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Human parvovirus PARV4 in clotting factor VIII concentrates

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Background and Objectives Parvoviruses are small non-enveloped DNA viruses, relatively resistant to virus inactivation procedures. The recently identified human parvovirus PARV4, including a related genotype 2 virus (also termed PARV5), has been found to be a contaminant of pooled plasma used in the manufacture of plasma-derived products. This report describes an investigation to determine whether PARV4 is present in clotting factor concentrates.

Materials and Methods Factor VIII concentrates manufactured in the past 30–35 years were screened for PARV4 and human parvovirus B19 (B19V) sequences. Viral loads in products testing positive for PARV4 were quantified using a consensus TaqMan assay designed to a highly conserved region. DNA sequence analysis was performed to confirm the genotypes present.

Results From a total of 175 lots of factor VIII concentrate, 28 of these contained PARV4 sequences, and in two lots both genotypes 1 and 2 were found to be present. The highest viral loads observed exceeded 10^5 copies per ml. The majority of factor VIII concentrates testing positive for PARV4 were manufactured in the 1970s and 1980s. Human B19V was also a frequent contaminant of these products.

Conclusions PARV4 was detected in 16% of factor VIII concentrates, particularly in older batches from the 1970s and 1980s. The significance in terms of the viral safety and potential transmission to recipients of these products is not yet known.

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Introduction

PARV4 was originally identified in plasma from a patient with symptoms of acute virus infection following high-risk behaviour for human immunodeficiency virus 1 (HIV-1) transmission, but subsequently confirmed to be HIV-1 negative [1]. This patient was an intravenous drug user, infected with hepatitis B virus (HBV), with a range of symptoms including fatigue, vomiting and diarrhoea, sore throat, neck stiffness and joint pains. Phylogenetic analysis showed that PARV4 did not closely resemble other known human or animal parvoviruses [1].

Parvovirus B19 (B19V) is the prototype human parvovirus, infecting erythroid progenitor cells leading to erythema infectiosum, aplastic crisis, arthropathy and hydrops fetalis [2]. B19V is normally transmitted via the respiratory route; however, transmission also occurs through the administration of contaminated blood products and solvent/detergent-treated plasma and can result in clinically apparent infection [3–6]. Since 2004, European regulations have required that manufacturers of certain plasma derivatives, including anti-D immunoglobulin and plasma pooled and treated for virus inactivation, screen pooled plasma for B19V by nucleic acid amplification techniques (NAT), and this has led to a reduction in the levels of B19V present in manufacturing start pools [7]. NAT screening for B19V has now been widely implemented by manufacturers.

We have recently demonstrated the presence of PARV4 and a related variant virus (termed PARV5), in pooled plasma used in the manufacture of plasma-derived medicinal products [8]. These viruses are frequently detected in 4–5% of these pools with viral loads of up to 10^6 copies per ml of plasma.

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In the case of blood donors, PARV4 and PARV5 have been found in approximately 2% of individuals and at a higher frequency in febrile patients [9]. Sequence analysis shows that PARV4 and PARV5 share ~92% nucleotide identity over a 4860-bp region [10], similar to the level observed between B19V genotypes 1–3 [11], to which PARV4 shares ~45% nucleotide identity. At the amino acid level, PARV4 and PARV5 sequences are more conserved, and this is especially the case for the second open reading frame (ORF2), encoding the viral capsid-like protein, such that PARV4 and PARV5 are likely to represent a single serotype [10]. This sequence analysis has led to the proposal that PARV4 and PARV5 should be referred to by a single virus name, PARV4, comprising genotypes 1 and 2 (previously PARV5). In this study, we have investigated the presence of PARV4 genotypes 1 and 2 in clotting factor VIII concentrates, manufactured over the past 30–35 years. We have also examined these products for the presence of B19V.

Materials and methods

Factor VIII concentrates

Coagulation factor VIII concentrate products received at the National Institute for Biological Standards and Control (NIBSC) were stored at 4 to −20 °C until analysis. A total of 175 lots of 12 factor VIII concentrate products, from 10 manufacturers (named A–J), were investigated. Products were manufactured over a 30- to 35-year period, with expiry dates ranging between 1974 and 2005. Factor VIII product details are further described in Table 1.

Nucleic acid extraction

Factor VIII concentrates were reconstituted in sterile distilled water according to the manufacturer's instructions. Total nucleic acid was extracted from 1 ml of reconstituted concentrate using the MagNA Pure LC instrument (Roche Applied Science, Mannheim, Germany) and was eluted in 50 μl as previously described [7].

Screening for PARV4 in factor VIII concentrates

Factor VIII concentrates were initially screened for the presence of PARV4 genotype 1 and 2 sequences using a gel-based polymerase chain reaction (PCR), using primers specific to ORF2 of PARV4 [9]. We have previously confirmed the specificity and sensitivity of these primers to be one to 10 copies of PARV4 sequences. The presence of PARV4 in factor VIII concentrates was confirmed by DNA sequence analysis of amplification products. Amplicons were purified using the QIAEX Gel Extraction kit (Qiagen, Hilden, Germany). Sequencing was performed using the BigDye Terminator v1·1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK), using the T7 promoter primer and the pUC/M13 reverse primer. Following removal of dye terminators, using the DyeEx 2·0 Spin Kit (Qiagen), sequencing reactions were run on an ABI 3130XL Genetic Analyser (Applied Biosystems).

Quantification of PARV4 in factor VIII concentrates

Following the initial screening of factor VIII concentrates for PARV4, viral loads in samples testing positive for these

Table 1 Detection of PARV4 and B19V in factor VIII concentrates

Product/ manufacturer	Expiry date	Number of lots tested	Purification process	Virus inactivation	Number of positive lots by PCR	
					PARV4	B19V
1/A	1974–1978	37	Precipitation	None	3	23
2/B	1976–1977	2	Precipitation	None	1	2
3/C	1976–1978	5	Precipitation	None	3	5
4/D	1977–1978	2	Precipitation	None	1	2
5/E	1977–1980	55	Precipitation	None	14	9
6/C	1985	1	Precipitation	Dry heat (68 °C, 72 h)	1	1
4/F	1985	1	Precipitation and adsorption	Wet heat (heptane) (60 °C, 20 h)	1	1
7/E	1985–1987	8	Precipitation and adsorption	Dry heat (68 °C, 72 h)	0	5
8/A	1986	4	Precipitation (plus further purification)	Steam treatment (60 °C, 10 h)	3	4
9/EGH	1997–2004	16	Monoclonal antibody	Pasteurization (60 °C, 10 h)	0	2
10/I	1998–2002	13	Monoclonal antibody	Solvent/detergent	0	7
11/I	1999–2003	13	Precipitation	Dry heat (80 °C, 72 h)	1	7
12/J	2001–2005	18	Affinity chromatography	Solvent/detergent, dry heat (80 °C, 72 h)	0	2
				Total number of positive lots/number of lots tested	28/175	70/175

viruses were determined using a real-time PCR assay designed to a highly conserved region of PARV4 as previously described [9,10]. The primers used in this assay are directed towards a region of ORF2 of PARV4 that is highly conserved between the two genotypes. A standard curve was generated from plasmid DNA containing the 103-bp ORF2 PCR product.

Detection of B19V DNA in factor VIII concentrates

Coagulation factor concentrates were additionally tested for the levels of B19V DNA using an in-house PCR assay as previously described [7]. This assay detects B19V genotypes 1–3.

DNA sequence analysis of a variable region of ORF1 of PARV4

Using a multiple sequence alignment of near full-length PARV4 genomes (GenBank accession no. DQ873386-91) [10], primers were designed to a variable region of the PARV4 genome. Primers PARV35F (5′ TTCCTACTGGATTTCTCTCCAACC 3′) and PARV596R (5′ GGTAAGGCAATAGCACCTTGAGG 3′) were used to amplify a 562-bp region of ORF1 of PARV4 (corresponding to nucleotides 317–878 of PARV4 genotype 1, GenBank accession no. AY622943, and nucleotides 151–712 of PARV4 genotype 2, GenBank accession no. DQ873390), from extracted factor VIII samples. Amplification reactions were performed using the proof-reading enzyme Phusion™ Hot Start DNA Polymerase (Finnzymes OY, Espoo, Finland) as described previously [8]. For thermal cycling, a T3 thermal cycler (Biometra, Göttingen, Germany) was used with the following cycling conditions: 98 °C for 30 seconds, followed by 45 cycles of 98 °C for 10 seconds, 59 °C for 30 seconds and 72 °C for 20 seconds. Amplicons were analysed by agarose gel electrophoresis and compared with known size markers. Amplification products were purified as before, and cloned into the pT7 Blue vector according to the manufacturer's instructions (Novagen, Darmstadt, Germany). Sequencing was performed as previously described and was analysed using the GCG software package, version 10·2 (University of Wisconsin, Madison, WI, USA). Sequences were aligned using Clustal W [12], and a neighbour-joining tree (nucleotide distance with Jukes–Cantor correction, pairwise gap deletion) with bootstrap resampling (100 replicates), was constructed using MEGA3 software [13].

Results

Contamination of factor VIII concentrates with human parvoviruses

A total of 175 lots of 12 factor VIII concentrate products, from 10 manufacturers, were examined for the presence of PARV4 and B19V DNA by PCR. The expiry dates on these lots ranged from 1974 to 2005. As shown in Table 1, 16% (28/175) of lots tested positive for PARV4, while 40% (70/175) of lots tested positive for B19V DNA. The majority of factor VIII products testing positive for PARV4 DNA had an expiry date of pre-1990 [23% (27/115) of lots expiring 1974–1989 tested positive for PARV4, while only 2% (1/60) of lots expiring 1990–2005 tested positive for PARV4 DNA] (Fig. 1). In contrast, there was no significant difference in the prevalence of B19V in factor VIII products expiring pre- and post-1990 [45% (52/115) of lots expiring 1974–1989 tested positive for B19V, while 30% (18/60) of lots expiring 1990–2005 tested positive for B19V DNA] (Fig. 1).

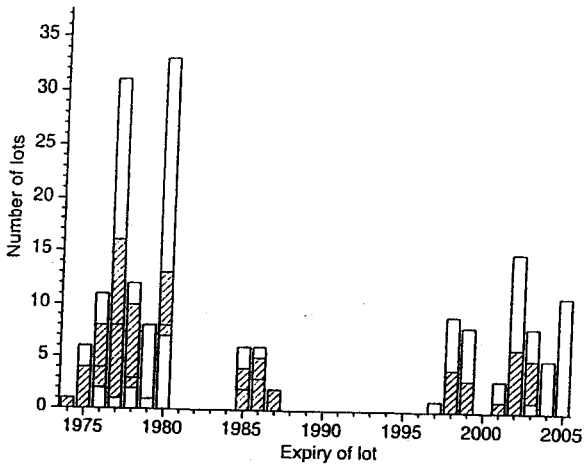

Fig. 1 Prevalence of parvoviruses PARV4 and B19V in factor VIII concentrates manufactured over the past 30–35 years. The number of lots testing positive for PARV4 (□), B19V (▨), both PARV4 and B19V (▨), and those testing negative for these viruses (□) are shown.

PARV4 ORF2 PCR products amplified by the gel-based assay were sequenced, and the majority determined to be of PARV4 genotype 2 (Table 2). In two factor VIII products both PARV4 genotype 1 and 2 sequences were amplified and sequenced. Viral loads of PARV4 in factor VIII products were determined by a consensus sequence real-time PCR assay [9], designed to detect a highly conserved region of ORF2 of PARV4. Viral loads ranged from < 100 to more than 3×10^5 copies per ml of product (Table 2), with the majority of contaminated lots containing 4–5 $\log_{10}$ PARV4 copies per ml of product (Fig. 2). The levels of B19V were as high as $2{\cdot}5 \times 10^8$ IU/ml of product (Table 2).

Manufacturing plasma pools relating to these factor VIII products were only available for the most recent factor VIII products. Factor VIII product number 28 (Table 2) had an expiry date of 2003, and was manufactured from two plasma pools 28A and 28B. Plasma pool 28A tested positive for PARV4 genotype 1 DNA by PCR with a viral load of $3{\cdot}3 \times 10^5$ copies per ml of plasma, while pool 28B tested negative for both PARV4 genotypes.

Table 2 Levels of PARV4 and B19V in factor VIII concentrates testing positive for PARV4 DNA

Factor VIII	Product/ manufacturer	Expiry date	PARV4 genotype[a]	PARV4 viral load (log_{10} genome copies per ml product)	B19V viral load (log_{10} IU/ml product)
1	1/A	1976	2	< 2·00[c]	8·40
2		1977	1	1·89	6·71
3		1977	1 & 2[b]	1·71	7·64
4	2/B	1977	2	3·11	2·59
5	3/C	1976	2	1·82	4·91
6		1977	2	3·28	5·33
7		1978	1	1·86	2·75
8	4/D	1977	2	2·48	2·22
9	5/E	1977	2	1·75	–
10		1977	2	4·10	2·39
11		1977	2	4·82	6·05
12		1978	2	4·15	–
13		1978	2	4·36	–
14		1979	2	2·66	–
15		1980	1	4·31	6·44
16		1980	1 & 2	3·01	–
17		1980	2	4·39	–
18		1980	2	5·49	–
19		1980	2	5·03	–
20		1980	2	2·37	–
21		1980	2	4·30	–
22		1980	2	2·00	–
23	4/F	1985	1	< 2·00[c]	4·57
24	6/C	1985	1	1·32	5·79
25	8/A	1986	1	4·08	7·15
26		1986	2	3·81	5·85
27		1986	2	4·53	4·36
28	11/I	2003	1	2·32	–

[a]Determined by sequencing of ORF2 amplification products.

[b]ORF2 amplification products were determined to be PARV4 genotype 1 sequences, while the amplified variable ORF1 region was determined to be PARV4 genotype 2.

[c]Factor VIII lot tested positive for PARV4 DNA by qualitative PCR but the viral load was below the level of quantification by real-time PCR, and was therefore given an arbitrary viral load of < 2 log_{10} genome copies per ml product.

–, product tested negative for B19V DNA.

Analysis of PARV4 sequences

Previous analysis of PARV4 sequences showed that ORF1 was slightly less conserved than ORF2 [10]. We therefore amplified and sequenced a 562-bp variable region at the 5′ end of ORF1 from 26/28 factor VIII concentrates testing positive for PARV4 sequences. It had not been possible to amplify the 562-bp variable ORF1 region of PARV4 from factor VIII products 7 and 9 (Table 2). Both PARV4 genotype 1 and 2 sequences were amplified from factor VIII product number 16 (Table 2). Phylogenetic analysis of these PARV4 sequences shows that they fall into two distinct genetic clusters, representing genotypes 1 and 2 (Fig. 3). Across the two genotypes, PARV4 nucleotide sequences amplified from factor VIII products differ from each other by greater than 11% over the region sequenced. Within each genotype, all PARV4 sequences amplified from factor VIII concentrates were greater than 99% homologous (at the nucleotide level, over the 515-bp region sequenced), despite products being manufactured over a 30- to 35-year period. In fact, several PARV4 genotype 1 and 2 sequences amplified from factor VIII products manufactured as early as the mid-1970s were 100% identical at the nucleotide level, over the 515-bp region sequenced, to the recently identified respective strains BR10749 (genotype 1) and BR10627 (genotype 2) [10].

Discussion

We recently demonstrated the presence of the newly identified human parvovirus PARV4 including the related genotype 2

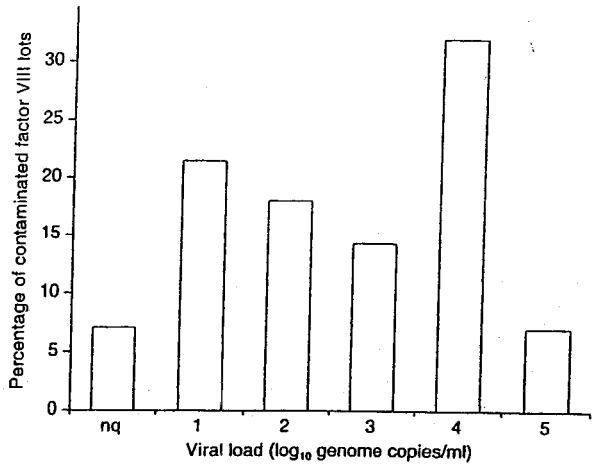

Fig. 2 Viral DNA loads of PARV4 (log_{10} genome copies per ml) in contaminated factor VIII concentrates. nq, not quantifiable.

virus (previously termed PARV5) in manufacturing plasma pools, with these viruses detected in approximately 5% of pools [8,9]. In this present study, we have detected PARV4 viruses in products derived from such plasma pools, specifically in coagulation factor VIII products, manufactured over the past 30–35 years. Information regarding the source of plasma used in the manufacture of products examined in this study was difficult to obtain as it is not provided with the products. These details could only be obtained for the most recent factor VIII product testing positive for PARV4 DNA. This factor VIII concentrate had an expiry date of October 2003, and was manufactured from two plasma pools in September 2000. Donations relating to these plasma pools were collected in or after July 1998 from paid donors from the USA. This suggests that viruses detected in these factor VIII products may date from up to 5 years prior to the expiry date on the product. Details from other manufacturers of recent factor VIII concentrates (testing negative for PARV4) also indicate that donations relating to these products were sourced up to 5 years prior to the expiry date.

The prevalence of PARV4 in factor VIII concentrates was found to be greater in products expiring pre-1990 than in those with an expiry date of post-1990. This difference in the prevalence of PARV4 in factor VIII products over time may reflect the introduction of blood safety measures from the mid-1980s in response to the HIV epidemic, in particular, the introduction of screening tests for HIV and hepatitis C virus (HCV) (in 1986 and 1991, respectively), and virus inactivation of manufacturing plasma pools (introduced in the mid-1980s). The screening of blood donations for HIV and HCV identified 'high-risk' donor groups, such as homosexual males and individuals with a history of intravenous drug use (IVDU), and these groups were subsequently excluded from donating blood [14]. Factor VIII products tested in this study

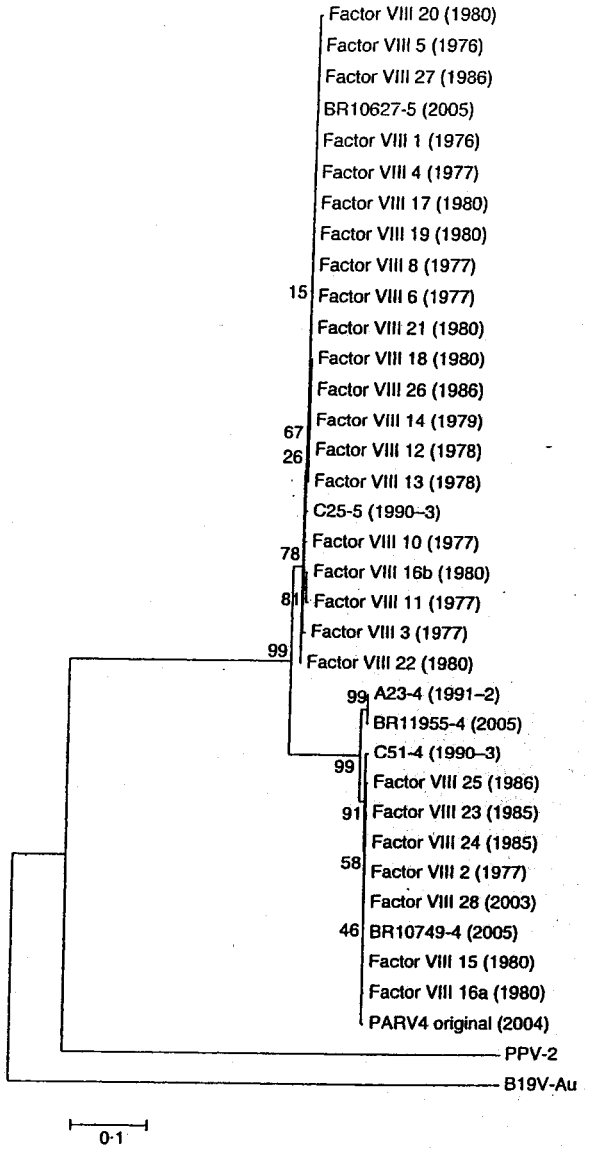

Fig. 3 Phylogenetic analysis of a 515-bp region of ORF1 of PARV4 amplified from factor VIII concentrates. Sequences are named according to factor VIII number and expiry of lot (Table 2). The alignment includes other recently sequenced strains of PARV4 genotype 1; PARV4 original (GenBank accession no. AY622943), BR10749-4 (GenBank accession no. DQ873386), BR11955-4 (GenBank accession no. DQ873388), A23-4 (GenBank accession no. DQ873389) and C51-4 (GenBank accession no. DQ873387); and PARV4 genotype 2, BR10627-5 (GenBank accession no. DQ873390) and C25-5 (GenBank accession no. DQ873391). The PARV4 original strain was sourced from the index case patient in 2004 [1]. Strains BR10749-4 and BR10627-5 were identified in our preliminary study of plasma pools [8], while the other strains were identified in further screening studies of manufacturing plasma pools [9]. Strains BR10749-4, BR11955-4 and BR10627-5 were from plasma samples received at NIBSC between 2004 and 2005, while A23-4, C51-4 and C25-5 were received at NIBSC 1990–1993. The alignment also includes the corresponding nucleotide sequences of porcine parvovirus 2 (PPV-2) (GenBank accession no. AB076669) and B19V-Au genotype 1 virus (GenBank accession no. M13178) as outgroups. Genetic distance and bootstrap values are indicated.

with an expiry date of post-1990 are likely to originate from plasma sourced from screened 'low-risk' blood donors. Therefore, the reduced prevalence of PARV4 in more recently manufactured factor VIII products may be a result of the removal of specific 'high-risk' donor populations.

Virus inactivation using a variety of heat treatments was introduced into the manufacturing process of existing coagulation factor products in the mid-1980s, before the implementation of HIV and HCV screening. The effectiveness of these treatments, for HCV particularly, varied greatly, depending on the duration and temperature of heating and whether the product is in liquid form or lyophilized [15,16]. Other virus inactivation procedures include solvent/detergent treatment, which is effective against enveloped viruses [17,18]. Animal parvoviruses, such as canine, bovine and porcine parvoviruses, and minute virus of mice, were used to investigate the effectiveness of virus inactivation of plasma prior to the development of cell culture-based assays for B19V. By virtue of their small size and absence of viral envelope, animal parvoviruses are relatively resistant to inactivation by a range of heat and chemical agents [19]. Based on studies using these model parvoviruses, B19V was also expected to be resistant to these virus inactivation strategies and unlikely to be effectively eliminated by dry heat and pasteurization [5]. However, recent studies using B19V cultures suggest that it is more susceptible to heat and low pH treatments than other animal parvoviruses [20–23]. Results here show that there was not a significant reduction in the prevalence of B19V DNA in factor VIII products manufactured after the introduction of virus inactivation procedures (B19V DNA was detected in 41% of products manufactured without virus inactivation measures vs. 39% of products manufactured using virus inactivation steps). However, it must be noted that virus inactivation procedures such as heat and low pH treatments do not physically remove viral DNA, which may still be detectable by NAT. The effect of virus inactivation procedures on PARV4 remains to be determined; however, the reduced prevalence of PARV4 in factor VIII products manufactured with virus inactivation (8% in virus inactivated products vs. 22% in products manufactured without virus inactivation) may suggest that these viruses are susceptible to virus inactivation treatments. The increased prevalence of PARV4 in factor VIII concentrates expiring in the late 1970s and mid-1980s may also result from epidemics of infection as has been observed for B19V [2]. Our investigation of recent and archived manufacturing plasma pools for PARV4 identified an increased prevalence of these viruses in plasma pools received from one manufacturer between 1991 and 1992, which may be the result of seasonal and/or epidemic variation [9].

PARV4 viral loads in these factor VIII concentrates were as high as 5 $\log_{10}$ per ml of product, while the levels of B19V were as high as 8 $\log_{10}$ per ml of product. The higher levels of contaminating PARV4 and B19V viruses were confined to the older factor VIII concentrates (expiring pre-1990). Considering that downstream purification and processing of manufacturing plasma pools will alter the viral loads present in subsequent plasma-derived products, viral loads in these factor VIII concentrates correlate well, albeit being approximately 1 $\log_{10}$ lower, with the levels of PARV4 and B19V detected in recent and archived plasma pools [8,9]. In these manufacturing plasma pools, the viral loads of these viruses typically range up to 6 $\log_{10}$ per ml of plasma for PARV4, and up to 9 $\log_{10}$ per ml of plasma for B19V.

In manufacturing plasma pool samples previously examined for the presence of PARV4, we found that genotypes 1 and 2 were detected in approximately equal proportions [8,9]. These samples were received at NIBSC for plasma pool testing between 2005 and 2006, but also included archived samples received between 1990 and 1993. In this present study, we detected a greater prevalence of PARV4 genotype 2 over genotype 1 in factor VIII concentrates manufactured in the past 30–35 years (21 products testing positive for PARV4 genotype 2 sequences vs. nine products testing positive for PARV4 genotype 1 sequences). As the majority of these PARV4-positive factor VIII products had expiry dates of pre-1990 and were likely to have been manufactured from blood donations collected before the mid-1980s, these results suggest a temporal change in the prevalence of PARV4 genotypes over the past 30–35 years. A similar temporal change in parvovirus genoprevalence has been suggested in the case of B19V genotypes 1 and 2, where both genotypes were equally detected in the tissues of individuals born in the 1950s or earlier, while genotype 1 viruses were predominantly detected in the tissues of individuals born in the 1960s and later [24]. Further evidence for a temporal succession of infection with PARV4 genotype 1 over genotype 2 has recently been reported in HIV infected patients [25].

Although positive PCR results do not necessarily reflect infectivity, the detection of PARV4 DNA in coagulation factor VIII concentrates in this study raises questions as to whether PARV4 has been transmitted parenterally to the recipients of such products. PARV4 was originally identified in an individual who was a daily injecting drug user and it is possible that he acquired the virus through this route [1]. In addition, we have identified an increased incidence in the detection of PARV4 in febrile patients, including IVDUs and homosexual men [9], and in individuals infected with HCV (including IVDUs) [26]. An increased prevalence of PARV4 in HIV-infected individuals has also recently been reported [25]. Nothing is yet known as to whether there is any pathology associated with PARV4 infection. Although the PARV4 index case patient had an acute viral infection syndrome, the lifestyle of this individual and an underlying infection with HBV make it impossible to determine whether PARV4 played a role in his symptoms [1]. The presence of PARV4 in pooled plasma from healthy blood donors suggests that infection

may cause subclinical infections, and the implications for the safety of blood and plasma-derived products such as factor VIII are still not known.

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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 2008年2月7日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり		研究報告の公表状況	The NEW ENGLAND JOURNAL of MEDICINE 2008 Feb 6 [Epub ahead of print]	公表国 オーストラリア	
販売名（企業名）	別紙のとおり					

研究報告の概要

問題点:脳出血で死亡した臓器提供者から腎臓と肝臓の提供を受けた後に死亡した3人の女性から発見されたウイルスは、遺伝子配列解析により、リンパ球性脈絡膜髄膜炎ウイルス様の新種のアレナウイルスと判明した。

2006年12月に3ヵ月間の前ユーゴスラビアの地方滞在からオーストラリアに帰国して10日後に脳出血で死亡した57才の臓器提供者から、腎臓と肝臓の提供を受けた63,64,44才の3人の女性が死亡した。女性たちは、移植直後の経過に変わったところはなかったが、その後脳症を伴う熱性疾患を発症し、提供を受けて4～6週後の2007年1月初めに死亡した。2人の患者から移植されたそれぞれの肝臓と腎臓のRNAの塩基配列を解析した結果、リンパ球性脈絡膜髄膜炎ウイルス（LCMV）様の新種のアレナウイルスと思われる遺伝子配列が検出された。また、PCR解析により、患者の腎臓、肝臓、血液及び髄液からウイルスの遺伝子断片が、免疫組織化学的解析により、移植された肝臓及び腎臓からウイルスの抗原が検出された。さらに、患者血清からは抗ウイルスIgM及びIgG抗体も検出された。

使用上の注意記載状況・その他参考事項等

記載なし

報告企業の意見	今後の対応
別紙のとおり	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗HBs人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤献血静注グロブリン“化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン“化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン20%化血研*、⑱アルブミン5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500注射用
報告企業の意見	LCMVは、アレナウイルス属に属するエンベロープに包まれた直径30～300nmの不定形粒子であり、二種類のマイナス一本鎖RNAを有する。げっ歯類を自然宿主とし、その糞尿や唾液、血液の曝露によってヒトに伝播する。LCMV感染症は多くは無症候性あるいは軽度であるが、妊婦では無菌性髄膜炎や脳炎、致死性の感染症を起こす危険がある。臓器移植患者におけるLCMVのヒト-ヒト感染は、過去にも報告がある。 弊所の血漿分画製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。 各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年8月30日)」に従い、ウシウイルス性下痢ウイルス（BVDV)、仮性狂犬病ウイルス（PRV)、ブタパルボウイルス（PPV)、A型肝炎ウイルス（HAV）または脳心筋炎ウイルス（EMCV）をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告したリンパ球性脈絡膜髄膜炎ウイルス（LCMV）は、エンベロープの有無、核酸の種類等からモデルウイルスとしてはBVDVが該当すると考えられるが、上記バリデーションの結果から、BVDVの除去・不活化効果を有することを確認している。 また、これまでに当該製剤によるLCMV感染の報告例は無い。 以上の点から、当該製剤はLCMVに対する安全性を確保していると考える。

*現在製造を行っていない

The NEW ENGLAND JOURNAL of MEDICINE

A New Arenavirus in a Cluster of Fatal Transplant-Associated Diseases

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ABSTRACT

BACKGROUND

Three patients who received visceral-organ transplants from a single donor on the same day died of a febrile illness 4 to 6 weeks after transplantation. Culture, polymerase-chain-reaction (PCR) and serologic assays, and oligonucleotide microarray analysis for a wide range of infectious agents were not informative.

METHODS

We evaluated RNA obtained from the liver and kidney transplants in two recipients. Unbiased high-throughput sequencing was used to identify microbial sequences not found by means of other methods. The specificity of sequences for a new candidate pathogen was confirmed by means of culture and by means of PCR, immunohistochemical, and serologic analyses.

RESULTS

High-throughput sequencing yielded 103,632 sequences, of which 14 represented an Old World arenavirus. Additional sequence analysis showed that this new arenavirus was related to lymphocytic choriomeningitis viruses. Specific PCR assays based on a unique sequence confirmed the presence of the virus in the kidneys, liver, blood, and cerebrospinal fluid of the recipients. Immunohistochemical analysis revealed arenavirus antigen in the liver and kidney transplants in the recipients. IgM and IgG antiviral antibodies were detected in the serum of the donor. Seroconversion was evident in serum specimens obtained from one recipient at two time points.

CONCLUSIONS

Unbiased high-throughput sequencing is a powerful tool for the discovery of pathogens. The use of this method during an outbreak of disease facilitated the identification of a new arenavirus transmitted through solid-organ transplantation.

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METHODS OF CLONING NUCLEIC ACIDS of microbial agents directly from clinical specimens offer new opportunities for the surveillance and discovery of pathogens. Molecular techniques have been used successfully in the identification of infectious agents such as the Borna disease virus, hepatitis C virus, Sin Nombre virus, human herpesviruses 6 and 8, *Bartonella henselae*, *Tropheryma whipplei*, West Nile virus, and the coronavirus associated with severe acute respiratory syndrome.[1]

The arenaviruses are enveloped, negative-strand RNA viruses in rodents; these viruses are most frequently transmitted to humans through exposure to infected urine. Infection with the prototype virus, lymphocytic choriomeningitis virus (LCMV), is typically asymptomatic or associated with mild, transient illness; however, LCMV has also been implicated in aseptic meningitis.[2] Human-to-human transmission of LCMV during pregnancy has been reported, and infection during the gestational period can result in fetal death, neurologic sequelae, and chorioretinopathy.[3] Fatal outbreaks of disease associated with human-to-human transmission of LCMV in recipients of solid-organ transplants have also been described.[4] We report the use of unbiased DNA sequencing in the discovery of a new LCMV-related arenavirus that caused fatal disease in three recipients of organs from a single donor.

METHODS

PATIENTS AND CLINICAL COURSE

Three women in Australia who were 63 years of age (Recipient 1), 64 years of age (Recipient 2), and 44 years of age (Recipient 3) received a liver transplant (Recipient 2) or kidney transplants (Recipients 1 and 3) from one male donor who was 57 years of age. The donor died of cerebral hemorrhage 10 days after returning to Australia from a 3-month visit to the former Yugoslavia, where he had traveled in rural areas. The immediate post-transplantation course in the three transplant recipients was unremarkable; however, febrile illnesses with varying degrees of encephalopathy developed in all three, and they died 4 to 6 weeks after transplantation (Table 1). Bacterial and viral cultures; polymerase-chain-reaction (PCR) assays for herpesviruses 1 through 8, lyssavirus, influenza A and B viruses, respiratory syncytial virus, picornavirus, adenovirus, human parainfluenza virus, flavivirus, alphavirus, hantavirus, polyomavirus, Crimean–Congo hemorrhagic fever virus, Rift Valley fever virus, toxoplasma, *Mycobacterium tuberculosis*, and *Mycoplasma pneumoniae*; and viral and panmicrobial oligonucleotide microarray analysis[4] revealed no candidate pathogens.

UNBIASED HIGH-THROUGHPUT SEQUENCING

RNA was extracted from the brain, cerebrospinal fluid, serum, kidney, and liver of Recipient 1, who had received a kidney transplant, and from the cerebrospinal fluid and serum of Recipient 2, who had received a liver transplant. As shown in Figure 1, after digestion with DNase I to eliminate human chromosomal DNA, RNA preparations were amplified by means of reverse-transcriptase PCR (RT-PCR) with the use of random primers.[5,6] Amplification products were pooled and sequenced with the use of the GSL FLX platform (454 Life Sciences), but DNA fragmentation was omitted.[7] After trimming to remove sequences derived from the amplification primer and after filtration to eliminate highly repetitive sequences, the data set was analyzed by subtracting fragments that matched human sequences, clustering nonredundant sequences,[8] and assembling them into contiguous sequences[9] for direct comparison with the GenBank databases of nucleic acids and proteins with the use of BLASTN and BLASTX software.[10] We analyzed the resulting alignments and assigned them to nodes in the National Center for Biotechnology Information taxonomy database, using a custom software application written in Perl (BioPerl version 5.8.5).

PCR QUANTITATION OF THE ARENAVIRUS BURDEN

RNA obtained from tissues, plasma, serum, and cerebrospinal fluid was reverse transcribed with the use of random hexamers. PCR was performed with the use of a SYBR Green assay (Applied Biosystems). The following cycling conditions were used: 50°C for 2 minutes and 95°C for 10 minutes, followed by 45 cycles at 95°C for 15 seconds and 60°C for 1 minute. Real-time PCR assays were performed with the following primer set: 5′AGTGCYTGCACAACATCGTTT3′ (forward) and 5′CAATGCCCAGCYTGACAAT3′ (reverse). Thermal cycling was performed with the use of an ABI 7500 real-time PCR system (Applied Biosystems).