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研究報告の概要

○過去の米国の集団においてヒトヘルペスウイルス-8が輸血を介して伝播したエビデンスはない

背景:ヒトヘルペスウイルス(HHV)-8はカポジ肉腫の原因ウイルスである。最近の試験では、ヒトヘルペスウイルス-8が輸血を介して伝播する証拠が時折発見されている。しかし、これらの研究は米国外で行われており、供血者-受血者の関連が確認されていないため、米国の血液バンクの方針に反映するには限りがある。

方法:1970年代に登録されたTransfusion-Transmitted Viruses Study(TTVS)の参加者にHHV-8血清学検査を行うことにより、米国における輸血を介したHHV-8伝播を調べた。

結果:HHV-8抗体陽性率は、供血者が2.8%(29/1023)、受血者が7.1%(96/1350)、輸血を受けず手術を受けた対照患者が7.7%(46/599)、カポジ肉腫を有する対照患者が96.3%(77/80)であった。1名の受血者はセロコンバージョンしたが(0.08%[1/1259])、この患者はHHV-8血清陽性血液をまったく投与されておらず、感染が輸血関連ではなかったことが示された。輸血を受けず手術を受けた対照患者の1例がセロコンバージョンした(0.18%[1/556])。セロコンバージョン率は、受血者が1000人年あたり1.6(95%信頼区間[CI]、1000人年につき0.04-8.9)、輸血を受けず手術を受けた対照患者が1000人年あたり3.6(95%CI、1000人年につき0.09-20.1)であった。

結論:輸血群および非輸血群のHHV-8セロコンバージョン率に統計学的な差はなく、過去の集団の特徴(例、白血球除去施行前)からは、現在の輸血を介する伝播が稀であることが示される。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
1970年代に登録された米国のコホートにおいて、ヒトヘルペスウイルス-8が輸血を介して伝播したエビデンスはなかったとの報告である。 HHV-8は脂質膜を持つ大型DNAウイルスである。これまで、本製剤によるHHV-8感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考える。	念のため今後も情報収集に努める。

Lack of Evidence for Human Herpesvirus–8 Transmission via Blood Transfusion in a Historical US Cohort

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(See the editorial commentary by Busch and Glynn, on pages 1564–6.)

Background. Recent studies have found evidence of occasional human herpesvirus (HHV)–8 transmission via blood transfusion. However, because these studies were conducted outside the United States or did not have linked donor-recipient pairs, they have a limited ability to inform US blood-banking policy.

Methods. We investigated HHV-8 transmission via blood transfusion in the United States by conducting HHV-8 serologic testing among participants of the Transfusion-Transmitted Viruses Study (TTVS), who enrolled during the 1970s.

Results. HHV-8 seroprevalence was 2.8% (29/1023) among blood donors, 7.1% (96/1350) among transfusion recipients, 7.7% (46/599) among surgical control patients who did not receive transfusions, and 96.3% (77/80) among control patients with Kaposi sarcoma. One transfusion recipient seroconverted (0.08% [1/1259]), but this patient did not receive any HHV-8–seropositive blood units, suggesting that the infection was not related to blood transfusion. One of the surgical control patients who did not receive transfusions also seroconverted (0.18% [1/556]). Rates of seroconversion were 1.6 per 1000 person-years (95% confidence interval [CI], 0.04–8.9 per 1000 person-years) for the transfusion recipients and 3.6 per 1000 person-years (95% CI, 0.09–20.1 per 1000 person-years) for the surgical control patients who did not receive transfusions ($P = .61$).

Conclusions. Rates of HHV-8 seroconversion in the transfusion and nontransfusion groups were not statistically different, and the historical nature of the cohort (e.g., before leukoreduction) suggests that any current transmission via blood transfusion is rare.

Human herpesvirus (HHV)–8 is necessary for the development of Kaposi sarcoma (KS), primary effusion lymphomas, and multicentric Castleman disease. Disease tends to occur, however, only in the presence of immunosuppression [1]. In the overall US population, HHV-8 seroprevalence is low (estimated at between 1% and 7% [2, 3]), but higher seroprevalences are found among men who have sex with men [4] and among persons with human immunodeficiency virus (HIV) infection or risk factors for HIV infection [5].

Initial studies found no evidence of HHV-8 transmission via blood transfusion [6–8]. However, these studies were limited by relatively small numbers of patients, many of whom received leukoreduced or acellular blood components. Later reports that HHV-8 infection was associated with injection drug use and, presumably, needle sharing [5, 9–12] led to larger-scale investigations of transmission via transfused blood [13–15]. These studies found evidence that HHV-8 was transmitted occasionally via blood transfusion, leading to renewed questions about the advisability of screening of blood for HHV-8 [16–19]. Nevertheless, all 3 studies had a limited ability to inform US blood-banking policy, either because they were conducted outside the United States or because they did not have linked donor-recipient pairs to prove transmission via transfusion.

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The findings and conclusions in this article have not been formally disseminated by the Centers for Disease Control and Prevention and should not be construed to represent any agency determination or policy.

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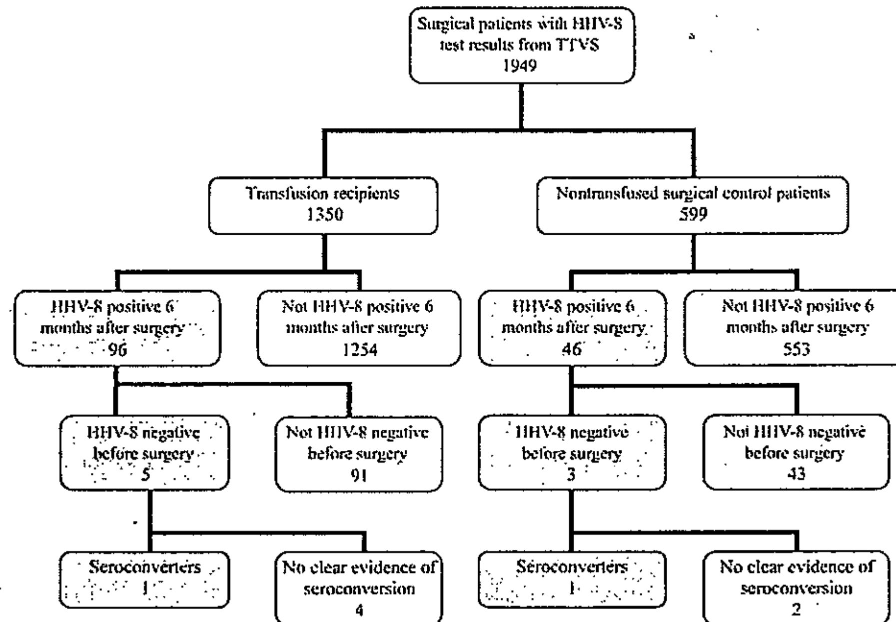

Figure 1. Testing algorithm and outcomes among the transfusion recipients and the surgical control patients who did not receive transfusions. All 4 blood donors for the 1 seroconverter who received a transfusion tested negative. HHV, human herpesvirus; TTVS, Transfusion-Transmitted Viruses Study.

To better evaluate the possibility of HHV-8 transmission via blood transfusion in the United States, we conducted HHV-8 serologic testing among participants of the Transfusion-Transmitted Viruses Study (TTVS). To our knowledge, this was the largest US study conducted with linked donor-recipient pairs and longitudinal follow-up specimens. The specimens were collected before the advent of several blood-safety improvements (such as HIV testing, more-stringent donor-deferral guidelines, transition to extended storage of blood components, and routine leukoreduction by filtration and apheresis techniques), making this study an important opportunity to detect HHV-8 transmission via blood transfusion in the United States.

METHODS

Study design and population. The TTVS was designed in the 1970s to prospectively identify cases of non-A, non-B hepatitis among a cohort of 1533 patients who had received transfusions and to create a repository for detecting the occurrence of virus transmission via blood transfusion [20]. The TTVS repository was funded by the National Heart, Lung, and Blood Institute (NHLBI) and is now housed at the NHLBI Biologic Specimen Repository. The TTVS repository has been used to demonstrate transmission of other viruses via transfusion, such as hepatitis B virus (HBV) and hepatitis C virus (HCV) [21–25]. TTVS participants consisted of blood donors, transfusion recipients (nearly all of whom underwent surgery), and surgical patients who did not receive transfusions (referred to hereafter as surgical control patients without transfusions). All transfusions occurred during the years 1974–1979. Donors could be linked to transfusion recipients, and both the transfusion recipients and the surgical control patients without transfusions had blood drawn before surgery and at multiple time points after surgery. The TTVS received institutional review board approval from the institutions at which it was performed. TTVS participants consented to future testing. The present analysis of HHV-8 was cleared by the Centers for Disease Control and Prevention (CDC) and the University of Southern California; specimens and associated data were delinked from participant identifiers so that the study did not fall under the category of human-subjects research.

For this study, we tested specimens from 1023 randomly selected TTVS blood donors (20.8% of the 4918 donors who had samples available), specimens from all transfusion recipients who had samples available 6 months after transfusion ($n = 1350$), and specimens from all surgical control patients without transfusions who had samples available 6 months after surgery ($n = 599$) (figure 1). To identify seroconverters, we tested the pretransfusion or presurgery specimens from all patients who were HHV-8 seropositive at 6 months. To determine the time of seroconversion, for all those who tested negative before surgery and positive 6 months after surgery, interim specimens were tested at monthly intervals. These serial specimens from individual patients were randomized and masked for testing. A small number of patients had specimens with repeated marginal reactivity; the specimens from these patients were grouped on the same slides and plates for retesting. We also tested any blood-donor speci-

Table 1. Human herpesvirus–8 seroprevalence in different groups in the Transfusion-Transmitted Viruses Study (TTVS).

Group	Proportion (%) positive
Control patients with KS[a]	77/80 (96.3)
Blood donors[b]	29/1023 (2.8)
Surgical control patients who did not receive transfusions	46/599 (7.7)
Transfusion recipients[c]	96/1350 (7.1)

NOTE. Data are the no. of positive specimens per the total no. tested. Specimens were considered positive if they were reactive at a dilution of 1:80 or greater by an immunofluorescence assay. KS, Kaposi sarcoma.

[a] Specimens from control patients with KS were randomly and blindly inserted among the other specimens.

[b] Donors were randomly selected from all the blood donors in the TTVS.

[c] Specimens were collected ~6 months after surgery.

mens (masked to the laboratory) that were linked to seroconverters but were not part of the initial sample of tested donors. As an additional control, 80 specimens from HIV-positive patients with KS were randomly and blindly inserted among specimens from study patients. To help evaluate the performance of the HHV-8 assay, we also tested serial specimens from 7 randomly selected HHV-8–positive (i.e., positive before and 6 months after surgery) and 57 randomly selected HHV-8–negative (i.e., negative before and 6 months after surgery) surgical patients (both those who had received transfusions and those who had not). To compute seroconversion rates, person-time was measured as the time from surgery until the 6-month visit.

Serologic analysis. Specimens were tested at the CDC for antibodies against HHV-8 by an immunofluorescence assay (IFA), as described elsewhere [3, 13, 14]. Specimens were considered positive if they were reactive at a dilution of 1:80 or greater. Specimens that were equivocal or negative at a dilution of 1:80 were classified as not positive. To avoid false identification of seroconverters, we chose a conservative a priori definition of seroconversion: negative (not equivocal) at a dilution of 1:40 before surgery and positive at a dilution of 1:80 after surgery at ≥2 consecutive time points. All specimens that tested positive at a dilution of 1:80 were also tested at a dilution of 1:160.

RESULTS

HHV-8 seroprevalences in the 4 different study populations are described in table 1. Nearly all specimens from control patients with KS were positive (96.3%). Blood donors had the lowest seroprevalence (2.8%), and the transfusion recipients and the surgical control patients without transfusions had similar seroprevalences 6 months after surgery (7.1% and 7.7%, respectively). For the 4918 donors linked to the 1350 transfusion recipients, the type of transfused units were whole blood (61.3%), unknown (17.9%), packed cells (17.8%), plasma (2.0%), other (0.8%), washed frozen (0.1%), and platelets (0.1%). Of the 142 patients who were seropositive 6 months after surgery (figure 1), 8 were seronegative at their presurgery visits and were considered potential seroconverters.

Serial specimen testing was done for the 8 potential seroconverters, with each having a total of 8 specimens tested (1A, 1B, and 2A–2F in figure 2). Of the 8 potential seroconverters, 2 (2D and 2F in figure 2) were clearly seropositive only at their last (6-month) visit, suggesting that their 6-month postsurgery specimen may have been mislabeled or had a false-positive result or that the patient may have acquired a community HHV-8 infection near the end of the follow-up period. Another 4 patients (2A–2C and 2E in figure 2) had mixed reactivities that did not meet our definition of seroconversion. The remaining 2 potential seroconverters (1A and 1B in figure 2) had serial test results that met our a priori criteria for seroconversion (figures 1 and 2). On the basis of these 2 seroconverters, we computed the risk of seroconversion as 0.08% (1/1259) (95% confidence interval [CI], 0.0%–0.44%) for the transfusion recipients and as 0.18% (1/556) (95% CI, 0.0%–1.0%) for the surgical control patients without transfusions. Rates of seroconversion were 1.6 per 1000 person-years (95% CI, 0.04–8.9 per 1000 person-years) for the transfusion recipients and 3.6 per 1000 person-years (95% CI, 0.09–20.1 per 1000 person-years) for the surgical control patients without transfusions. The difference in rates was not statistically significant ($P = .61$). Rates of seroconversion determined using a more relaxed definition (i.e., negative at a dilution of 1:80 before surgery and positive at a dilution of 1:80 six months after surgery) were similar between the 2 groups (5.2% [5/96] for the transfusion recipients vs. 6.5% [3/46] for the surgical control patients without transfusions; $P = .72$) (figure 1).

The seroconverter who had undergone transfusion received a unit of blood from each of 4 donors (2 U of whole blood and 2 U of packed cells), none of whom was HHV-8 seropositive. Applying the HHV-8 seroprevalence of 2.8% to the 4918 donors who gave blood to the 1350 transfusion recipients, we estimate that ~138 seropositive units were transfused, 128 (92.9%) of which would have been given to HHV-8–seronegative transfusion recipients, none of whom seroconverted.

Serial testing was also done for patients whose serostatus was constant before surgery and 6 months after surgery (either positive or negative at both time points). For these 64 patients, serial HHV-8 testing results are shown in figure 2 (3A–3G and 4A–4H) and table 2. For the 7 HHV-8–positive patients, all serial specimens were positive at dilutions of 1:80 or greater at all visits. For the 57 HHV-8–negative patients, nearly all test results were negative, although a few were equivocal and 2 were positive (table 2).

DISCUSSION

In the present study—the largest US study to analyze HHV-8 infection among transfusion recipients and their linked donors—we found no evidence that HHV-8 is transmitted via

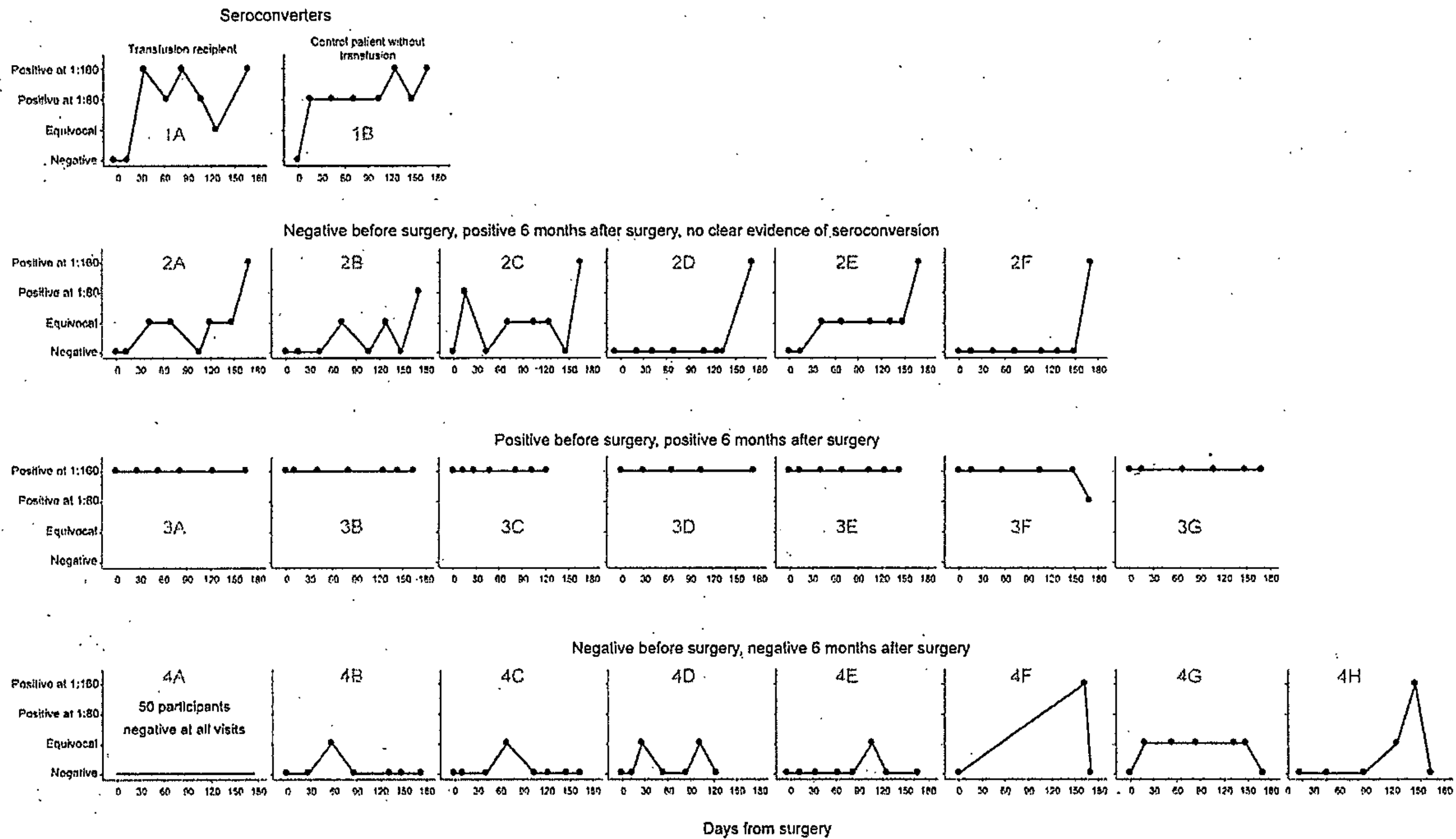

Figure 2. Human herpesvirus–8 serial test results among Transfusion-Transmitted Viruses Study surgical patients.

Table 2. Consistency of human herpesvirus–8 test results among serial specimens from patients in the Transfusion-Transmitted Viruses Study.

Group	Patients tested,[a] no.	No. of visits with specimen results that were			
		Negative	Equivocal	Positive at 1:80	Positive at ≥1:160
Positive before surgery and positive 6 months after surgery	7	0	0	1	43
Negative before surgery and negative 6 months after surgery	57	379	11	0	2

NOTE. Included are test results for specimens from the presurgery visits and from all follow-up visits through 6 months after surgery (mean no. of visits, 6.8).
[a] Patients were selected to be positive and negative control subjects for the seroconversion study.

blood transfusion. Two study patients met our a priori definition of seroconversion—they were negative by the IFA at a dilution of 1:40 before surgery and had at least 2 consecutive positive IFA results at a dilution of 1:80 after surgery. One seroconverter received only HHV-8–seronegative blood, and the other seroconverter was a surgical control patient without a transfusion.

The study design did not allow us to determine the cause of seroconversion in these 2 patients. It is conceivable that the patient who underwent transfusion received blood from an HHV-8–infected donor who was in the so-called window period—that is, not yet HHV-8 seropositive but with newly acquired HHV-8 circulating in the blood. Alternatively, the seroconverters might have experienced community-acquired infections or nosocomial infections unrelated to transfusion.

The lack of evidence in this historical cohort suggests that the current risk of HHV-8 transmission via blood transfusion is very low. Even if we assume that the one seroconverter who received a blood transfusion was infected via the transfusion, current practices make it much less likely that such transmission would occur now compared with when the TTVS specimens were collected. Since the 1970s, blood banks have stricter donor-deferral guidelines [26], and tests that screen out blood positive for HIV, HBV, and HCV may also screen out blood positive for HHV-8, given that there are shared risk factors for infection among HHV-8 and these other viruses [5, 27]. Moreover, leukoreduction, which became commonplace in the mid-1990s, is likely to reduce the risk of HHV-8 transmission via transfusion, because HHV-8 is highly cell associated [7, 16, 28]. Similarly, the current increased use of red blood cell components, which are stored for up to 42 days at 4°C, is likely to reduce HHV-8 transmission because such storage conditions are known to decrease the infectivity of transfusion-transmissible herpesviruses, such as cytomegalovirus. However, it is worth noting that the seroprevalence of HHV-8 among TTVS blood donors is very similar to more recent estimates [3], suggesting that HHV-8 is endemic at low levels in the United States.

Our results are consistent with those from previous studies of HHV-8 transmission via blood transfusion in the United States—the risk to current transfusion recipients is very low, but rare transmission cannot be ruled out [6–8, 13]. For example, Pellett et al. [3] found that HHV-8 seroprevalence among blood donors was low (~3.5%), and HHV-8 DNA was not detected in the blood of seropositive donors. Although in another historical cohort we identified 2 possible transfusion-related HHV-8 seroconversions, that study was not able to show a linkage to seropositive donor blood [13]. Given the safety improvements created by current blood donation and transfusion practices, a cohort containing thousands of linked donor-recipient pairs, such as the NHLBI RADAR (REDS [Retrovirus Epidemiology Donor Study] Allogenic Donor and Recipient) repository [29], would be required to rule out rare transmission events.

In contrast with these US results, HHV-8 has been shown to be transmitted via blood transfusion in Uganda [14], with ~3% of HHV-8–seropositive units causing infection. If there were a comparable risk in the TTVS, we would have expected to see ~3.8 (3% of 128) infections resulting from blood transfusion, rather than the zero that we observed (for the difference between the observed vs. the expected, $P = .035$). The transfusion risk may be higher in Uganda because of a higher prevalence of immunosuppression, a higher risk of exposure and reinfection, and a higher frequency of viremia among HHV-8–seropositive individuals. In addition, donor-deferral guidelines in Uganda were less stringent, testing for HCV was not done, and leukoreduction was not performed. Furthermore, blood was often stored for short periods of time, perhaps allowing virus to remain viable.

The lack of evidence for HHV-8 transmission via blood transfusion is unlikely to be explained by assay deficiencies. We used an IFA and a dilution (1:80) that have been validated and used in previous studies [13, 14] and that have been shown to have high sensitivity and specificity. The assay detected HHV-8 in 96.3% of specimens from control patients with KS (specimens were randomly and blindly inserted among the TTVS specimens), including KS specimens that had been found to have relatively low levels of antibodies by other assays [30, 31]. Our low seroprevalence among blood donors (2.8%) was consistent with the findings of other studies [2, 3] and suggested high assay specificity. The higher seroprevalences among the transfusion recipients and the surgical control patients without transfusions (7.1% and 7.7%, respectively) were consistent with their older age and health status (i.e., surgical patients may be less healthy than the general population). Furthermore, our results for longitudinal follow-up specimens were highly coherent, with results remaining consistent throughout follow-up among postsurgery specimens for >95% of the presurgery specimens with a positive or

negative result (table 2). For the small number of patients with incoherent longitudinal reactivity patterns, a few explanations may pertain. First, a single positive serum specimen among a series of negative specimens (e.g., 2D, 2F, 4F, and 4H in figure 2) is likely the result of nonspecific reactivity or a specimen-labeling error. Second, up-and-down reactivity patterns (e.g., 2A–2C and 2E in figure 2) may be the result of periodic nonspecific reactivity or, more likely, low levels of HHV-8 antibody fluctuating above and below the lower limit of detection of the assay.

Screening of blood donors for HHV-8, if warranted, faces important technical challenges. Currently, there is no consensus on a standard HHV-8 assay that has known high sensitivity and specificity. The IFA used in the present study is time-consuming and could not be readily standardized across laboratories in the implementation of a screening program. Enzyme-linked immunosorbent assay formats, which might be more amenable to the high throughput demanded by a screening program, may be less sensitive. The main challenge is that the HHV-8 antibody response in healthy individuals is relatively weak, and most of the current assays have inadequate sensitivity and specificity.

In conclusion, the present study does not provide evidence of transmission of HHV-8 via blood transfusion in the United States. Rates of seroconversion in the transfusion and nontransfusion groups were not statistically different, and the historical nature of the cohort suggests that any current transfusion transmission is rare. However, much larger studies would be required to rule out rare transmission events. Nevertheless, if such transmission is shown to occur in the United States, universal screening of blood donors may not be warranted, because HHV-8 seldom causes disease in immunocompetent populations. If suitable assays become available, screening of blood for HHV-8 may be beneficial for immunosuppressed populations. However, the challenges associated with reliably detecting HHV-8 antibody or HHV-8 DNA in a healthy blood-donor population remain a substantial barrier, one that must be crossed before the costs and benefits of HHV-8 blood screening can be appropriately weighed.

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医薬品
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識別番号・報告回数			報告日	第一報入手日 2009年8月10日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②乾燥抗 HBs 人免疫グロブリン ③ポリエチレングリコール処理抗 HBs 人免疫グロブリン		研究報告の公表状況	Nature Medicine(online) 2009; 15(8): 871-872	公表国 カメルーン	
販売名（企業名）	① ヘブスブリン筋注用 200 単位（ベネシス） ②ヘブスブリン筋注用 1000 単位（ベネシス） ③ヘブスブリン IH 静注 1000 単位（ベネシス）					

研究報告の概要

ゴリラ起源の新型のヒト免疫不全ウイルス：
我々は、カメルーンの首都ヤウンデ近郊に住んでいた 62 歳の女性が、2004 年に渡仏した際に HIV 感染が発覚し、彼女から連続的に採取した血液分析により、HIV-1(SIVgor)に密接に関連した新型のヒト免疫不全ウイルスを同定した。
新型のヒト免疫不全ウイルスは、密接にゴリラ・サル免疫不全ウイルス（SIVgor）に関係があり、他の HIV-1 系統で組換えの証拠を示さない。これまでに知られているチンパンジー由来のウイルス（種を超え交差伝播した(SIVcpzPtt)）とは異なり、最近ヒト感染に必要な生物学的特性の多くを持っている SIV（SIVgor）が野生のゴリラ（Gorilla gorilla gorilla）で発見されている。
発見された新型 HIV-1 変異株は現在確認されている HIV の 3 つの亜種（M、N および O）とは異なっている。我々は HIV-1 グループ P と称することを提案する。
Strain RBF168（subject number）は血清学的そして非特異的な分子試験で古典的な HIV-1 の挙動を示すことより、気づかれずにカメルーンやその他の地域ですでに感染が広がっている可能性があることを示唆する。
結論として、我々の知見はゴリラがチンパンジーに加えて、HIV-1 の有望な起源であることを示す。
この新しい HIV-1 系統の発見は、特に西中央アフリカは全ての既存の HIV-1 グループの起源であることより、新しい HIV 変異株の出現を継続して見守る必要があることを強調する。

使用上の注意記載状況・その他参考事項等

代表としてヘブスブリン IH 静注 1000 単位の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びろ過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
新たに発見された亜型ウイルス(HIV-1 グループ P)は、カメルーンからパリに移住した 62 歳の女性の血液サンプルから発見されたという報告である。 HIV-1ウイルスは、レトロウイルス科レンチウイルス属に属し、成熟ウイルスの粒子直径約100nmのエンベロープを持つ一本鎖RNAウイルスである。HIV-1は塩基配列により3群に分類され、グループM（Major）、グループO（Outlier）、グループN（non-M/non-O）に分けられるが、世界的に分布しているウイルスの多くがグループMに属している。現在、原料血漿に実施されているスクリーニング(抗体検査、ミニプールNAT)によりこの新たなHIVが検出可能か否かは不明であるものの、もし原料血漿にHIV-1グループPが混入したとしても、HIV-1をモデルウイルスとしたウイルスバリデーション試験成績から、製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えるものではないと考えるので、特段の措置はとらない。

BRIEF COMMUNICATIONS

nature medicine

A new human immunodeficiency virus derived from gorillas

Jean-Christophe Plantier[1], Marie Leoz[1], Jonathan E Dickerson[2], Fabienne De Oliveira[1], François Cordonnier[3], Véronique Lemée[1], Florence Damond[4], David L Robertson[2] & François Simon[5]

We have identified a new human immunodeficiency virus in a Cameroonian woman. It is closely related to gorilla simian immunodeficiency virus (SIVgor) and shows no evidence of recombination with other HIV-1 lineages. This new virus seems to be the prototype of a new HIV-1 lineage that is distinct from HIV-1 groups M, N and O. We propose to designate it HIV-1 group P.

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HIV-1, the virus principally responsible for the AIDS pandemic, arose through cross-species transmission of a retrovirus (SIVcpz*Ptt*) found in chimpanzees (*Pan troglodytes troglodytes* (*Ptt*))[1,2]. Another SIV (SIVgor), recently discovered in wild-living gorillas (*Gorilla gorilla gorilla*)[3], has many of the biological properties necessary for human infection[4]. We have now identified a new human immunodeficiency virus closely related to SIVgor in a Cameroonian woman. This new HIV-1 variant is distinct from the three established groups of HIV-1, namely M (major or main), N (non-M, non-O) and O (outlier)[5,6].

Since 2001, a French network of reference laboratories has been monitoring HIV genetic diversity. Infection with an unusual variant is suspected when RNA viral load assays or molecular tests are negative in an individual with acquired immunodeficiency naive of antiretroviral therapy. As part of these surveillance activities, we analyzed serial samples from a 62-year-old woman (subject number RBF168) who was found to be HIV seropositive in 2004, shortly after moving to Paris from Cameroon (**Supplementary Methods**). Several HIV-1 screening tests were all reactive, and western blotting with HIV-1 group M proteins showed weak reactivity against the envelope glycoprotein 120 and no reactivity against Gag p18 protein (**Supplementary Methods** and **Supplementary Fig. 1**). She currently has no signs of AIDS, remains untreated and has a stable $CD4^+$ cell count of about 300 cells per mm^3 (**Supplementary Fig. 2**). Her viral load has been consistently high since diagnosis (4.4 to 5.3 log copies per ml) in nonspecific group M and O PCR commercial assays (LCx HIV RNA Quantitative and Real*Time* HIV1, Abbott) and in an in-house real-time RT-PCR assay[7] (**Supplementary Fig. 2**). The virus replicates in cultured human donor peripheral blood mononuclear

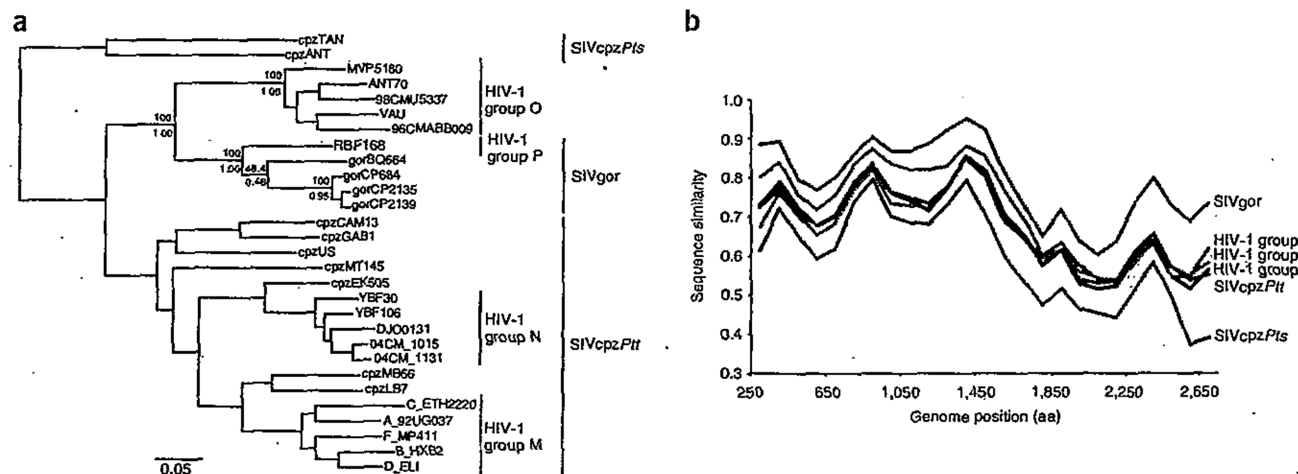

Figure 1 Evolutionary relationship of strain RBF168 to HIV-1, SIVcpz and SIVgor. (a) Maximum likelihood phylogeny inferred from concatenated amino acid alignments corresponding to the partial sequences available for SIVgorBQ664 (ref. 4); 1,052 amino acid positions remained after stripping gap-containing sites. The support values (indicated for key nodes only) in black above the branches are from 1,000 maximum likelihood bootstraps (shown as percentages), whereas posterior probabilities from amino acid Bayesian analysis are shown in blue below the branches (shown as proportions). (b) Average sequence similarity (250 amino acid windows, 100-amino-acid increments) of RBF168 with representative strains of HIV-1 groups M, N and O, SIVgor, SIVcpz from *Pan troglodytes schweinfurthii* (SIVcpz*Pts*) and SIVcpz*Ptt* across the concatenated translated gene sequence alignments. Similar results were obtained with the nucleotide sequence alignment (data not shown).

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cells and is easily isolated from both the subject's plasma and peripheral blood mononuclear cells (**Supplementary Methods**). Her viral load cannot, however, be quantified with a group M–specific commercial assay (Amplicor Monitor v1.5, Roche) or with an academic assay (Generic HIV charge virale, Biocentric[8]) (**Supplementary Fig. 2**). We did not obtain amplification with complementary group M–specific PCRs (**Supplementary Methods**). We initially suspected HIV-1 group O infection, endemic in western central Africa, especially in view of the subject's Cameroonian origin. However, amplification with our usual group O primers failed (**Supplementary Methods** and **Supplementary Table 1**), leading us to search for a divergent virus by using a nonspecific extra-long RT-PCR method. We successfully amplified the viral genome with this approach, allowing us to fully sequence it (**Supplementary Methods, Supplementary Fig. 5** and **Supplementary Table 2**).

Evolutionary analysis of the near-complete genome sequence (**Supplementary Methods**) shows that the RBF168 strain is most closely related to SIVgor (**Fig. 1a** and **Supplementary Fig. 3**), and similarity plotting confirms that this relationship is maintained in all regions of the genome (**Fig. 1b**). Before the discovery of strain RBF168, HIV-1 group O was the lineage most closely related to SIVgor, but it is too divergent to be directly derived from current SIVgor strains[4]. As strain RBF168 clusters significantly with SIVgor strains (see support values on tree, **Fig. 1a** and **Supplementary Fig. 3**), the most likely explanation for its emergence is gorilla-to-human transmission of SIVgor (**Supplementary Fig. 4a,b**). Similar to the proposed chimpanzee origin for the HIV-1 group O and SIVgor lineage[4], we cannot rule out the possibility that SIVcpz gave rise to strain RBF168, either indirectly by transmission to gorillas and then to humans (**Supplementary Fig. 4a,b**) or directly by transmission to humans and also to gorillas (**Supplementary Fig. 4c**). Detection of RBF168-like viruses in chimpanzees would be needed to confirm this possibility.

Strain RBF168 thus represents a new HIV-1 variant and is the prototype of a new human lineage that we designate as putative group P, pending the identification of further human cases, in keeping with nomenclature guidelines[6]. The human case described here does not seem to be an isolated incident, as before coming to Paris the subject had lived in the semiurban area of Yaoundé, the capital of Cameroon, and reported no contact with apes or bush meat (**Supplementary Methods**), and the variant's high level of replication *in vivo* and ready isolation in culture indicate that it is adapted to human cells. This efficient replication of RBF168 is rather unexpected, given the absence of an arginine (or lysine) at position 30 in the Gag protein, considered a signature of human-specific adaptation of HIV-1 (ref. 9). Contrary to most HIV-1 strains (apart from group M subtype C), but like SIVgor and all SIVcpz*Ptt* strains[9], RBF168 has a methionine at this amino acid position.

The human prevalence of this new lineage remains to be determined. Strain RBF168 shows typical HIV-1 behavior in serological and nonspecific molecular tests, suggesting that it could be circulating unnoticed in Cameroon or elsewhere. HIV screening tests and molecular tools have improved markedly over the past two decades, enabling the distinct HIV types and groups to be detected. This increased sensitivity, however, may paradoxically mask the circulation of divergent strains. Indeed, new variant infections can now be detected only by monitoring discrepancies between immunological status and virological results in molecular assays. Currently, there is no simple detection algorithm based on existing serological and molecular tools, and, therefore, only nucleotide sequencing can identify further HIV-1 group P strains.

In conclusion, our findings indicate that gorillas, in addition to chimpanzees, are likely sources of HIV-1. The discovery of this novel HIV-1 lineage highlights the continuing need to watch closely for the emergence of new HIV variants, particularly in western central Africa, the origin of all existing HIV-1 groups.

Accession codes. The near full-length sequence of strain RBF168 has been submitted to GenBank under accession number GQ328744.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS
J.-C.P., M.L. and F.D.O. conceived of and designed the experiments. M.L., F.D.O. and V.L. performed the molecular and serological experiments. J.E.D. and D.L.R. performed the computational analysis. F.C. managed the subject and collected epidemiological data. J.-C.P., V.L. and F.D. monitored the subject's virologic status. J.-C.P., M.L., J.E.D., F.D.O., D.L.R. and F.S. wrote the paper.

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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
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販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○乳児への食物の噛み与え行為：HIV伝播のリスク要因の可能性
目的：一部の保護者は乳児に食物を噛んで与えているが、一般的に、当該行為と離乳期間におけるHIV伝播とは関連付けられていなかった。我々は、この行為に関連すると考えられる米国のHIV伝播3症例について述べる。
患者と方法：9、15、39ヵ月齢の小児においてHIV感染3症例が診断された。臨床症状の発症により検査を実施し、他の伝播経路を除外するため詳細な調査を行った。また、envのC2V3C3またはgp41領域とgag領域をコードするp17を用いて、症例および疑わしい感染源から得られたウイルスの系統発生学的比較を行った。
結果：2症例は、HIVに感染した母親から授乳は行われず、米国のHIV検査ガイドラインによってHIVの周産期伝播は否定されていた。第3の症例では、小児の世話を手伝った叔母がHIVに感染していたが、母親は感染していなかった。3例ともHIV感染者が世話をし、食物を噛んで与えていた。2例では、食物を噛み与えた大人に口腔内出血があったことが報告された。系統樹解析により、3例中2例において、世話をしていたHIV感染者からの噛み与えにより感染したという疫学的結論が支持された。
結論：この報告症例は、噛み与えとHIV感染とを関連づける有力な証拠を提供するものである。これは、重大な世界的影響を有する未報告の伝播ルートであり、これまで授乳によると考えられてきた乳児の生後HIV伝播報告の説明となり得る。噛み与えリスクおよび歯周病などの修飾因子についての理解が深まるまでは、HIV感染あるいはリスクのある保護者や出産を控えた親に対して、噛み与え行為について質問し、安全性が高く実行しやすい離乳食を指導するよう医療提供者に勧める。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
HIVに感染した保護者が食物を噛み与えることによって乳児がHIVに感染したことが、系統樹解析により支持されたとの報告である。 これまで、本製剤によるHIV感染の報告はない。また本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれている。さらに最終製品についてHIV-NAT陰性であることを確認している事から本製剤の安全性は確保されていると考える。	今後も情報の収集に努める。なお、日本赤十字社ではHIV抗体検査にこれまでの凝集法と比べてより感度の高い化学発光酵素免疫測定法(CLEIA)を導入したことに加え、20プールNATについてもHIV-2及びHIVグループOの検出が可能な新NATシステムを導入し、陽性血液を排除している。

⑨

Practice of Feeding Premasticated Food to Infants: A Potential Risk Factor for HIV Transmission

WHAT'S KNOWN ON THIS SUBJECT: Although some caregivers are known to premasticate food for infants, usually during the weaning period, HIV transmission has not been linked to this practice.

WHAT THIS STUDY ADDS: The reported cases provide compelling evidence linking premastication to HIV infection, a route of transmission not previously reported that has important global implications including being a possible explanation for some of the reported cases of "late" HIV transmission in infants, so far attributed to breastfeeding.

OBJECTIVES: Although some caregivers are known to premasticate food for infants, usually during the weaning period, HIV transmission has not been linked to this practice. We describe 3 cases of HIV transmission in the United States possibly related to this practice.

PATIENTS AND METHODS: Three cases of HIV infection were diagnosed in children at ages 9, 15, and 39 months; clinical symptomatology prompted the testing. A thorough investigation to rule out alternative modes of transmission was conducted. In addition, phylogenetic comparisons of virus from cases and suspected sources were performed by using the C2V3C3 or gp41 region of *env* and the p17 coding region of *gag*.

RESULTS: In 2 cases, the mothers were known to be infected with HIV, had not breastfed their children, and perinatal transmission of HIV had previously been ruled out following US HIV testing guidelines. In the third case, a great aunt who helped care for the child was infected with HIV, but the child's mother was not. All 3 children were fed food on multiple occasions that had been premasticated by a care provider infected with HIV; in 2 cases concurrent oral bleeding in the premasticating adult was described. Phylogenetic analyses supported the epidemiologic conclusion that the children were infected through exposure to premasticated food from a caregiver infected with HIV in 2 of the 3 cases.

CONCLUSIONS: The reported cases provide compelling evidence linking premastication to HIV infection, a route of transmission not previously reported that has important global implications including being a possible explanation for some of the reported cases of "late" HIV transmission in infants, so far attributed to breastfeeding. Until the risk of premastication and modifying factors (eg, periodontal disease) are better understood, we recommend that health care providers routinely query children's caregivers and expecting parents who are infected with HIV or at risk of HIV infection about this feeding practice and direct them to safer, locally available, feeding options. *Pediatrics* 2009; 124:658–666

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KEY WORDS

HIV, feeding, premastication, prechewed, child

ABBREVIATIONS

MTCT—mother-to-child transmission
CDC—Centers for Disease Control and Prevention
PCR—polymerase chain reaction
EIA—enzyme immunoassay
EBV—Epstein-Barr virus

The views in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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The availability of antiretroviral medications, the appropriate use of cesarean delivery, and the avoidance of breastfeeding have dramatically reduced the incidence of mother-to-child transmission (MTCT) of HIV in the United States and other developed nations. Every diagnosis of HIV infection in a child, especially in the developed world, should prompt us to identify missed opportunities for diagnosis and intervention to prevent MTCT.[1,2] Although the practice of premasticating food for children, usually during the weaning period, has been described in various parts of the world,[3–8] including the United States, HIV transmission has not been linked to this practice. We report 3 cases of pediatric HIV infection that are likely to have resulted from a child, who was not infected with HIV, receiving premasticated (prechewed) food from an adult who was infected with HIV.

PATIENTS AND METHODS

Local health departments investigated the 3 cases through interviews with the available involved adults and through review of medical charts. Blood specimens from the children, available family caregivers, and the sexual partner of a deceased caregiver were sent to the Centers for Disease Control and Prevention (CDC) for HIV nucleic acid extraction, polymerase chain reaction (PCR) amplification, and genetic sequencing of the C2V3C3 or gp41 coding regions of *env* and the p17 coding region of *gag*.[9] These regions are commonly used for phylogenetic comparison of HIV sequences to determine relatedness between strains when transmission is suspected. Phylogenetic analysis has been widely used in transmission cases, both epidemiologic and forensic, and both person-to-person and transmission chains.[9] In brief, sequences were edited with Sequencher 3.1 software (Gene Codes, Madison, WI) and aligned with the SE-Al 1.0 sequence alignment editor.[10] The Modeltest 3.04 program[11] was used with each alignment to test for a statistically justified model of DNA substitution for use in the phylogenetic tree-building program by using neighbor-joining methodology implemented in *PAUP***.[12] Because of the epidemiologic focus of this report, phylogenetic analysis has been used to either support or fail to support the conclusions of the epidemiologic investigations. Available family caregivers consented to specimen collection and participation in the investigation. In addition, consent to report deidentified case details was obtained from the mothers of the children in cases 1 and 3. Case 1, who is now an adolescent, provided his assent as well. Unfortunately, Case 2 and his mother, as well as the great-aunt of case 1, have died.

RESULTS

Case 1 (Miami, FL)

In 1993, a previously healthy 15-month-old black boy was seen by a pediatrician for recurrent diarrhea and otitis media. The results of a first-generation HIV-1 antibody test (enzyme immunoassays [EIAs]) (Bio-Rad Laboratories, Hercules, CA) and Western blots performed on specimens from the child at 15, 16, and 19 months of age were positive. PCR-based tests for HIV were not available for clinical care at that time. The results of EIAs performed on specimens from the mother (21 years old) at these same 3 intervals were negative.

The mother reported that when the child was aged 9 to 14 months, she and the infant had lived with a maternal great-aunt (33 years old) infected with HIV. During this time, the great-aunt helped care for the child and fed him food that she had premasticated. The mother noted that on more than 1 occasion, the great-aunt's gingiva were bleeding when she premasticated the child's food, and the mother saw blood mixed with the prechewed food; however, at that time, the mother was unaware of the great-aunt's HIV diagnosis. The great-aunt died of sepsis and pneumonia related to *Streptococcus pneumoniae* when the child was ~14 months of age (~1 month before the child's first positive EIA test result). She was not reported to be on antiretroviral medications and had an absolute CD4 count of ~270 cells per μL on more than 1 occasion during the 6 months before her death.

The great-aunt had been in a 12-year sexual relationship with a male intravenous drug user who was HIV-infected. The mother stated that he did not use intravenous drugs in the house while she and the child resided there. She did not recall seeing needles in the house (and thus did not believe that the child could ever have been stuck by one) and did not believe that the child had ever been sexually abused by her great-aunt's sexual partner. In addition, there was no history of him ever feeding the child premasticated food.

HIV phylogenetic analysis was performed on the HIV-1 sequences of the great-aunt's sexual partner because clinical specimens from the great-aunt had not been banked before her death. Phylogenetic analysis of the HIV-1 sequences from the child and the great-aunt's sexual partner showed no phylogenetic clustering, suggesting that these 2 viral strains were not epidemiologically linked (Fig 1). However, the history of premastication in the absence of known risk factors for HIV transmission and the possibility that the great-aunt's HIV strain was from a source other than her sexual partner suggested that the great-aunt was the possible source of the child's HIV infection.

Case 2 (Miami, FL)

A black child born to a mother (36 years old) infected with HIV was followed up in the University of Miami

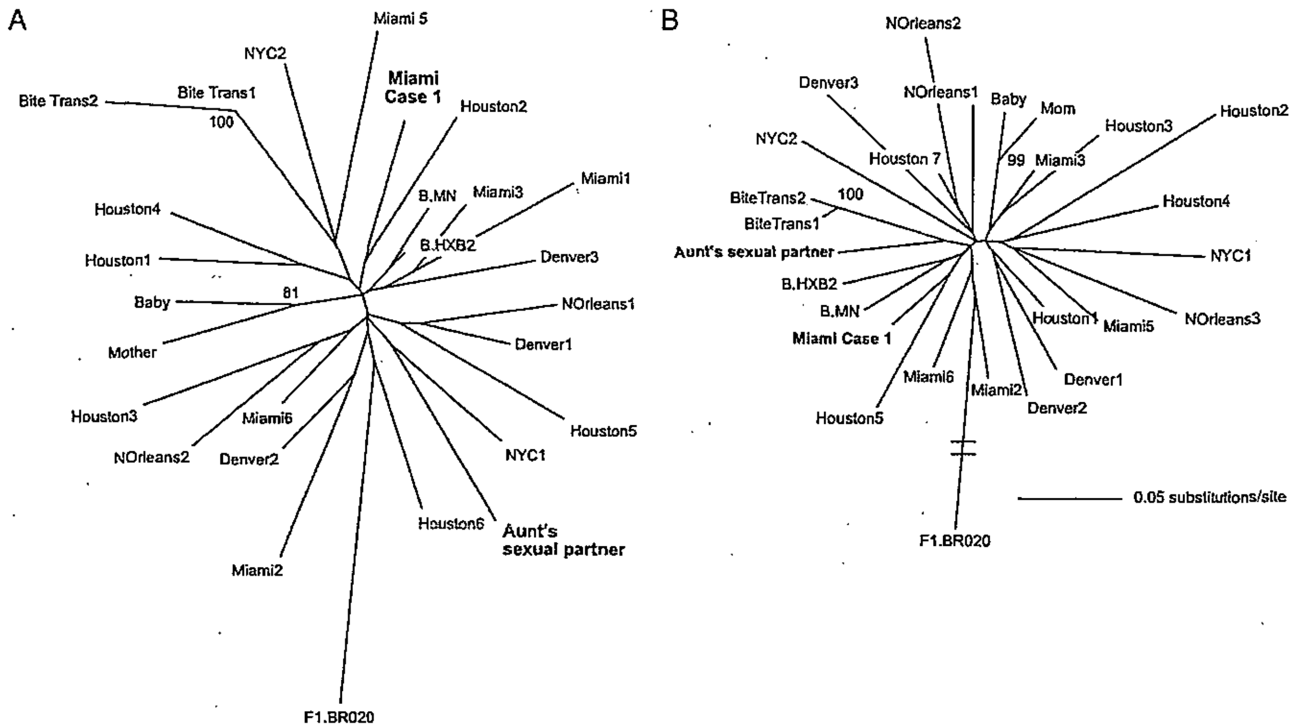

FIGURE 1

Case 1 (Miami). A, Phylogenetic relationship of the HIV sequences derived from case 1 and the great-aunt's sexual partner, 5 unrelated subtype B strains from Miami (Miami 1–3, 5, and 6), 15 unrelated subtype B strains from US cities other than Miami (Denver 1–3, Houston 1–6; New Orleans 1 and 2; New York City 1 and 2, B.MN, and B.HXB2), 1 subtype F strain (F1.BR020), and 2 epidemiologically related transmission pairs (bite transmission 1 and 2; infant and mother). Shown is a neighbor-joining tree of the gp17 region of *gag*, only bootstrap values of >70% are indicated. B, Phylogenetic relationship of the HIV sequences derived from case 1 and the great aunt's sexual partner, 4 unrelated subtype B strains from Miami (Miami 2, 3, 5, and 6), 16 unrelated subtype B strains from US cities other than Miami (Denver 1–3, Houston 1–5 and 7, New Orleans 1–3, New York City 1 and 2, B.MN, and B.HXB2), 1 subtype F strain (F1.BR020), and 2 epidemiologically related transmission pairs (bite transmission 1 and 2; infant and mother). Shown is a neighbor-joining tree of the C2V3C3 region of *env*; only bootstrap values of >70% are indicated. In A and B, US subtype B sequence strains were used as references along with subtype F as an outgroup. Sequences from 2 epidemiologically related transmission pairs were also included (bite transmission 1 and 2; infant and mother). Phylogenetic analysis shows no clustering or epidemiological relatedness between the virus from case 1 (Miami) and the virus from the great aunt's sexual partner.

Pediatric HIV Screening Clinic until 1993, when HIV-1 infection was ruled out on the basis of negative results from first-generation HIV-1 antibody test (EIAs) (Bio-Rad Laboratories) performed when the child was aged 20 and 21 months. PCR-based tests for HIV were not available for clinical care at that time. The child had normal immunoglobulin levels and a normal CD4 count (absolute count: 1700 cells per μL) at the time of the negative EIA results. Neither the mother nor child received perinatal antiretroviral prophylaxis. In 1995, at age 39 months, the child was seen by a pediatrician for anemia and recurrent submandibular lymphadenitis with abscess caused by *Mycobacterium fortuitum*. The mother's history of AIDS and intranasal cocaine abuse without intravenous drug abuse, combined with the child's clinical presentation, prompted the pediatrician to order an HIV-1 EIA (Bio-Rad Laboratories), a confirmatory Western blot, and p24 antigen testing for the child: all results were positive. A concurrent CD4 count of 24 cells per μL (1%) indicated severe immunosuppression.

The mother reported feeding the child premasticated table food but could not recall details regarding the child's age or her own oral health during the time she prechewed the child's food.

Phylogenetic analysis of the mother's and the child's HIV-1 sequences supported the epidemiologic conclusion that the mother was the source of the child's HIV-1 infection (Fig 2).

Case 3 (Memphis, TN)

In 2004, a 9-month-old black girl was seen in an emergency department because of fever, jaundice, nosebleed, oral thrush, and failure to thrive. HIV-1 infection was diagnosed based on an ultrasensitive HIV-1 RNA PCR of >100 000 copies per mL (Cobas Amplicor HIV-1 Monitor 1.5 test [Roche Molecular Systems, Inc, Branchburg, NJ]; dynamic range of detection: 50–100 000 copies per mL). Given the mother's history of chronic HIV infection since 1995, this child had previously been screened for perinatal infection. Three

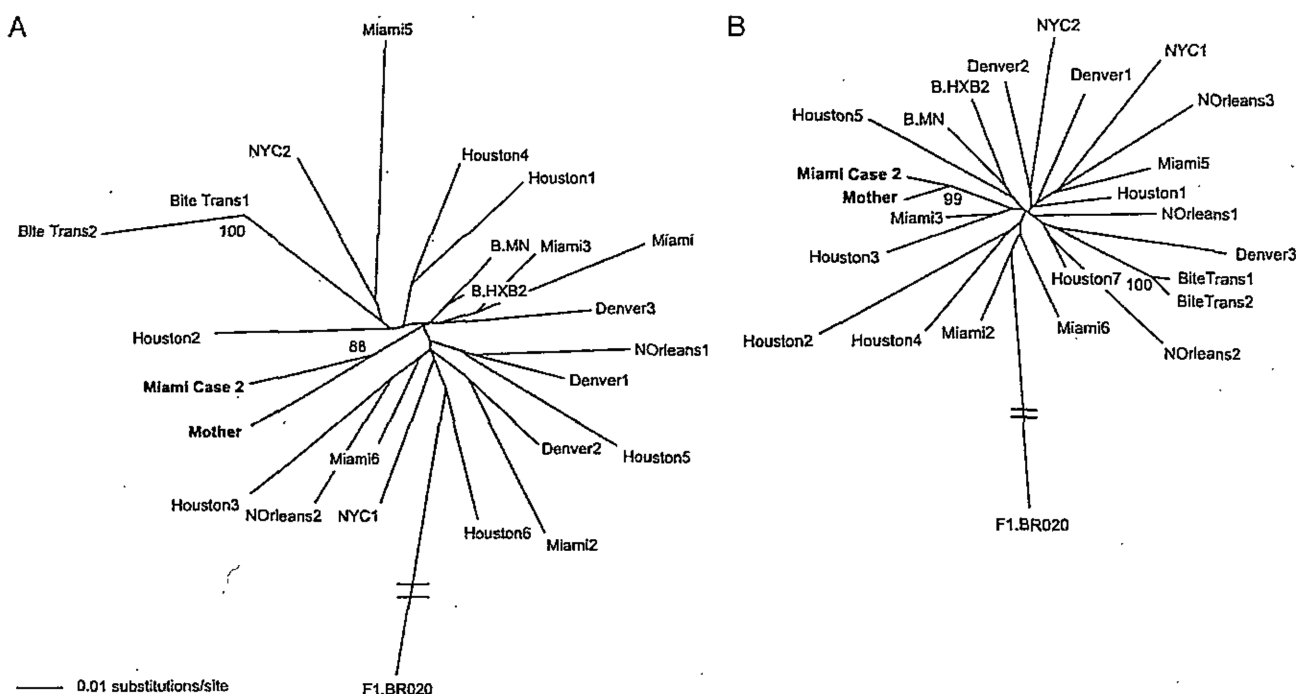

FIGURE 2
Case 2 (Miami). A, Phylogenetic relationship of the HIV sequences derived from the mother-infant pair of case 2, 5 unrelated subtype B strains from Miami (Miami 1–4 and 6), 15 unrelated subtype B strains from US cities other than Miami (Denver 1–3, Houston 1–6, New Orleans 1 and 2, New York City 1 and 2, B.MN, and B.HXB2), 1 subtype F strain (F1.BR020), and 1 epidemiologically related human bite-transmission pair (bite transmission 1 and 2). Shown is a neighbor-joining tree of the gp17 region of *gag*; only bootstrap values of >70% are indicated. In A and B, US subtype B sequences were used as reference strains along with a subtype F as an outgroup. Sequences from an epidemiologically related transmission pair were also included (bite transmission 1 and 2). Phylogenetic analysis shows strong clustering, with an 88% bootstrap support for the epidemiological relatedness between the virus from case 2 (Miami) and the child's mother. B, Phylogenetic relationship of the HIV sequences derived from the mother-infant pair of case 2 from Miami, 4 unrelated subtype B strains from Miami (Miami 2, 3, 5, and 6), 16 unrelated subtype B strains from US cities other than Miami (Denver 1–3, Houston 1–5 and 7, New Orleans 1–3, New York City 1 and 2, B.MN, and B.HXB2), 1 subtype F strain (F1.BR020), and 1 epidemiologically related human bite-transmission pair (bite transmission 1 and 2). Shown is a neighbor-joining tree of the C2V3C3 region of *env*; only bootstrap values of >70% are indicated. Phylogenetic analysis shows strong clustering with a 99% bootstrap support for the relatedness between the virus from case 2 (Miami) and the child's mother.

standard quantitative HIV RNA viral loads (Cobas Amplicor HIV-1 Monitor 1.5 test; dynamic range of detection: 400–750 000 copies per mL) were performed at 41, 60, and 118 days of life. Results of all 3 tests were negative (no copies of HIV RNA detected).

The mother (31 years old) had not adhered to highly active antiretroviral therapy during pregnancy. During pregnancy, she was started on nevirapine, stavudine, and lamivudine and was later switched to once-a-day ritonavir-boosted atazanavir and tenofovir because of poor compliance. Her viral load on the day before delivery was 35 100 copies per mL. The child was delivered at 35 weeks' gestation via cesarean delivery because of the mother's high blood pressure and edema. The mother received intravenous zidovudine before her cesarean delivery. The mother reported that she gave the infant oral zidovudine during the first 6 weeks of life and that the infant did not breastfeed.

At ~8 months of age, the child was seen for low-grade fever and was diagnosed with oral candidiasis and a nonspecific viral infection. In the following week, a red blotchy rash developed on the child's face, arms, and legs; the pediatrician ascribed the rash to allergic dermatitis.

A clinician who routinely queried caregivers about infant care feeding practices, including premastication, determined that the mother had intermittently offered the child prechewed meats from ~120 days of life until the child's current illness. The mother reported that during the period that she prechewed the child's food, she had intermittently bleeding gums and mouth sores that later resolved spontaneously or with medications for oral thrush. During this same period, the mother's adherence to highly active antiretroviral therapy was poor, her HIV viral load was 499 000 copies per mL, and her CD4 count was 100 cells per μL (6%).

Phylogenetic analysis of the mother's and the child's HIV-1 sequences sup-