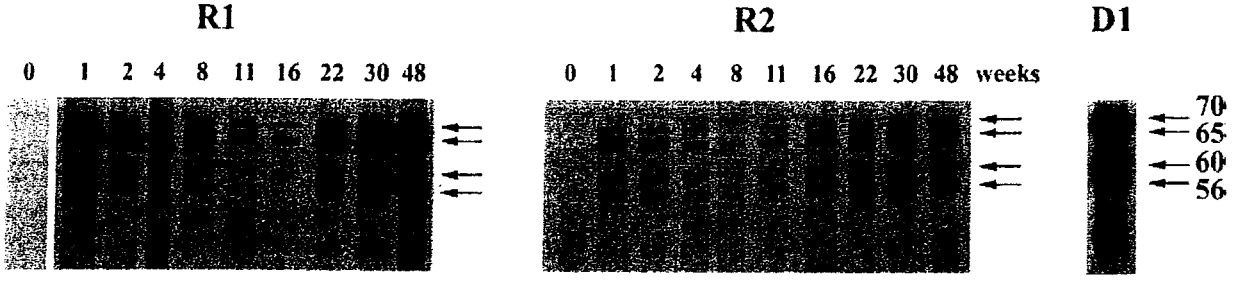

Fig. 1. Detection of SFV-specific antibodies by Western blot analysis. Monkey plasma samples, obtained on day of blood transfusion (Week 0) and at various weeks after transfusion (except Week 16, where serum was used), were incubated with immunoblot strips containing lysate prepared from SFV-2-infected *M. dunni* cells and proteins visualized as described under Materials and methods. A 5-second exposure of the autoradiogram is shown. The molecular masses of prominently visible, SFV-specific proteins, calculated from standard markers (MultiMark, Novex, San Diego, CA), are indicated in kilodaltons.

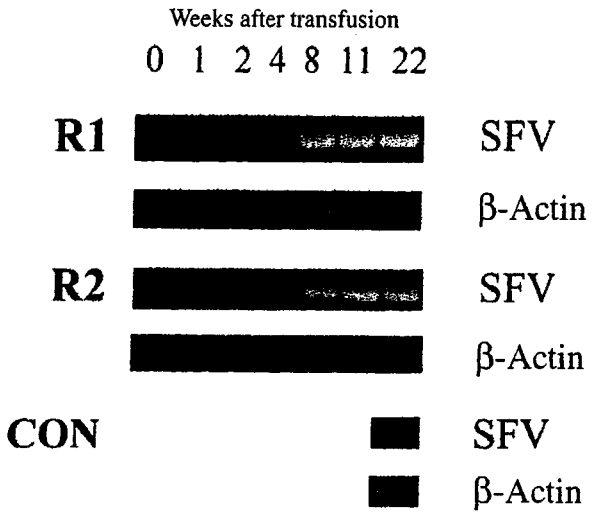

Fig. 2. Detection of SFV in monkey PBMNCs by PCR assay. SFV-specific primers were used to analyze PBMNC DNA as described under Materials and methods. DNA samples were prepared from PBMNCs that were obtained on the day of blood transfusion (Week 0) and at the indicated times after transfusion. PCR amplification with β-actin primers confirmed presence of DNA in the samples. CON = control.

the 22-week sample of R1 and R2 was confirmed by nucleotide sequence analysis. As shown in Fig. 3, the SFV sequences in R1 and R2 were identical to the SFV in D1. SFV-specific sequences were not detected in R3 and R4 at any time up to 30 weeks (the last tested time), including early time after transfusion, where passive antibodies were present (data not shown). The control animal was negative by PCR with SFV primers.

SFV isolation from monkey PBMNC

To determine whether the SFV sequences detected in R1 and R2 were associated with an infectious virus, monkey PBMNCs from Week 11 and Week 22 after transfusion were cocultured with *M. dunni* cells. The cultures were monitored for replicating SFV by the appearance of CPE in the cell monolayer and by RT production in cell-free supernatant. The RT results, shown in Fig. 4, indicate earlier virus isolation with the Week 11 sample from both R1 and R2, with culture termination due to extensive CPE at Day 14: in the case of the Week 22 sample, there was a slightly delayed kinetics of virus isolation with culture termination on Day 16. This difference in the kinetics of virus isolation was also evidenced by CPE detection in the cocultures, which was seen on Day 9 in the case of the Week 11 sample and on Day 11 with the Week 22 sample. The kinetics of virus isolation with PBMNC from the day of blood transfusion for D1 showed that CPE was seen on Day 11 with culture termination on Day 18. No virus was detected in PBMNC from R1 and R2 on the day of transfusion nor at any time from the control animal. There was no evidence of virus isolation from PBMNC of R3 and R4 at any time point tested including 1 year after blood transfusion, the last tested time; virus was isolated from D2 on the day of blood transfer (data not shown).

The identity of the viruses isolated in the coculture experiments with the Week 11 sample from R1 and R2 was confirmed by PCR amplification and nucleotide sequence analysis: the results indicated sequence identity with SFV-D1 (data not shown).

Characterization of donor monkeys

The selection of D1 and D2 as donors was initially based on the results of earlier infectivity studies, which demonstrated that the SFVs isolated from the PBMNC of D1 and D2 were distinct in their replication kinetics and CPE development: SFV-D1 had high replication and rapid CPE as compared with SFV-D2 (data not shown). To further investigate the differences in SFV transmission by blood transfusion with D1 and D2, the neutralizing antibody titer and PBMNC viral load were determined on stored samples from the animals. The results indicated a neutralizing antibody endpoint titer of 1:50 for D1 and 1:800 for D2. PBMNC viral load analysis indicated 32.4 IUPM for D1 and 3.8 IUPM for D2. Additionally, a retrospective analysis the CBC differential count indicated that the WBC count in D2 was about half of that in D1.

DISCUSSION

The identification of SFV-seropositive blood donors has raised safety concerns regarding SFV transmission by blood transfusion. A study analyzing recipients of blood components such as RBCs, filtered RBCs (WBC-reduced), PLTs, and fractionated plasma from one SFV-infected donor demonstrated absence of virus transmission,[16] however, PBMNC, which are known targets of SFV infection, were not examined and the results are limited by the sample size. Based on a theoretical risk the CDC has been counseling infected people not to donate blood.[16] To evaluate the potential risk of SFV transmission by blood and blood products, we have initially determined virus transmission by whole-blood transfusion in a monkey model. Blood was transferred from two donor animals that were naturally infected with SFVs that had distinct replication kinetics and nucleotide sequences. Interestingly, SFV transmission only occurred with D1: antibodies developed at 16 to 22 weeks and persisted approximately 1 year after transfusion (the last time tested); SFV sequences were detected by PCR at 8 weeks after transfusion, and infectious virus was isolated from PBMNC at Week 11 and Week 22. The lack of virus transmission with blood transfusion from D2 was unexpected because SFV has an

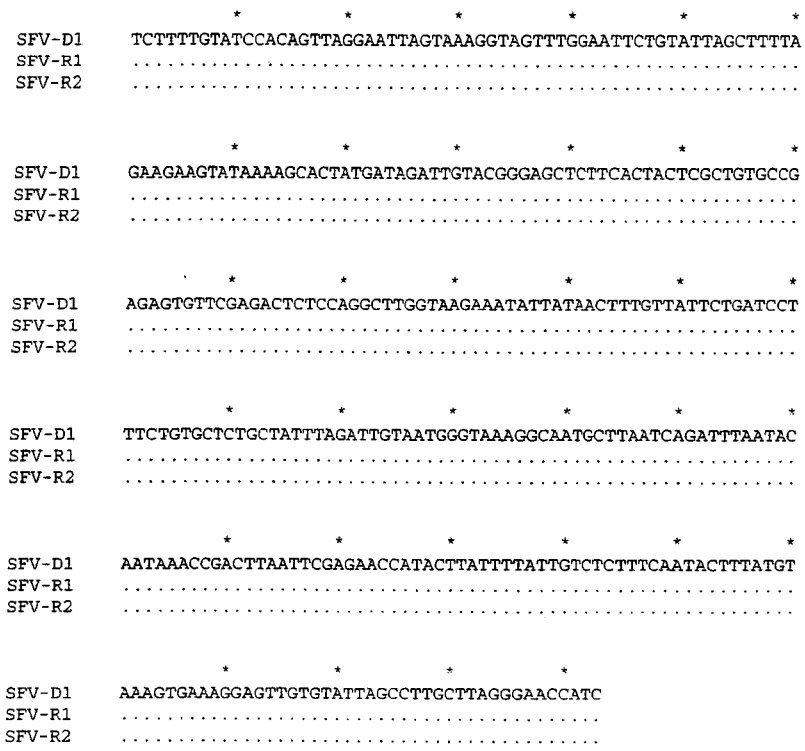

Fig. 3. Nucleotide sequence identification of SFV sequences in blood recipient monkeys. Nucleotide sequences of SFV in R1 and R2 (designated as SFV-R1 and SFV-R2, respectively) were determined from DNA fragments that were PCR-amplified from PBMNC at 22 weeks after transfusion (shown inFig. 2). Sequence comparison with SFV in the donor animal (SFV-D1) are shown: dots indicate base identity; asterisks indicate base count.

exceptionally broad host range and tissue tropism and is easily transmitted in NHPs, albeit via the saliva.[3] Different factors may contribute to retrovirus transmission such as virus load in the inoculum and fitness of the donor virus. Additionally, neutralizing antibodies have been shown to block SHIV infection of macaques.[25,26] Antibody analysis of D1 and D2 indicated a significantly higher neutralizing antibody endpoint titer in D2 compared to D1 (1:800 versus 1:50, respectively) suggesting that neutralizing antibodies may play a role in SFV transmission. Studies are under way to investigate the contribution of antibody titer in the failure of SFV transmission by D2. The results of these studies may provide insight regarding factors involved in SFV transmission and in assessing the risk of virus transmission by blood donors.

High viral load is an important determinant of virus transmission in HIV-1 infection.[27] In the case of SFV infection, the virus largely infects lymphocytes and monocytes,[3,14,15] and it is believed that virus is mostly cell-associated with no detectable virus in the plasma. Therefore, we initially determined the PBMNC viral load of D1 and D2: the results indicated that the IUPM was 32.4 and 3.8, respectively. Interestingly, this is similar to the PBMNC viral load reported in chronic infection with SIV in African green monkeys[28] and HIV-1 in humans.[29] Although the blood transfer volume was the same (10 mL), based on the CBC differential, it was found that D1 had twice the number of WBC as D2: thus approximately 29×10^6 PBMNC were transfused in case of D1 and 15×10^6 in case of D2 so that the approximate number of infected cells transferred by D1 was 940 cells and 57 by D2. Additional studies will be performed to determine whether the PBMNC viral load represents the total number of infected cells in blood and the contribution of plasma viral load, if any, in SFV transfusion transmission. It should be noted that virus fitness[30] may play an important role in virus transmission from D1 based upon in vitro studies indicating that SFV-D1 had earlier replication kinetics and more rapid CPE development than SFV-D2 (data not shown). The relationship between virus fitness and SFV transmission will be investigated to assess the risk of infection by blood transfusion.

Interestingly, virus isolation occurred with more rapid kinetics with the Week 11 PBMNC samples from R1 and R2 than with the Week 22 samples (Fig. 4). Furthermore, the kinetics of virus isolation from PBMC of chronically infected D1 was similar to that of Week 22 samples. This result suggests a higher PBMNC viral load early after infection, with a subsequent lower set point in long-term infection. To evaluate the kinetics of virus infection in vivo, longitudinal analysis of PBMNC viral load will be done on stored samples, including quantitative analysis by TaqMan PCR. Additionally, corresponding plasma samples will be tested for evidence of any SFV viremia. Analysis of PBMNC and plasma viral load may identify a high-risk window period of SFV transmission by blood transfusion. It is noteworthy that the apparent reduction in viral load in the Week 22 PBMNC samples coincided with the increase in SFV-specific antibodies (Table 1 and Fig. 1), thereby suggesting a potential role of neutralizing antibodies in reducing virus replication.

The consequences of cross-species transmission of retroviruses are unpredictable and may not be noticed for an extended period until there is a clinical outcome. This is most effectively evidenced by HIV-1, which was discovered in 1983 due to the AIDS epidemic[31] more than 50 years after the initial cross-species infection with SIV.[32,33] The lack of disease associated with SFV in any spe-

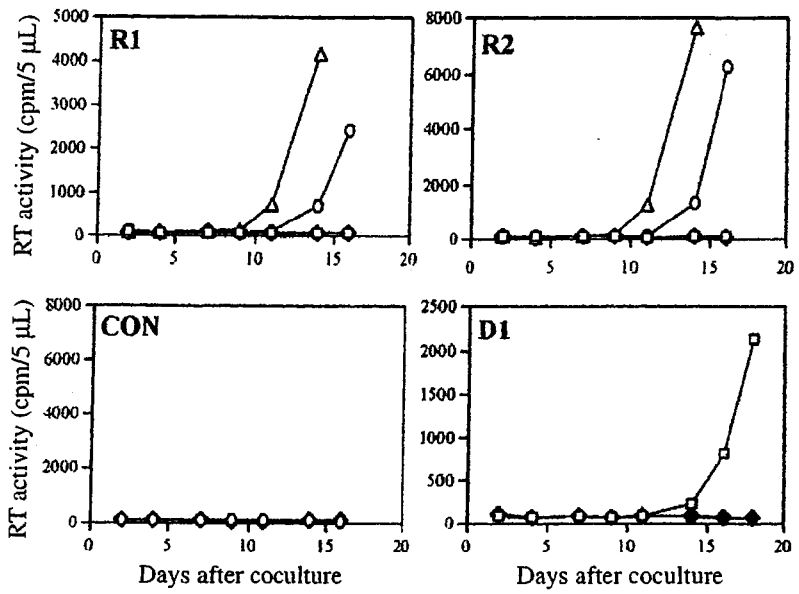

Fig. 4. SFV isolation from monkey PBMNC. PBMNC of R1 and R2, obtained on the day of blood transfer and at Weeks 11 and 22 after transfusion (2.0×10^6-2.3×10^6) were PHA-stimulated and cocultured with *M. dunni* cells, until the cultures were terminated due to extensive CPE. PBMNC from the control animal (CON: Week 22; 2.4×10^6) and donor D1 (day of blood transfusion; $<2.0 \times 10^6$) were PHA-stimulated, and cocultures set up as controls for PBMNC from negative and positive monkey, respectively. *M. dunni* cells without monkey PBMNC were included as cell culture control. Filtered supernatant was collected during the culture period and assayed for RT activity (mean ± standard deviation was calculated from two spots). Day of blood transfusion, □; Week 11 after transfusion, △ Week 22 after transfusion, ○; *M. dunni* control, ◆.

cies is an enigma,[3,4] especially since foamy viruses can be highly cytopathic in cells in vitro.[4] Due to the stable integration and long-term persistence of infectious viral sequences in the host genome, SFV might have an unexpected clinical outcome. Thus, similar to other retroviruses of public health impact, it is prudent take appropriate measures to avoid SFV exposure and infection.

The absence of known disease and lack of transmission in humans does not negate health concerns related to SFV infection in humans due to insufficient data. Demonstration of SFV infection by blood transfusion in a monkey model indicates potential risk for virus transmission in humans. The results support consideration of appropriate safeguards against exposure to SFV, or any other simian agent, through the human blood supply.

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