医薬品
医薬部外品　研究報告　調査報告書
化粧品

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
				2004.4.6	該当なし	

一般的名称	人血小板濃厚液	研究報告の公表状況	J Infect Dis. 2004; 189(6):1018-1023.	公表国 ブラジル
販売名(企業名)	濃厚血小板「日赤」　(日本赤十字社) 照射濃厚血小板「日赤」　(日本赤十字社) 濃厚血小板HLA「日赤」　(日本赤十字社) 照射濃厚血小板HLA「日赤」　(日本赤十字社)			

研究報告の概要

リューシュマニア・パラサイトがヒト皮膚リューシュマニア症（ACL）の臨床的治癒後に消失するか否かは不確定である。最近、感受性の高い分子学的手法によりリューシュマニア・パラサイトを患者瘢痕標本において直接同定することが可能となった。ブラジル北東部においてACLを治療し、臨床的治癒が認められた患者32人の瘢痕をPCR法、組織培養および組織病理学的検査を用いて分析した。*Leishmania*（*Viannia*）に特異的DNAが患者32人中30人（93.7%）の瘢痕で検出された。瘢痕3例の標本においては、リューシュマニア・パラサイトは培養物から分離され、PCR法の結果もまた、それら標本3例で陽性であった。組織病理学的検査ではパラサイトは検出されず、わずかな炎症性病巣が4例で観察され、線維性変化が全例に存在した。本結果はACLの臨床的に治癒してもパラサイトが完全に排除されることはむしろ、稀であることを示唆する。特にリューシュマニア症と後天性免疫不全症候群との重感染発生率の上昇とともに、リューシュマニア症の臨床的進行、再発および伝播とパラサイトの持続の密接な関連については、さらなる研究を行うに値する。

報告企業の意見	今後の対応
現在まで、日本において輸血によるリューシュマニア感染は報告されていないが、イラク旅行者及び長期滞在者によってリューシュマニアが日本に持ち込まれる可能性があることについては、報告済みである。本論文は、リューシュマニア・パラサイトを患者瘢痕標本において直接同定することが可能となったことを示すものである。	日本赤十字社は、問診時に海外渡航歴と既往歴を質問しているが、あらたにリューシュマニア流行地域への渡航歴およびリューシュマニア症の既往歴に対する問診マニュアルを改訂した。今後も、リューシュマニア感染について情報収集に努める。

使用上の注意記載状況・その他参考事項等

濃厚血小板「日赤」
照射濃厚血小板「日赤」
濃厚血小板HLA「日赤」
照射濃厚血小板HLA「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

MAJOR ARTICLE

Persistence of *Leishmania* Parasites in Scars after Clinical Cure of American Cutaneous Leishmaniasis: Is There a Sterile Cure?

Mitzi G. Mendonça,[1a] Maria E. F de Brito,[2] Eduardo H. G. Rodrigues,[2] Valdir Bandeira,[1] Márcio L. Jardim,[1] and Frederico G. C. Abath[2]

[1]Departamento de Dermatologia, Universidade Federal de Pernambuco, and [2]Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz, Recife, Brazil

Background. It is uncertain whether *Leishmania* parasites ever disappear after clinical cure of American cutaneous leishmaniasis (ACL). Recently, sensitive molecular techniques have allowed the identification of *Leishmania* parasites directly in specimens from patients' scars.

Methods. Scars of 32 patients from northeastern Brazil who were treated and clinically cured of ACL were analyzed by use of polymerase chain reaction (PCR), culture, and histopathologic examination.

Results. DNA specific for *Leishmania (Viannia)* was detected in scars of 30 (93.7%) of 32 patients. In specimens from 3 of the scars, *Leishmania* parasites could be isolated by culture; PCR results also were positive for those 3 specimens. No parasites were found by histopathologic examination, and fibrotic alterations were present in all cases, with slight inflammatory foci observed in 4 of the cases studied.

Conclusions. The results suggest that clinical cure of ACL is rarely associated with sterile cure. The implications of persistence of parasites for the clinical evolution, relapse, and transmission of leishmaniasis deserves further studies, particularly with the increasing incidence of coinfection with leishmaniasis and acquired immunodeficiency syndrome.

American cutaneous leishmaniasis (ACL) is an anthropozoonotic disease caused by protozoans of the genus *Leishmania* that infect the vertebrate host after a bite by infected phlebotomus insects of the genus *Lutzomya*. Human infections may be inapparent or display a wide clinical spectrum, ranging from localized, sometimes self-healing cutaneous lesions to severe mutilating mucocutaneous lesions to diffuse cutaneous leishmaniasis. In Brazil, ACL is widely distributed from south of the Amazon basin to the southeast [1]; most cases are caused by *Leishmania (Viannia) braziliensis* [2]. Leishmaniasis caused by *L. (V.) braziliensis* is distinguished from other forms of leishmaniasis by its chronicity, latency, and tendency to metastasize, resulting in recurrent lesions with the potential for mucosal involvement [3].

A hallmark of infections with certain viruses (e.g., herpesviruses), intracellular bacteria (e.g., *Mycobacteria, Coxiella,* and *Chlamydia* species), or protozoa (e.g., *Trypanosoma cruzi)* is the long-term persistence of the pathogen after clinical cure of the disease. Various immunologic mechanisms have been proposed as viral or microbial survival strategies that promote persistence, such as modulation of host cell antimicrobial activities, synthesis of inhibitory cytokines, impairment of T cell activation, or retreat of the pathogen into cells that do not elicit an immune response [4–7]. In experimental cutaneous leishmaniasis, live parasites have been demonstrated in various strains of mice after clinical cure by chemotherapy [8, 9].

Sensitive molecular techniques have allowed the identification of *Leishmania* parasites directly in clinical specimens obtained from patients. In human ACL, the persistence of *Leishmania* parasites after treatment and clinical cure has been demonstrated by the detection

Received 12 June 2003; accepted 5 September 2003; electronically published 2 March 2004.

Presented in part: XXXVII Congresso da Sociedade Brasileira de Medicina Tropical, Salvador, Brazil, 11–15 March 2001.

Financial support: Fundação Oswaldo Cruz (internal funds); Conselho Nacional de Desenvolvimento Científico e Tecnológico.

[a] M.G.M. was an M.Sc. fellow at the Departamento de Medicina Tropical, Universidade Federal de Pernambuco, at the time that this work was completed.

Reprints or correspondence: Dr. Frederico G. C. Abath, Dept. de Imunologia, Centro de Pesquisas Aggeu Magalhães, Av. Prof. Moraes Rego s/n, Cidade Universitária, 50670-420 Recife, Brazil (fabath@cpqam.fiocruz.br).

The Journal of Infectious Diseases 2004; 189:1018–23

0022-1899/2004/18906-0009$15.00

of DNA in peripheral blood [10] and scars of patients [11]. However, to our knowledge, isolation of viable parasites has been restricted to 2 case reports [12]. Although the focus of the present study is on the persistence of *L. (V.) braziliensis*, this persistence has also been described for other *Leishmania* species [13, 14]. In the present study, we extended these observations to show that DNA specific for *Leishmania (Viannia)* could be detected in specimens from the scars of 30 of 32 patients clinically cured of ACL, and, in some of these specimens, evidence for the viability and infectivity of the parasite is provided, strongly suggesting that persistence of parasites is the rule, rather than the exception, in leishmaniasis.

SUBJECTS, MATERIALS, AND METHODS

Study area and patients. A total of 63 cutaneous biopsy specimens were obtained from 2 groups of subjects: 32 patients clinically cured of ACL living in the Amaraji Municipality and neighboring regions (Pernambuco State, Brazil), a region where *L. (V.) braziliensis* is endemic, and 31 patients with cutaneous lesions caused by nonleishmanial diseases, including tuberculosis, sporotricosis, epidermoid carcinoma, leprosy, tropical ulcer, zygomycosis, epidermal cyst, amyloidosis, and several types of nevi (control group). Patients in the first group were clinically cured of ACL after receiving meglumine antimonate chemotherapy (10 mg/kg/day intramuscularly [im] for 20 days, repeated if necessary). The project was approved by the ethics committee of Centro de Pesquisas Aggeu Magalhães–Fundação Oswaldo Cruz, and all the enrolled subjects provided written, informed consent. The criteria for inclusion in the group of patients clinically cured of ACL were as follows: (1) a previous diagnosis of ACL based on clinical and epidemiological evidence (i.e., the presence of typical lesions, compatible epidemiological history, and clinical response to specific treatment) and direct detection of the parasite (microscopic smear examination, histopathologic examination, or isolation by culture); (2) healing of lesions with the presence of scar for at least 6 months; and (3) the absence of lesions suggestive of active disease or relapse. Patients who did not provide written consent, those who presented with concomitant debilitating diseases, and those <15 years old were excluded from the present study.

Patients' samples. Four-to-6-mm skin-punch biopsy specimens were obtained from the scar site, under sterile conditions and local anesthesia (3% prilocaine cloridrate). Specimens were divided into 3 samples. The first sample was processed by use of polymerase chain reaction (PCR), the second sample was used for histopathologic examination of tissue sections, and the third sample was used for in vitro and in vivo culture. Patients' samples were obtained during 1995–2000 in the field or at the outpatient facility of a reference hospital (Hospital das Clínicas, Universidade Federal de Pernambuco, Recife).

Standard diagnostic procedures. The tissue sample for histopathologic examination was fixed in 10% buffered formalin, and 5-μm sections were stained with hematoxylin-eosin. In vitro culture was performed by inoculating tissue fragments in tubes containing culture medium (4% Bacto-blood agar base [Difco Beckton Dickinson] and 10% rabbit blood) incubated at 26°C. After preliminary microscopic observation for 5 days, the samples were transferred to fresh medium and observed carefully for at least 8 weeks before being designated as negative by culture. For in vivo isolation, biopsy material was triturated and then inoculated into hamsters either intraperitoneally or intradermally in the hindfeet. Three months later, the hamsters were killed, and spleen and skin fragments were inoculated into culture medium. Indirect immunofluorescence (IIF) was performed by use of promastigotes of *L. (V.) braziliensis*, exactly as described elsewhere [27]. The presence of body and flagellum fluorescence at a 1:40 serum dilution was considered to be a positive reaction.

PCR. DNA was purified for PCR by use of the GenomicPrep Cells and Tissue DNA Isolation Kit (Amersham Pharmacia Biotech), according to the instructions of the manufacturer. For each DNA isolation, frozen tissue samples of ~20 mg were used. After purification, the DNA was suspended in 100 μL of 10 mmol/L Tris plus 1 mmol/L EDTA [pH 8.0] and stored at −20°C until use. Two microliters of the DNA solution was added to the PCR mixture. A PCR-based system specific for *Leishmania (Viannia)* was used [15]. A 25-μL PCR mixture was prepared containing 10 mmol/L Tris-HCl, 50 mmol/L KCl, 0.1 mg/mL gelatin, 1.5 mmol/L MgCl, 0.2 mmol/L each dNTP, 25 pmol of each appropriate primer, and 2.5 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech). Amplification was performed on a Perkin Elmer model 4800 thermocycler. PCR programs were run for 35 cycles, each consisting of annealing for 1 min at 65°C, extension for 1 min at 72°C, and denaturation for 1 min at 94°C. The tubes were heated for 4 min at 94°C before cycling. Several negative controls (no DNA added) and positive controls (containing 100 pg and 10 pg of *L. (V.) braziliensis* genomic DNA) were included each time PCR was performed, to detect false results caused by contamination or variation in sensitivity. Products (10 μL) were separated by electrophoresis in agarose gels, and ethidium bromide–stained gels were visualized and photographed over a UV light by use of the Polaroid MP4+ System [16]. The *Leishmania (Viannia)*–specific PCR amplifies a 750-bp band and is able to detect 10 fg of promastigote genomic DNA [17]. This amplicon is unique to this subgenus and represents a single linearized minicircle.

Characterization of isolates by enzyme electrophoresis and monoclonal antibodies (MAbs). Analysis of enzymic loci were performed as described elsewhere [18], and a battery of

23 MAbs were used to characterize the isolates, as described elsewhere [19].

RESULTS

Characteristics of the clinically cured patients. Table 1 shows detailed characteristics of each patient. The patients cured of ACL were 15–62 years old (mean, 35 years). Most of them (65.6%) were male and engaged in agricultural activity (75%). The scar age ranged from 6 months to 11 years (mean, 3 years). Eight (25%), 21 (65.6%), and 3 (9.4%) of the 32 patients had scars with ages of 6–17 months, 18–66 months, and >66 months, respectively. All the patients had scars suggestive of previous cutaneous leishmaniasis. Most of the scars were atrophic lesions, round or oval in shape, with smooth, shiny surfaces (figure 1). In general, scars were hypo- or hyperpigmented, hairless, and depressed. Figure 1 is representative of the gross shape presented by the scars.

Histopathologic examination of the scars. *Leishmania* parasites were not found in any of the scars examined by use of stained tissue sections. Fibrotic processes compatible with healing were seen in all cases. However, residual inflammatory foci were observed in only 4 samples. Such inflammatory reactions were considered to be slight and were composed predominantly of mononuclear cells (figure 2). No association was found between age of scars and characteristics of the inflammatory reaction.

Isolation and characterization of Leishmania ***parasites from scars.*** *Leishmania* parasites were isolated from 3 of 32 specimens from scars of patients clinically cured of ACL (range of scar age, 1–6 years). In these 3 cases, PCR results were positive. The isolates were characterized by MAbs and isoenzymes, confirming the diagnosis of *L. (V.) braziliensis* (serodeme 1). The isolates were inoculated in golden-hamsters, resulting in manifestations indicative of leishmaniasis.

Detection of Leishmania **(Viannia)*-specific DNA in the scars.*** DNA specific for the subgenus *Leishmania (Viannia)* was detected in specimens from scars of 30 (93.7%) of 32 patients clinically cured of ACL. For 1 of the patients, biopsy was performed twice, first in 1997 and again in 1999; PCR results were positive on both occasions. PCR results were negative for the 31 patients with nonleishmanial cutaneous lesions (100% specificity). Figure 2 is representative of the results obtained. For the 2 patients for whom PCR results were negative, their scars were aged 1 and 3 years, respectively. The presence of a 750-bp band indicates amplification of the target DNA.

Table 1. Demographic characteristics of the 32 patients clinically cured of American cutaneous leishmaniasis examined in the present study.

Patient	Sex	Age, years	Scar age, months	Presence of inflammatory foci	PCR result	Isolation by culture[a]	IIF
1	M	20	12	No	Pos	Neg	Neg
2	M	49	36	No	Pos	Neg	Pos
3	M	15	14	No	Pos	Neg	Pos
4	M	57	36	No	Pos	Neg	Pos
5	F	30	60	No	Pos	Neg	Neg
6	F	24	132	No	Pos	Neg	Neg
7	F	22	36	No	Pos	Neg	Neg
8	F	23	36	No	Pos	Neg	Neg
9	M	28	36	No	Pos	Neg	Neg
10	F	29	60	No	Pos	Pos	Neg
11	M	29	60	Yes	Pos	Neg	Neg
12	M	60	60	No	Pos	Neg	Neg
13	F	45	66	No	Pos	Neg	Neg
14	M	19	15	No	Pos	Neg	Neg
15	M	30	17	No	Pos	Neg	Neg
16	M	17	24	Yes	Pos	Neg	Neg
17	M	18	15	No	Pos	Pos	Neg
18	M	47	24	No	Pos	Neg	Neg
19	M	62	72	No	Pos	Pos	Neg
20	F	37	36	Yes	Pos	Neg	Neg
21	M	27	24	No	Pos	Neg	Neg
22	M	62	29	No	Pos	Neg	Neg
23	M	23	36	No	Pos	Neg	Neg
24	M	59	29	Yes	Pos	Neg	Neg
25	M	25	60	No	Pos	Neg	Pos
26	F	15	12	No	Pos	Neg	Neg
27	F	52	18	No	Pos	Neg	Neg
28	F	43	24	No	Neg	Neg	Neg
29	F	20	36	No	Pos	Neg	Pos
30	M	58	12	No	Neg	Neg	Neg
31	M	16	18	No	Pos	Neg	Pos
32	M	57	6	No	Pos	Neg	Pos

NOTE. IIF, indirect immunofluorescence; neg, negative; PCR, polymerase chain reaction; pos, positive.

[a] Isolation by culture refers to both in vitro and in vivo culture.

DISCUSSION

Several authors have previously raised the question of whether *Leishmania* parasites ever disappear after clinical cure of ACL [3, 9, 20]. However, because of the sensitivity limitations of culture in detecting viable parasites, the evidence for persistence of parasites after clinical cure of ACL in humans has been restricted, to our knowledge, to 2 case reports [12]; in 1 of those cases, the attempt to maintain the culture by inoculation into a hamster was unsuccessful, precluding the characterization of the parasite and raising doubts about infectivity. In areas where leishmaniasis is endemic, exogenous reinfection may explain recurrent lesions. Nonetheless, reactivation is the most

likely mechanism of recurrent lesion involving genotypically and phenotypically identical, sequentially isolated strains [3]. Infection by *L. (V.) braziliensis* is characterized by chronicity, latency, and a tendency to metastasize in mutilating lesions of the nasopharyngeal mucosa. Mucosal disease has generally been described in association with healed cutaneous lesions caused by *L. (V.) braziliensis* and has been attributed to the spread of persistent infection via the blood or lymphatic dissemination [3, 21]. Recrudescence of the disease in immunocompromised hosts has been demonstrated repeatedly and results in mucocutaneous or visceral disease, particularly in patients with HIV infection, which corroborates the occurrence of persistent inapparent infection by various *Leishmania* species, including those usually associated with cutaneous leishmaniasis [14, 22–26]. This phenomenon is particularly important in view of the increasing coexistence of AIDS and leishmaniasis, which is the result of the ruralization of the former and the urbanization of the latter. Thus, indirect clinical evidence for the persistence of *Leishmania* parasites in human ACL has been provided by the occurrence of spontaneous reactivation [20], reactivation in immunosuppressed patients [24], and persistence of significant immune responses long after cure [21]. In this regard, we had shown previously that serum samples obtained from patients clinically cured of ACL recognize certain leishmanial antigens, suggesting that parasites persisted in those patients [27]. In the present study, 7 of 32 clinically cured patients were positive for *Leishmania* parasites by IIF.

The use of highly sensitive diagnostic approaches based on the detection of DNA suggests that the persistence of parasites is much more frequent than previously realized [11]. Indeed, DNA detection techniques are both sensitive and specific for diagnosis in situations in which the organisms are scarce, such as in lesions of ACL and, in particular, in scars after clinical cure. Because several studies implicate *L. (V.) braziliensis* as the only causative agent of ACL in Pernambuco, Brazil [17], we used primers specific for the subgenus *Leishmania (Viannia)* in PCR, to detect parasite DNA in scars of clinically cured patients. The patients were from the Pernambuco State in northeastern Brazil, where studies of the characterization of circulating strains of the parasite and of the long-term clinical evolution of ACL are relatively scarce.

It has been shown that mice experimentally infected with *L. major* that recover from infection still harbor infective parasites many months later [8]. The lymph nodes draining the initial site of infection are, by far, the most consistent source of persistent parasites. The parasite can also be found in blood, as judged by the fact that *Leishmania (Viannia)* DNA was detected by use of PCR in blood samples obtained from patients who were clinically cured of ACL after treatment [21]. In 1 case report, DNA of *L. (V.) braziliensis* was found in the blood of a woman who had been cured of ACL 30 years previously and who had lived in areas where leishmaniasis is not endemic since then [28]. More recently, in a study of patients from endemic foci in Rio de Janeiro (southeastern Brazil) treated with pentavalent antimony either intralesionally or im, at a dose of 5 or 20 mg/kg/day for 30 days, analysis of paraffin-embedded

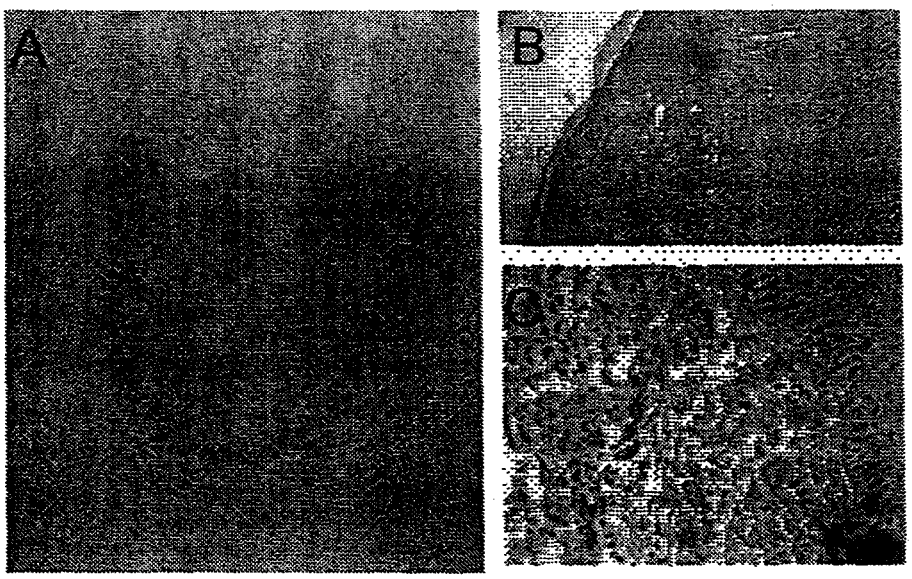

Figure 1. Clinical aspect and histopathologic features of scars of patients cured of American cutaneous leishmaniasis. *A*, Round scar with marginal hyperpigmentation, central hypopigmentation, and slightly rough surface; *B*, irregular interpapillary spaces, with slight collagen deposition (magnification, ×50); *C*, higher magnification (×100) of the histologic section in panel *B*, showing dilated blood vessels and a few foci of mononuclear cells in the derma. Sections were stained with hematoxylin-eosin.

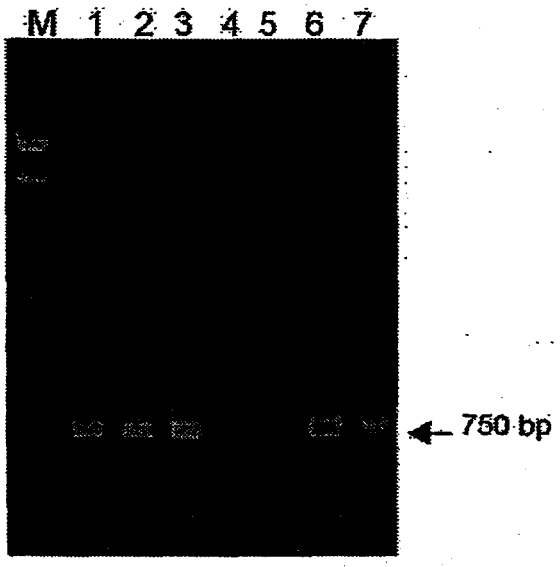

Figure 2. Representative agarose gel of polymerase chain reaction products obtained from biopsy specimens, with primers specific for the subgenus *Leishmania (Viannia)*. *Lanes 1–3*, scars from patients clinically cured of American cutaneous leishmaniasis; *lane 4*, scars from patients clinically cured of other diseases of nonleishmanial etiologies; *lane 5*, negative control; and *lanes 6* and *7*, positive controls containing 100 and 10 pg of parasite genomic DNA, respectively. M, molecular weight markers (λ DNA digested with *HindIII*; 23.1, 9.4, 6.6, 4.4, 2.3, and 0.56 kb). *Arrow*, 750-bp diagnostic band.

tissue by PCR followed by hybridization detected *Leishmania (Viannia)* DNA in scars of 16 (80%) of 20 patients, with the period after clinical cure of ACL ranging from 21 days to 8 years [11]. We extended the results of that study by analyzing frozen biopsy specimens from a larger sample of patients; we detected *Leishmania (Viannia)* DNA in a very high percentage of scars (93.7%; 30/32 patients) aged 6 months to 11 years. One of the factors explaining the higher rate of positivity for detection of DNA in the present investigation could be the fact that we worked with recently frozen biopsy specimens. Moreover, *L. (V.) braziliensis* was isolated by culture from 3 patients cured of ACL (also positive by PCR) and subsequently was inoculated in golden hamsters, resulting in manifestations compatible with leishmaniasis. Thus, we have formally demonstrated, in 3 cases, persistence, viability, and infectivity of the parasite, even 6 years after clinical cure. Of note, in vitro culture of parasites from punch biopsy or aspirate specimens of active lesions has had variable success rates [29, 30] and could be expected to be even less sensitive in detecting the parasite from scars, where the parasites are much scarcer. There was no association between time after healing and the results of PCR and culture.

Although it is possible that small amounts of DNA remain in scar tissue, there are several arguments against the persistence of this biological macromolecule in an metabolically active milieu. Even in situations in which relatively large amounts of DNA are injected, such as in DNA vaccine experiments, the vast majority of nucleic acids are degraded within the first weeks after injection [31]. In addition, investigations to achieve radical cure of experimental cutaneous leishmaniasis showed that, at week 13 after chemotherapy with pamidronate, results of both PCR and culture detection of parasite DNA were negative [32]. Thus, the detection of specific *Leishmania (Viannia)* DNA by PCR in scars many months after clinical cure of disease strongly suggests the persistence of the parasite in these sites.

Fibrotic processes compatible with healing were found in all scars, although residual inflammatory foci were observed in some samples, not necessarily those from which parasites were isolated by culture. This result is in agreement with a systematic histopathologic study of human ACL before and after treatment in which the authors concluded that clinical cure did not always coincide with histopathologic cure [33].

The demonstration of persistence of parasites after clinical cure of ACL raises several issues with regard to clinical evolution, epidemiology, and control of leishmaniasis. It is relevant to investigate whether immunocompromised, clinically cured patients can act as reservoirs of leishmaniasis in areas of active transmission. In the mouse model of leishmaniasis, the persistence of even a low number of parasites in the skin after healing has been shown to maintain the host as a long-term reservoir of infection for vector sand flies [4]. Although rodent models for cutaneous leishmaniasis may not correspond to the clinical situation among humans, these data suggest that attempts should be made to achieve complete elimination of the parasite. In this direction, there have been some studies of mice suggesting that bisphosphonate pamidronate could be a useful lead compound for the synthesis of new drugs against the disease [32]. In addition, mice treated during latency with anti–interleukin-10 receptor antibodies achieved sterile immunity in the skin and draining lymph nodes and were no longer at risk of reactivation of disease, suggesting new therapeutic approaches for leishmaniasis [4]. Nevertheless, one could speculate that persistence of the parasite would be beneficial for patients living in areas where leishmaniasis is endemic, by maintaining a T cell memory. If this is true, complete elimination of the parasite would make the patient more vulnerable to reinfection. It is possible that mechanisms dependent on both the immune response of the host and on genotypic and phenotypic features of the parasite play a role in the persistence of parasites. Persistent parasites in an immune host would presumably have to escape the host's immune response by clonal selection or molecular changes resulting from mutational or recombinational processes. In at least 1 study using the murine model, persistent *L. major* isolates retained the characteristics

of the parental clone [8]. All these issues are of importance in strategies for control of leishmaniasis and require further study.

Acknowledgments

We thank Elisa Cupolillo and Edna Ishikawa, for typing our isolates by enzyme electrophoresis and monoclonal antibodies, respectively; and David Freedman and Siddhartha Mahanty, for valuable suggestions and comments.

References

1. World Health Organization. Special Programme for Research and Training in Tropical Diseases. Leishmaniasis section. Available at: http://www.who.int/tdr/index.html. Accessed 10 June 2003.
2. Grimaldi G, David JR, McMahon-Pratt D. Identification and distribution of New World *Leishmania* species characterized by serodem analysis using monoclonal antibodies. Am J Trop Med Hyg 1987; 36:270–87.
3. Saravia NG, Weigle K, Segura I, et al. Recurrent lesion in human *Leishmania braziliensis* infection—reactivation or reinfection? Lancet 1990; 336:398–402.
4. Belkaid Y, Hoffmann KF, Mendez S, et al. The role of interleukin IL-10 in the persistence of *Leishmania major* in the skin after healing and the therapeutic potential of anti–IL-10 receptor antibody for sterile cure. J Exp Med 2001; 194:1497–506.
5. Bogdan C, Donhauser N, Doring R, Rollighoff M, Diefenbach A, Rittig MG. Fibroblast as host cells in latent leishmaniasis. J Exp Med 2000; 191:2121–9.
6. Murray PJ. Defining the requirements for immunological control of mycobacterial infections. Trends Microbiol 1999; 7:366–72.
7. Bogdan C, Rollinghoff M. The immune response to *Leishmania*: mechanisms of parasite control and evasion. Int J Parasitol 1998; 28:121–34.
8. Aebischer T, Moody SF, Handman E. Persistence of virulent *Leishmania major* in murine cutaneous leishmaniasis: a possible hazard for the host. Infect Immun 1993; 61:220–6.
9. Rossel RA, Duran RJ, Rossel O, Rodriguez AM. Is leishmaniasis ever cured? Trans R Soc Trop Med Hyg 1992; 86:251–3.
10. Delgado O, Guevara P, Silva S, Belfort E, Ramirez JL. Follow-up of a human accidental infection by *Leishmania (Viannia) braziliensis* using conventional immunologic techniques and polymerase chain reaction. Am J Trop Med Hyg 1996; 55:267–72.
11. Schubach A, Haddad F, Oliveira-Neto MP, et al. Detection of *Leishmania* DNA by polymerase chain reaction in scars of treated human patients. J Infect Dis 1998; 178:911–4.
12. Schubach A, Marzochi MC, Cuzzi-Maya T, et al. Cutaneous scars in American tegumentary leishmaniasis patients: a site of *Leishmania (Viannia) braziliensis* persistence and viability eleven years after antimonial therapy and clinical cure. Am J Trop Med Hyg 1998; 58:824–7.
13. le Fichoux Y, Quaranta JF, Aufeuvre JP, et al. Occurrence of *Leishmania infantum* parasitemia in asymptomatic blood donors living in an area of endemicity in southern France. J Clin Microbiol 1999; 37:1953–7.
14. Morales MA, Cruz I, Rubio JM, et al. Relapses versus reinfections in patients coinfected with *Leishmania infantum* and human immunodeficiency virus type 1. J Infect Dis 2002; 185:1533–7.
15. de Bruijn MHL, Barker DC. Diagnosis of New World leishmaniasis: specific detection of species of the *Leishmania braziliensis* complex by amplification of kinetoplast DNA. Acta Trop 1992; 52:45–58.
16. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
17. Rodrigues EHG, Brito MEF, Mendonça MG, et al. Evaluation of PCR for diagnosis of American cutaneous leishmaniasis in an endemic area of Northeastern Brazil. J Clin Microbiol 2002; 40:3572–6.
18. Cupolillo E, Grimaldi J, Momem H. A general classification of New World *Leishmania* using numerical zymotaxonomy. Am J Trop Med Hyg 1994; 50:296–311.
19. Silveira FT, Ishikawa IAY, De Souza AAA, Lainson R. An outbreak of cutaneous leishmaniasis among soldiers in Belém, Pará State, Brazil, caused by *Leishmania (Viannia) lindebergi* n. sp.: a new leishmanial parasite of man in the Amazon region. Parasite 2002; 9:43–50.
20. Oliveira-Neto MP, Mattos M, Souza CSF, Fernandes O, Pirmez C. Leishmaniasis recidiva cutis in New World cutaneous leishmaniasis. Int J Dermatol 1998; 37:846–9.
21. Coutinho SG, Pirmez C, Da-Cruz AM. Parasitological and immunologica follow-up of American tegumentary leishmaniasis patients. Trans R Soc Trop Med Hyg 2002; 96(Suppl 1):S173–8.
22. Silva ES, Pacheco RS, Gontijo CMF, Carvalho IR, Brazil RP. Visceral leishmaniasis caused by *Leishmania (Viannia) braziliensis* in a patient infected with human immunodeficiency virus. Rev Inst Med Trop Sao Paulo 2002; 44:145–9.
23. Ramos-Santos C, Hernandez-Montes O, Sanchez-Tejeda G, Monroy-Ostria A. Visceral leishmaniosis caused by *Leishmania (L.) mexicana* in a Mexican patient with human immunodeficiency virus infection. Mem Inst Oswaldo Cruz 2000; 95:729–33.
24. Sampaio RNR, Salaro CP, Resende P, de Paula CDR. Leishmaniose tegumentar americana associada a AIDS: relato de quatro casos. Rev Soc Bras Med Trop 2002; 35:651–4.
25. Czechowicz RT, Millard TP, Smith HR, Ashton RE, Lucas SB, Hay RJ. Reactivation of cutaneous leishmaniasis after surgery. Br J Dermatol 1999; 141:1113–6.
26. Ferreira MS, Borges AS. Some aspects of protozoan infections in immunocompromised patients—a review. Mem Inst Oswaldo Cruz 2002; 97:443–57.
27. Brito MEF, Mendonça MG, Gomes YM, Jardim ML, Abath FGC. Dynamics of the antibody response in patients with therapeutic or spontaneous cure of American cutaneous leishmaniasis. Trans R Soc Trop Med Hyg 2001; 95:203–6.
28. Guevara P, Ramirez JL, Rojas E, Scorza JV, Gonzalez N, Anez N. *Leishmania braziliensis* in blood 30 years after cure [letter]. Lancet 1993; 341:1341.
29. Aviles H, Belli A, Armijos R, Monroy FP, Harris E. PCR detection and identification of *Leishmania* parasites in clinical specimens in Ecuador: a comparison with classical diagnostic methods. J Parasitol 1999; 85: 181–7.
30. Weigle KA, de Davalos M, Heredia P, Molineros R, Saravia NG, D'Alessandro A. Diagnosis of cutaneous and mucocutaneous leishmaniasis in Colombia: a comparison of seven methods. Am J Trop Med Hyg 1987; 36:489–96.
31. Nichols WW, Ledwith BJ, Manam SW, Troilo PJ. Potential DNA vaccine integration into host cell genome. Ann NY Acad Sci 1995; 772:30–9.
32. Rodriguez N, Bailey BN, Martin MM, Oldfield E, Urbina JA, Docampo R. Radical cure of experimental cutaneous leishmaniasis by the bisphosphonate pamidronate. J Infect Dis 2002; 186:138–40.
33. Botelho ACC, Tafuri WL, Genaro O, Mayrink W. Histopathology of human American cutaneous leishmaniasis before and after treatment. Rev Soc Bras Med Trop 1998; 31:11–8.

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	厚生労働省処理欄
一般的名称	血液		研究報告の公表状況	Transfusion 2002;42:886-891.	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
米国赤十字血液センター及びCDCにより行われたレトロスペクティブな調査結果の報告。 サル泡沫状ウイルス(SFV)感染が職業上ヒト以外の霊長類と接触した作業者で確認され、感染日以降に供血を行っていた。輸血を介したSFVのヒト・ヒト感染とその病原性は現在まで調査されていない。この供血者からの複数の受血者が特定され、これら受血者の血液検体でSFVについてウエスタンブロット法およびPCR法で検査を行った。 赤血球輸血を受けた1名と新鮮凍結血漿輸血を受けた別の1名は、レトロウイルス感染と関係なく死亡していた。血小板輸血を受けた1名は検査できなかった。供血後3～35日後に赤血球輸血を受けた2名と白血球除去赤血球輸血を受けた1名、血小板輸血を受けた1名の計4名は、受血後19カ月から7 年後の検査でSFV陰性であった。 2回行われた供血血漿から製造された25%アルブミン製剤1ロット、加熱人血漿蛋白製剤3ロットの検査ではウエスタンブロット法もRT-PCR法でも陰性であった。 輸血を介したSFV感染伝播は、SFV感染供血者からの受血者4名では確認されなかった。感染した供血者からの血漿成分を含む血漿分画製剤はSFV陰性であったが、感染か確認された人は血液等を提供しないように助言する。	サル泡沫状ウイルス(SFV)による感染はヒト以外の霊長類で広くみられる。 本文献は、2004年3月に入手した情報(ProMed 情報、記事番号 20040323-0080)の元文献である。

報告企業の意見	今後の対応
SFV 感染者由来の血球製剤による感染の伝播はみられず、また、当該血漿を含む原料から製造されたアルブミン製剤を PCR 法等により検査した結果も陰性であったことから、現時点で対応の必要はないと考える。	今後もSFVの感染伝播等に関する情報に注意していく。

Simian foamy virus infection in a blood donor

R.S. Boneva, A.J. Grindon, S.L. Orton, W.M. Switzer, Vedapuri Shanmugam, A.I. Hussain, V.B. Bhullar, M.E. Chamberland, Walid Heneine, T.M. Folks, and L.E. Chapman

BACKGROUND: Infections with simian foamy virus (SFV) are widely prevalent in nonhuman primates. SFV infection was confirmed in a worker, occupationally exposed to nonhuman primates, who donated blood after the retrospectively documented date of infection. Human-to-human transmission of SFV through transfusion and its pathogenicity have not been studied.

STUDY DESIGN AND METHODS: Recipients of blood from this donor were identified and blood samples from such recipients were tested for SFV infection by Western blot and PCR assay.

RESULTS: One recipient of RBCs and another recipient of FFP had died; retroviral infections were not implicated. One platelet recipient could not be tested. Recipients of RBCs (two), a WBC-reduced RBC unit (one), and a platelet unit (one) tested SFV-negative 19 months to 7 years after transfusion. Tested recipients had transfusions 3 to 35 days after blood donation. Samples of one lot of albumin and three lots of plasma protein fraction (manufactured from recovered plasma from two donations) tested negative both for antibodies and for viral RNA.

CONCLUSION: SFV transmission through transfusion was not identified among four recipients of cellular blood components from one SFV-infected donor. Derivatives containing plasma from that donor tested negative for SFV.

Seropositivity to simian foamy virus (SFV) has been identified in at least 17 humans exposed to nonhuman primates.[1-5] Fifteen of these SFV-seropositive persons were identified through CDC surveillance of workers occupationally exposed to nonhuman primates, their biological materials, or simian viruses in the laboratory.[3-5] Of those 15, 10 provided additional samples for testing. Persistent seropositivity by Western blot, identification of two conserved SFV proviral DNA sequences in PBMNCs by PCR, and sequence analysis provide evidence for persistent SFV infection in all.[5] All 10 infected workers are men and report good health after 2 to 24 years of documented SFV infection; six wives were tested and are SFV negative.[3,6] Four of the workers had donated blood after the retrospectively determined date of seroconversion.[5] This raised concerns about the safety of blood transfusions from SFV-seropositive donors.

Foamy viruses (FVs), including SFV, belong to the *Spumavirus* genus in the family Retroviridae. FVs cause cytopathic effects with syncytium formation in vitro but appear to be nonpathogenic in vivo. They establish persistent infections in many animal species including nonhuman primates, cats, and cows. SFVs infect 70 to 90 percent of captive nonhuman primates,[1,7-9] in which they appear to cause latent infection in almost all organs while minimal viral replication occurs in the oral mucosa.[10]

ABBREVIATIONS: ARCBS = American Red Cross Blood Services; FV(s) = foamy virus(es); HFV = human foamy virus; SFV(s) = simian foamy virus(es); SFV_{AFG} = SFV from African green monkeys; SFV_{CPZ} = SFV from chimpanzees.

From the Division of AIDS, STD, and TB Laboratory Research, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA; American Red Cross Blood Services, Southern Region, Atlanta, GA; and the American Red Cross, Holland Laboratory, Rockville, MD.

Address reprint requests to: Roumiana S. Boneva, MD, PhD, DASTLR/NCID, Mail stop G-19, CDC, 1600 Clifton Road NE, Atlanta, GA 30333; e-mail: rboneva@cdc.gov.

Received for publication December 4, 2001; revision received March 8, 2002, and accepted March 12, 2002.

TRANSFUSION 2002;42:886-891.

After a FV, referred to as human foamy virus (HFV), was isolated from a nasopharyngeal carcinoma specimen from an African man,[11] early studies raised questions about possible associations between HFV infection and a variety of human diseases (e.g., thyroiditis, amyotrophic lateral sclerosis).[12-16] However, later investigations using more precise diagnostic methods have been unable to confirm either endemic human infection or disease associated with FVs.[1,17-22] Further, HFV is closely related phylogenetically to SFV from chimpanzees (SFV_{CPZ}), suggesting a possible zoonotic infection.[23] Currently SFVs are not considered pathogenic,[24,25] and, because they infect a broad range of human cells, including human cell lines,[8] they are viewed by some investigators as potential candidates for gene transfer therapy.[26,27]

This report presents the results of a retrospective study jointly conducted by the American Red Cross Blood Services (ARCBS) and CDC. The purpose of this study was to determine if SFV was transmitted to recipients of blood components from one SFV-infected person whose donations were traceable.

MATERIALS AND METHODS

Donor

The donor has worked with nonhuman primates for 21 years, during which time he was exposed to body fluids from nonhuman primates and bitten by a chimpanzee. Serum, plasma, and PBMNCs from the donor were tested using methods described below and previously.[3,5,28] Seropositivity by Western blot was confirmed on four serial samples collected between 1998 and 2000 (Fig. 1). SFV DNA was detected by PCR in the donor's PBMNCs in all four samples (two of which are shown in Fig. 2A), and SFV was successfully cultured from PBMNCs on two of three serial attempts,[29] indicating persistent infection. Phylogenetic analysis of the cultured virus indicated a chimpanzee-like strain.[5] Testing of archived serum documented seropositivity at least since 1981. RT-PCR on donor plasma from 1998 and 2000 was negative (Fig. 2B). He donated blood six times between 1992 and 1997, during the retrospectively determined period of seropositivity.

Recipient identification and enrollment

Written informed consent was obtained from all participants. The study protocol was approved by the institutional Review Boards of the CDC and the ARCBS. The ARCBS searched blood center disposition records for components from all donations from the SFV-infected donor. It then identified and notified all hospitals to which these blood components were consigned. These hospitals identified the recipients of blood components (e.g., RBCs, WBC-reduced [filtered] RBCs, platelets, and

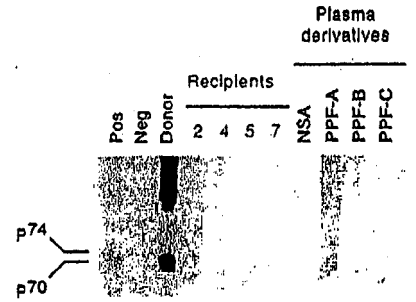

Fig. 1. Western blot analysis for SFV antibodies. Lane 1, positive control serum (Pos) from an SFV-infected chimpanzee; Lane 2, negative control serum (Neg); Lane 3, plasma from SFV-infected donor (Donor); Lanes 4 through 7, plasma from four recipients of blood components (Recipients 2, 4, 5, and 7, respectively, Table 1); Lanes 8 through 11, normal serum albumin (NSA) and three lots of purified protein fraction (PPF-A, -B, and -C).

FFP) from the donor and contacted the recipients' clinicians. The recipients themselves were contacted by their physicians or, with hospital approval, by an ARCBS designee, if a current physician could not be identified. After obtaining the recipient's (or a parent's) informed consent, a blood sample was drawn and tested by methods described below. The blood samples from children were drawn at a time when they were undergoing other blood tests for routine clinical follow up.

Plasma from this donor had also been used for production of pooled plasma products. The ARCBS identified consignees of such plasma, who made nontransfused products available for testing.

Laboratory methods

Recipients' plasma samples and available donor plasma derivatives were screened for SFV antibodies by a modified Western blot assay, which uses mixed antigens from SFV from African green monkeys, SFV_{AGM}, and SFV_{CPZ}. Blots were visualized by chemiluminescence according to previously described methods.[4] Test results were considered positive if the samples were reactive to the diagnostic gag precursor proteins p70 and p74.[1,4,28] The endpoint dilution at which donor's plasma samples from 2000 tested positive by Western blot was 1 in 6400.

DNA lysates prepared from recipient's PBMNCs were tested for SFV sequences by investigational nested PCR

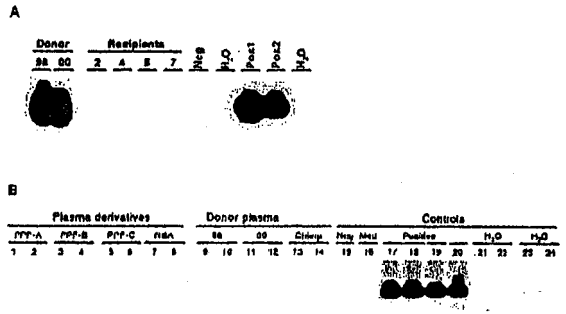

Fig. 2. Analysis for SFV integrase sequences. (A) Proviral nested PCR. Lanes 1 and 2, DNA lysates from PBMNCs collected from the donor in 1998 (98) and 2000 (00); Lanes 3 through 6, DNA lysates from PBMNCs of Recipients 2, 4, 5, and 7 (Table 1); Lanes 7 and 8, negative DNA (Neg) and water (H_2O) controls, respectively; Lanes 9 and 10, positive DNA controls from SFV_{AGM}-infected canine thymocyte cells, representing DNA equivalents of 15 (Pos1) and 1.5 (Pos2) cells diluted in a background of DNA from 150,000 human PBMNCs; Lane 11, negative water (H_2O) control for the nested PCR reactions. (B) Nested RT-PCR. Lanes 1 through 8, duplicate results (two lanes/product) for donor plasma derivatives: purified protein fraction A (PPF-A), purified protein fraction B (PPF-B), purified protein fraction C (PPF-C), and normal serum albumin (NSA); Lanes 9 through 12, duplicate results for donor plasma from 1998 (98) and 2000 (00); Lanes 13 and 14, duplicate results for an SFV-infected chimpanzee (Chimp); Lanes 15 and 16, negative human plasma (Neg) and media (Med) controls; Lanes 17 through 20, SFV RNA from SFV_{AGM} tissue culture supernatant diluted with media from 10^{-3} to 10^{-6} (positive), respectively; Lanes 21 through 24, duplicate results for water-only controls (H_2O) for the reverse-transcription and PCR, respectively.

using primers for two conserved SFV proviral genes (integrase and polymerase) as previously described.[3] The proviral integrase PCR assay has previously been reported to have a molecular sensitivity of 1 to 10 SFV genomes per 10^5 cells.[1]

Available plasma derivative products and donor plasma samples were tested for SFV RNA sequences by RT-PCR. RNA was extracted from plasma samples by the use of a kit (QIAamp viral RNA mini kit, Qiagen, Valencia, CA) and was then reverse transcribed in duplicate test samples by using the first-round integrase antisense primer. PCR amplification was performed by using the first-round integrase sense primer followed by nested PCR and detection of the amplified product as described previously.[3]

RESULTS

Tracing of blood donations, blood components, and pooled plasma products

Six donations between 1992 and 1997 resulted in the production of 11 blood components: four components of RBC, two of WBC-reduced RBC, two of platelets, and three of FFP. These 11 components were distributed to 7 hospitals. Additionally, plasma recovered from two donations (December 1993 and September 1994) underwent fractionation, and subfractions were sent to two manufacturers of plasma derivatives. Some of these derivatives were marketed internationally. Fraction IV_1 and IV_4 from a donation in 1993 went to Company A for production of albumin and another plasma derivative. Intermediate paste from Fractions I, II, and III from a 1994 donation went to Company B. At the time of the investigation, most plasma derivatives were not available for testing. However, samples from one lot of 25-percent normal serum albumin and three lots of plasma protein fraction had expired unused but had not yet been discarded and were available for testing.

Blood components

We could not obtain disposition records from consignees for 2 of the 11 blood components (Table 1, Components 3 and 8). Two other components, RBC from a 1994 donation and FFP from a 1997 donation, expired and were used for production of nontransfusable reagents (Table 1, Components 7 and 11).

The recipients of seven blood components were identified. The physician of Recipient 1, who received platelets in 1992 (Table 1, Component 1), decided that testing was not in the patient's best interest and declined to contact that recipient. Two recipients had died (Table 1, Components 4 and 9). Recipient 3 died in 1997, 4 years after receiving an RBC unit component donated in 1993. The identified cause of death was chronic osteomyelitis and Crohn's disease. Recipient 6 died in 1995 on the day of transfusion of FFP.

Four recipients were tested (Table 1, Components 2, 5, 6, and 10; corresponding to Recipients 2, 4, 5, and 7, respectively; Fig. 1). Recipient 2, a 72-year-old, and Recipient 4, a 41-year-old, received RBC in association with surgical procedures in 1992 and 1994, respectively. Both Recipients 2 and 4 tested negative for seroreactivity to SFV and for SFV DNA in PBMNCs 7 years after each transfusion.

Recipient 5, a 52-day-old infant with Down Syndrome, a congenital heart defect, and hypothyroidism (Table 1, Component 6), received a platelet component in

TABLE 1. Summary of the investigation on the blood donations and transfusions from the SFV-infected donor

Component number	Blood component; donation date	Recipient number; age (yrs) at transfusion	Diagnosis/condition	Date of transfusion	Time (days) from donation to transfusion	Outcome
1	Platelets; September 1992	1; Unknown	Unknown	September 1992	5	Patient not available for testing.
2	RBCs; September 1992	2; 72	Surgery	September 1992	5	Recipient SFV-negative (by Western blot and PCR) 7 years after transfusion.
3	FFP; June 1993	Unknown	Unknown	Unknown	Unknown	Record unavailable.
4	RBCs; June 1993	3; 57	Chronic osteomyelitis; Crohn's disease	June 1993	4	Patient died 4 years after transfusion. No illness suggestive of retrovirus infection.
5	RBCs; December 1993	4; 41	Surgery for multiple injuries	January 1994	35	Recipient SFV-negative (by Western blot and PCR) 7 years 1 month after transfusion.
6	Platelets; December 1993	5; 52	Cardiac surgery	December 1993	3	Recipient SFV-negative (by Western blot and PCR) 8 years 11 months after transfusion.
7	RBCs; September 1994					RBC used for reagent manufacturing; not for transfusion.
8	RBCs; WBC-reduced June 1995	Unknown	Unknown	Unknown	Unknown	Record unavailable. WBC-reduced RBCs sent for outpatient transfusion.
9	FFP; June 1995	6; Unknown	Unknown	Unknown	Unknown	Patient died on the day of transfusion.
10	WBC-reduced RBCs; December 1997	7; 4.5	Hematologic disorder	December 1997	8	Recipient SFV-negative (by Western blot and PCR) 1 year 9 months after transfusion.
11	FFP; December 1997					Plasma used for reagent manufacturing; not for transfusion.

1993. Five years and 11 months after the transfusion, the child tested negative for SFV.

Recipient 7, a 4.5-year-old child (Table 1, Component 10), received a WBC-reduced RBC component in 1997 because of a hematologic disorder. A BMT was performed 11 months after the transfusion. Recipient 7 also tested negative for SFV 1 year and 9 months after the transfusion (10 months after the BMT). Blood samples before BMT were not available for testing.

Pooled plasma derivatives tested negative by both RT-PCR and Western blot (Figs. 1 and 2).

DISCUSSION

Our investigation found no evidence of SFV transmission through blood transfusion. Using serologic and molecular methods, we were unable to detect either antibodies or viral DNA in four recipients of blood components from one SFV-infected donor.

SFV is believed to be cell associated. SFV DNA has been detected in PBMNCs, and SFV has been isolated from PBMNCs of infected nonhuman primates and humans,[1,3,5,6,29-31] including the donor reported here.[5] Early, limited attempts to culture SFV from whole blood from nonhuman primates[5] or from serum of experimentally infected rabbits[5] have been unsuccessful, possibly both because of strong neutralizing antibodies and because viral replication appears to occur primarily in the oral mucosa.[10] RT-PCR did not detect viral RNA in the donor's plasma. In these respects, SFV appears to be more similar to HTLV, which is not readily found in plasma, than to HIV, which replicates in lymphocytes and is released into the plasma.

Thus, SFV could be expected to be present in any blood component that contains WBCs. The number of WBCs varies among different component types; for example, RBCs contains 10^8 WBCs per unit, whereas platelet components contain approximately 10^7 WBCs per unit. Standards require that WBC-reduced RBCs contain fewer than 5×10^6 WBCs per unit.

Because all tested recipients received cellular components, the absence of SFV transmission may indicate that transfusion is a relatively inefficient mechanism of infection. However, the small number of recipients evaluated precludes any meaningful estimates of the rate of transmission of SFV by transfusion.

Another factor that may influence transmission is the survival time of SFV in stored blood components. For example, the risk of HTLV transmission decreases with increasing duration of storage of blood components. HTLV appears to be transmitted only through cellular components stored no longer than 10 days.[32] Although the survival time of SFV in blood components is not known, it is of interest that three of the four recipients we evaluated received cellular components stored 8 days or less.

Recipient 7, the child with the hematologic disorder, underwent marrow ablation, including total body irradiation, followed by BMT 11 months after the transfusion. Such therapy would be expected to ablate the patient's WBCs. No specimens collected from Recipient 7 before the BMT were available for testing. The tested blood sample, which was collected from Recipient 7 9 months after BMT, should have contained only cells from the marrow donor. Therefore, we can not determine whether the current absence of SFV infection in Recipient 7 is due to an absence of SFV transmission initially or was related to the marrow ablation.

Not surprisingly, we found no evidence of SFV in the plasma derivatives we evaluated. SFV was not detected in the donor's plasma. Plasma derivatives are pooled products containing plasma from thousands of individual donors per lot. Thus, in a given lot, an individual donor's plasma may be diluted up to 300,000 times. The massive dilution of this donor's plasma in the production of pooled plasma derivatives makes it unlikely that either SFV antibodies or SFV viral expression products would be detected in the final product even if they had been present in the donor's plasma.

Identifying blood donors who are persistently infected with nonhuman primate viruses highlights the potential for introduction of new viruses into the blood supply. Although our results do not support SFV transmission through blood transfusion, the findings of this study should be interpreted cautiously because of the limited sample size. Ongoing implementation of universal WBC reduction offers the potential benefit of further decreasing the risk of transmission of leukotropic agents for which blood donors cannot be tested,[33] including SFV. Additional studies are needed to evaluate the survival of SFV in blood components and the impact of WBC reduction measures on SFV presence in blood components. Because transmission of SFV through transfusion of blood products and components remains theoretically possible, we counsel persons confirmed to be infected with SFV to not donate blood or other biological materials.

ACKNOWLEDGMENTS

The authors thank Jay Epstein, MD, and Indira Hewlett, PhD, of the Office of Blood Research and Review, CBER, FDA, for assistance during the investigation and critical review of the manuscript.

REFERENCES

1. Schweizer M, Turek R, Hahn H, et al. Markers of foamy virus infections in monkeys, apes, and accidentally infected humans: appropriate testing fails to confirm suspected foamy virus prevalence in humans. AIDS Res Hum Retroviruses 1995;11:161-70.
2. Schweizer M, Falcone V, Gange J, et al. Simian foamy virus isolated from an accidentally infected human individual. J Virol 1997;71:4821-4.
3. Heneine W, Switzer WM, Sandstrom P, et al. Identification of a human population infected with simian foamy viruses. Nature Med 1998;4:403-7.
4. Sandstrom PA, Phan KO, Switzer WM, et al. Simian foamy virus infection among zoo keepers. Lancet 2000;355:551-2.
5. Switzer WM, Vedapuri S, Sandstrom P, et al. Increased rate of simian foamy virus infection among persons occupationally exposed to nonhuman primates (abstract). Fourth International Conference on Foamy Viruses, Atlanta, GA, March 14-16, 2002.
6. Boneva RS, Switzer WM, Spira T, et al. Human infection with simian foamy viruses: preliminary results from a long-term follow-up study (abstract). Fourth International Conference on Foamy Viruses, Atlanta, GA, March 14-16, 2002.
7. Neumann-Haefelin D, Fleps U, Renne R, et al. Foamy viruses. Intervirology 1993;35:196-207.
8. Hooks JJ, Gibbs CJ. The foamy viruses. Bacteriol Rev 1975; 39:169-85.
9. Meiering CD, Linial ML. Historical perspective of foamy virus epidemiology and infection. Clin Microbiol Rev 2001;14:165-76.
10. Falcone V, Leopold J, Clotten J, et al. Sites of simian foamy virus persistence in naturally infected African green monkeys: latent provirus is ubiquitous, whereas viral replication is restricted to the oral mucosa. Virology 1999;257:7-14.
11. Achong B, Mansell PWA, Epstein MA, et al. An unusual virus in cultures from a human nasopharyngeal carcinoma. J Natl Cancer Inst 1971;46:299-307.
12. Stancek D, Stanchekova-Gressnerova M, Janotka M, et al. Isolation and some serological and epidemiological data on the viruses recovered from patients with subacute thyroiditis de Quervain. Med Microbiol Immunol 1975;161: 133-44.

13. Cameron KR, Birchall SM, Moses MA. Isolation of foamy virus from a patient with dialysis encephalopathy. Lancet 1978;2:796.
14. Werner J, Geldeblom H. Isolation of foamy virus from patients with de Quervian thyroiditis. Lancet 1979;2:258-9.
15. Westarp ME, Kornhuber HH, Rossler J, et al. Human spuma retro-virus antibodies in amyotrophic lateral sclerosis. Neurol Psych Brain Res 1992;1:1-4.
16. Lagaye S, Vexiau P, Morozov V, et al. Human spumaretroviruses-related sequences in the DNA of leukocytes from patients with Grave's disease. Proc Natl Acad Sci U S A 1992;89:1070-74.
17. Schweizer M, Turek R, Reinhardt M, et al. Absence of foamy virus DNA in Graves' disease. AIDS Res Hum Retroviruses 1994;10:601-5.
18. Heneine W, Musey VC, Sinha SD, et al. Absence of evidence for human spumaretrovirus sequences in patients with Graves' disease (Letter). J AIDS 1995;9:99-101.
19. Ali M, Taylor GP, Pitman RJ, et al. No evidence of antibody to human foamy virus in widespread human populations. AIDS Res Hum Retroviruses 1996;12:1473-83.
20. Rosener M, Hahn H, Kranz M, et al. Absence of serological evidence for foamy virus infection in patients with amyotrophic lateral sclerosis. J Med Virol 1996;48:222-6.
21. Winkler IG, Flügel RM, Asikainen K, Flower RL. Antibody to human foamy virus not detected in individuals treated with blood products or in blood donors. Vox Sang 2000; 79:118-9.
22. Butera ST, Brown J, Callahan ME, et al. Survey of veterinary conference attendees for evidence of zoonotic infection by feline retroviruses. J Am Vet Med Assoc 2000;217: 1475-9.
23. Herchenroder O, Renne R, Loncar D, et al. Isolation, cloning and sequencing of simian foamy viruses from chimpanzees (SFVcpz): high homology to human foamy virus (HFV). Virology 1994;201:187-99.
24. Weiss RA. Reverse transcription: foamy viruses bubble on. Nature 1996;380:201.
25. Linial M. Why aren't foamy viruses pathogenic? Trends in Microbiology. 2000;8:284-89.
26. Bieniasz PD, Erlwein O, Aguzzi A, et al. Gene transfer using replication-defective human foamy virus vectors. Virology 1997;235:65-72.
27. Nestler UM, Heinkelein M, Lucke M, et al. Foamy virus vectors for suicide gene therapy. Gene Ther 1997;4: 1270-7.
28. Hussain AI, Shanmugam V, Bhullar VB, et al. Diagnosis of SFV infection in nonhuman primates and occupationally exposed humans by using a combined antigen Western blot assay (abstract). Fourth International Conference on Foamy Viruses, Atlanta, GA, March 14-16, 2001.
29. Switzer WM, Shanmugam V, Bhullar V, et al. Virus recovery from the oral mucosa and evidence of viral quasispecies and tissue compartmentalization in an SFV_{CPZ}-infected person (abstract). Fourth International Conference on Foamy Viruses, Atlanta, GA, March 14-16, 2001.
30. Callahan ME, Switzer WM, Matthews AL, et al. Persistent zoonotic infection of a human with simian foamy virus in the absence of intact orf-2 accessory gene. J Virol 1999;73: 9619-24.
31. Von Laer D, Neumann-Haefelin D, Heeney JL, Schweizer M. Lymphocytes are the major reservoir for foamy viruses in peripheral blood. Virology 1996;221:240-4.
32. Donegan E, Lee H, Operskalski EA, et al. Transfusion transmission of retroviruses: human T-lymphotropic virus types I and II compared with human immunodeficiency virus type 1. Transfusion 1994;34:478-83.
33. FDA. Draft guidance for industry: pre-storage leukocyte reduction of whole blood and blood components intended for transfusion. Rockville, MD: US Department of Health and Human Services, Food and Drug Administration, Federal Register 2001.