医薬品 研究報告 調査報告書

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一 般 的 名 称	別紙のとおり		研究報告の公表 状況	Novel Parvovirus and Related Variant in Human Plasma Emerging Infectious Diseases Vol. 12, No. 1, January 2006	公表国		
販 売 名（企 業 名）	別紙のとおり					米国	

研究報告の概要	（問題点：医薬品製造用としてプールされた血漿サンプルから新しいパルボウイルス（PARV4）の遺伝子が検出された。） 　PCR 法により、急性 HIV 感染の症状を示す患者の血漿から新しいパルボウイルス（PARV4）の遺伝子が検出されたが、遺伝子のみの検出であり、ウイルス本体は未だ見つかっていない。また、PARV4 の患者数、PARV4 疾患における役割、あるいは動物宿主からヒトに感染したのかどうか等、一切分かっていない。 　本研究報告では、血漿から製造される医薬品製造用としてプールされた血漿をサンプルとして、PCR 法で PARV4 遺伝子の検出を行った。その結果、137 の血漿プール（9 社の合計）のうち、7 つが PARV4 及びそのバリアントである PARV5 に陽性反応を示した。 　採血時には無症状であった健康なヒトからの献血により血漿は得られた。PARV4 を検出するためのより特異的で感度の高い測定法によって、この新しいウイルスのヒトでの疾患での役割の更なる解析と汚染された血液と血液製剤による感染の関係を調べることができるであろう。	使用上の注意記載状況・ その他参考事項等
		別紙のとおり 　代表製剤として、献血アルブミン 20 “化血研” の「使用上の注意」の記載状況を示す。

報告企業の意見	今後の対応
別紙のとおり	現時点においては、特段の対応は不要と考えるが、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。

Novel Parvovirus and Related Variant in Human Plasma

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We report a novel parvovirus (PARV4) and related variants in pooled human plasma used in the manufacture of plasma-derived medical products. Viral DNA was detected by using highly selective polymerase chain reaction assays; 5% of pools tested positive, and amounts of DNA ranged from <500 copies/mL to >10^6 copies/mL plasma.

Using a sequence-independent polymerase chain reaction (PCR) amplification method, we recently identified a new parvovirus in plasma from a patient with exposures and symptoms consistent with acute HIV infection, but who was HIV RNA negative (1). Phylogenetic analyses of sequence data suggest that this virus, termed PARV4, is only distantly related to previously known human or animal members of the family *Parvoviridae*, including members of the *Erythrovirus* genus known to infect humans, such as parvovirus B19. Infection with parvovirus B19, although frequently asymptomatic, may result in erythema infectiosum, arthropathy, pregnancy complications (e.g., hydrops fetalis), transient aplastic crisis, and disease in immunocompromised patients (2). Parvovirus B19 is most frequently transmitted through the respiratory route or vertically from mother to fetus. However, blood- and plasma-derived medical products, particularly clotting factors, contaminated with parvovirus B19 can also transmit the virus (3). Manufacturers of plasma derivatives screen minipools by using nucleic acid amplification techniques (NAT), which has enabled levels of erythrovirus DNA to be substantially reduced in start pools; for certain products, screening is now a regulatory requirement (4). This study examined pooled human plasma for fractionation to detect PARV4 DNA sequences.

The Study

Samples of manufacturing plasma pools submitted to the National Institute for Biological Standards and Control

for testing for hepatitis C virus RNA were stored at −70°C until analysis, in compliance with European regulatory requirements. Manufacturing pools were sourced from donations collected in Europe and North America and received during the previous 6 months. Total nucleic acid was extracted from plasma pools as described previously (4) before analysis for PARV4 DNA.

Using multiple sequence alignments of human erythroviruses and comparison with the sequence for PARV4 (1), we designed highly selective primers to the open reading frame 1 (ORF1) of PARV4, homologous to the non-structural proteins of other parvoviruses. Primers PV4ORF1F (5′-AAGACTACATACCTACCTGTG-3′) and PV4ORF1R (5′-GTGCCTTTCATATTCAGTTCC-3′) amplify a 220-bp region of ORF1. The specificity of these primers was confirmed by PCR using a cloned fragment of the ORF1 region alongside erythrovirus control material (Figure 1A). Each PCR contained 1× PCR buffer II (PE Applied Biosystems, Warrington, UK), 200 µmol/L each deoxynucleoside triphosphate, 2 mmol/L $MgCl_2$, 10 pmol each primer, and 2.5 U AmpliTaq Gold DNA polymerase (PE Applied Biosystems) in a final volume of 50 µL. For thermal cycling, a T3 thermal cycler (Biometra, Göttingen, Germany) was used with the following cycling conditions: 95°C for 9 min, followed by 45 cycles of 96°C for 30 s, 55°C for 30 s, and 72°C for 1 min. Amplicons were analyzed by agarose gel electrophoresis and compared to known size markers. The PARV4 control sequences (nucleotides 1293–1833 of ORF1, GenBank accession no. AY622943) were cloned into the vector pT7 Blue according to the manufacturer's instructions (Novagen, Darmstadt, Germany). The sensitivity of these PCR reactions was 1–10 copies of PARV4 sequences. DNA extracted from 137 pools was screened for PARV4 ORF1 sequences by PCR using 5 µL extracted DNA. Results, summarized in Table 1, show that 7 of 137 plasma pools screened with these primers tested positive for PARV4 DNA sequences and those of a related variant, known as PARV5. Typical results from pools and control plasmid samples are shown in Figure 1B. DNA sequence analysis showed that PARV5, over the region amplified, shares ≈92% nucleotide identity with PARV4 (Figure 2). Further sequence analysis around the primer-binding sites showed that the primers were 100% homologous in both genotypes. This level of relatedness is similar to that seen for the different erythrovirus genotypes (7).

The levels of PARV4 in the positive plasma pools were determined by real-time PCR using the screening primers from the ORF1 region of PARV4. Amplification reactions were performed on the LightCycler instrument using the LightCycler FastStart DNA Master[PLUS] SYBR green I kit (Roche Applied Science, Mannheim, Germany) in accordance with the manufacturers' instructions. A standard

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A   B
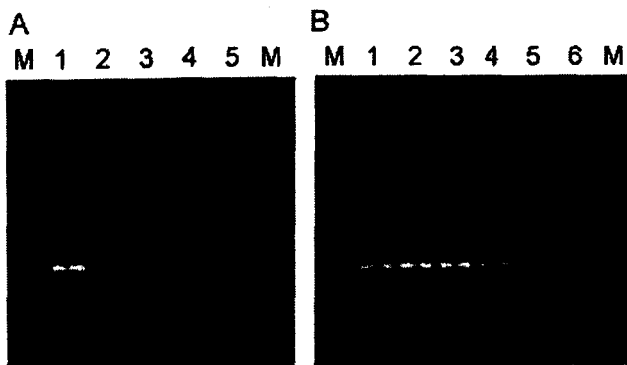M 1 2 3 4 5 M   M 1 2 3 4 5 6 M

Figure 1. A) Specificity of primers for PARV4. Samples in lanes 1–5 were amplified by using primers directed to open reading frame 1 (ORF1) of PARV4. Template DNA in lane 1 was a plasmid subclone of the PARV4 ORF1 region. In lane 2, the template DNA was derived from parvovirus B19 International Standard (99/800, National Institute for Biological Standards and Control, South Mimms, UK) as representative of genotype 1 erythrovirus sequences; in lane 3, the template DNA was derived from a genotype 2 erythrovirus plasmid clone (A6; obtained from K. Brown, National Heart, Lung and Blood Institute, Bethesda, MD, USA); in lane 4, the template DNA was derived from a genotype 3 erythrovirus plasmid clone (D91.1; obtained from A. Garbarg-Chenon, Hôpital Trousseau, Paris, France). Template DNA in the erythrovirus samples (lanes 2–4) was adjusted to give ≈$10^{5.5}$ copies of each genotype per reaction. Lane 5, no template control. Polymerase chain reaction (PCR) products were analyzed on a 2.5% agarose gel alongside PCR Markers (M) (Promega, Madison, WI, USA). B) Screening manufacturing plasma samples for PARV4. Samples in lanes 1–6 were amplified by using primers directed to the ORF1 region of PARV4. Template DNA in lanes 1 and 2 consisted of 1 × 10^2 and 1 × 10^3 copies of the ORF1 subclone of PARV4. In lane 3, the template DNA was derived from a plasma pool containing 3.9 × 10^6 PARV4 genome copies/mL plasma; in lane 4, the template DNA was derived from a plasma pool containing <500 PARV4 genome copies/mL plasma; in lane 5, the template DNA was derived from a plasma pool that tested negative for PARV4 sequences. Lane 6, no template control. PCR products were analyzed on a 2.5% agarose gel alongside PCR Markers (M) (Promega).

curve was generated from the cloned plasmid DNA containing the ORF1 fragment of PARV4. Levels of PARV4 DNA were as high as 3.9 × 10^6 copies/mL plasma, although several pools contained <500 copies/mL plasma (Table 2).

Plasma pools found positive for PARV4 sequences were tested for the levels of erythrovirus DNA as described previously (4). Only 2 of the PARV4-positive pools contained any human erythrovirus DNA, and these were at low levels (Table 2). Of the plasma pools found to be positive for PARV4 sequences, blood products from only 2 were available for further analysis. Both products were immunoglobulin preparations, and in neither case could PARV4 sequences be detected.

Table 1. Analysis of plasma pools for PARV4 and PARV5

Manufacturer	No. positive/no. analyzed
A	5/12
B	0/7
C	0/9
D	2/6
E	0/14
F	0/21
G	0/50
H	0/16
I	0/2

Conclusions

This report is the first to describe novel parvovirus sequences in pooled human plasma for fractionation. PARV4 was originally identified in a patient with acute viral infection syndrome coinfected with hepatitis B virus (1). As yet, nothing is known about the prevalence of PARV4, its possible role in human disease, or whether PARV4 was transmitted to the original patient from an unidentified animal host.

Although PARV4 shares limited homology with human erythroviruses, the latter are frequent contaminants of plasma, pooled and used for fractionation (3). Levels of PARV4 DNA ranged from <500 copies/mL to >10^6 copies/mL
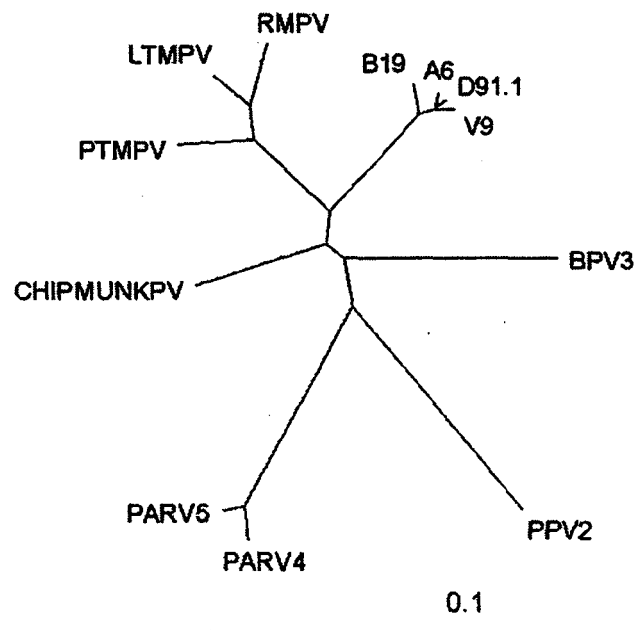

Figure 2. Phylogenetic analysis of a 178-bp sequence of ORF1 of PARV4 and PARV5 (GenBank accession no. DQ112361) with other members of the Parvoviridae subfamily. The alignment includes the members of the Erythrovirus genus (parvovirus B19 [5]) and related viruses such as V9 (6), D91.1 (7), and A6 (8), as well as the closely related viruses infecting the cynomolgus macaque (LTMPV) (9) and rhesus (RMPV) and pig-tailed macaques (PTMPV) (10). Two other viruses tentatively assigned to the group include a parvovirus isolated from chipmunks (11); BPV3, a novel bovine parvovirus (BPV3) (12); and porcine parvovirus 2 (PPV2) (13). Analysis was performed by using the program ClustalW (14).

Table 2. Viral loads in plasma pools that tested positive for PARV4 or PARV5 sequences

Positive pool	Manufacturer	PARV4 viral load (genome copies/mL plasma)	Human erythrovirus viral load (IU/mL plasma)
1	A	5×10^5	Negative
2	D	<500	Negative
3	A	$3.9 \times 10^{6*}$	140
4	A	<500*	340
5	A	$2.1 \times 10^{4*}$	Negative
6	A	<500*	Negative
7	D	Not determined	Not determined

*Sequences contaminating plasma pool represent PARV5 and not PARV4.

plasma. If a single donation with a high PARV4 count was responsible for the contamination of such a pool, the levels of virus DNA in the original donation would have been in the order of 10^9 or 10^{10} copies/mL plasma, given the volume of the start pool. Because erythroviruses are small, nonenveloped, and relatively resistant to virus inactivation procedures, manufacturers of plasma-derived products have used NAT to exclude high-titer donations from manufacturing start pools. Before such measures were introduced, more than half of production start pools contained erythrovirus DNA, some with titers of 10^9 copies/mL plasma (4; S. Baylis, unpub. data). The prevalence of PARV4 and PARV5 and the titers observed in the pools examined in this study are much lower than the usual prevalence and titers observed with erythroviruses. Because of PARV4's insufficient homology with human erythroviruses, current methods of NAT are unlikely to identify donations positive for PARV4.

The availability of highly specific reagents for PARV4 and PARV5 will assist in further studies to elucidate their possible role in human disease. The detection of PARV4 and PARV5 in plasma may have been caused by an epidemic at the time of plasma donation. In a recent study that screened for enteroviruses in human plasma, seasonal changes were observed in the frequency and level of viremia (15). Studies to examine the epidemiology of PARV4 and PARV5 infection will help address issues such as these.

In summary, PARV4, a novel parvovirus, and PARV5, a related variant, have been identified in plasma used in the manufacture of blood products. Plasma is obtained from healthy persons, who at the time of donation are asymptomatic, despite being viremic for PARV4 or PARV5. Highly specific and sensitive assays to detect PARV4 will facilitate further analysis of the role of this novel virus in human disease and the implications of virus transmission by contaminated blood and blood products.

Note

After this article was submitted for publication, human bocavirus, a novel parvovirus, was identified in respiratory tract samples (16). PARV4 and PARV5 are distinct from human bocavirus. For example, comparison of PARV4 (AY622943) with

human bocavirus strains ST and ST2 (DQ000495 and DQ000496) shows nucleotide identity of 41% and 40%, respectively.

Acknowledgment

We thank Nita Shah for technical assistance.

Dr Fryer is a scientist at the National Institute for Biological Standards and Control. Her work focuses on the quality of blood and blood products with respect to transfusion-transmitted infections.
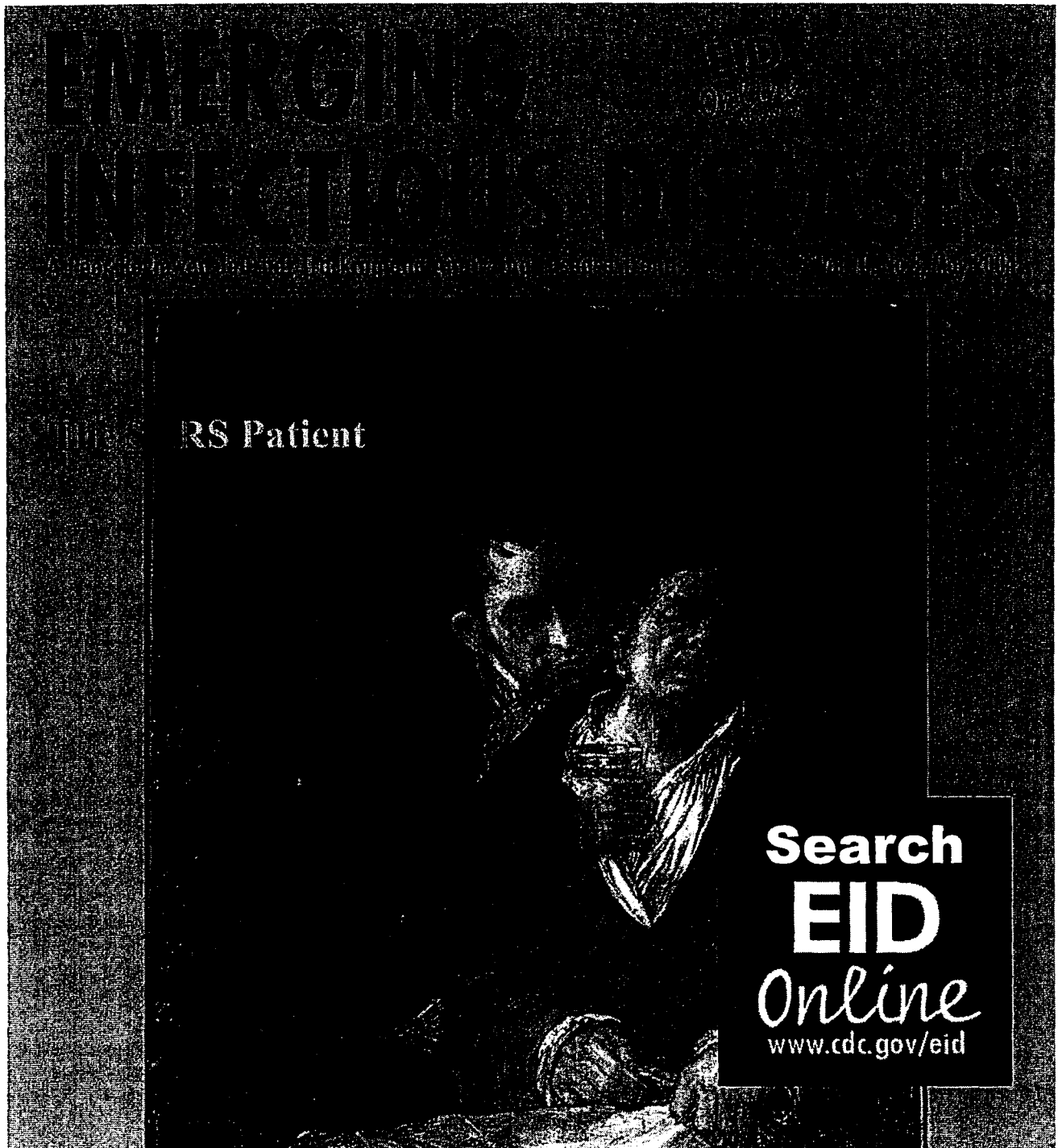
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医薬品　　研究報告　調査報告書

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
一般的名称	－		研究報告の公表状況	Transfusion (2005) 45, 1811-1815.	公表国		
販売名（企業名）	－				米国		

研究報告の概要	血液悪性腫瘍患者は血液製剤を必要とすることが多く、パルボウイルス B19 は本経路で伝播することが知られている。 6ヵ月間にわたり1つの血液疾患病棟の患者全員に投与された合計 2,123 血液製剤(966 単位の赤血球(RBCs)、630 の濃厚血小板製剤、235 の免疫グロブリン製剤、206 単位の新鮮凍結血漿、52 のアルブミン製剤、17 の同種末梢血前駆細胞(PBPC)または骨髄製剤、12 のアンチトロンビンⅢ製剤、4 の濃厚顆粒球、および 1 のフィブリノゲン製剤)について、パルボウイルス B19DNA が存在するかどうかを施設内のリアルタイムポリメラーゼ連鎖反応法(PCR、TaqMan 法)によりレトロスペクティブに検討した。 B19DNA は血液疾患患者に投与された血液製剤の 1% (2,123 血液製剤中 21 血液成分、1 の免疫グロブリン製剤、4 のアルブミン製剤、1 のフィブリノゲン製剤、3 の RBCs、2 の濃厚血小板製剤、7 の新鮮凍結血漿、3 の PBPC)で検出された。 この試験期間を通して、患者 114 名がこの病棟で治療を受け、そのうちの 14 名(12%)が B19DNA 陽性の血液成分の投与を受けたが、症候性の感染症には至らなかった。	使用上の注意記載状況・その他参考事項等 **慎重投与（次の患者には慎重に投与すること）** ・溶血性・失血性貧血の患者［ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。］ ・免疫不全患者・免疫抑制状態の患者［ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。］ **重要な基本的注意** (1)本剤の原材料となる・・[スクリーニング項目、不活化・除去工程]・・投与に際しては、次の点に十分注意すること。 1)血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。 **妊婦、産婦、授乳婦等への投与** 妊婦又は妊娠している可能性のある婦人には治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。［妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。］

報告企業の意見	今後の対応
B19DNA 陽性の血液製剤(血漿分画製剤を含む)投与による感染に関するレトロスペクティブ調査の報告であり、調査範囲内では症候性の感染症はなかった。 弊社血漿分画製剤は最終製品において B19DNA 陰性であることを確認している。	今後ともパルボウイルス B19 に関する血漿分画製剤の安全性に関する情報に留意していく。

TRANSFUSION COMPLICATIONS

Exposure of hematologic patients to parvovirus B19 as a contaminant of blood cell preparations and blood products

Annelie Plentz, Joachim Hahn, Antje Knöll, Ernst Holler, Wolfgang Jilg, and Susanne Modrow

BACKGROUND: Patients with hematologic malignancies often require blood products, and parvovirus B19 is known to be transmitted by this route. Primary infection with parvovirus B19 shows a wide variety of disease manifestation. In immunocompromised patients, symptoms are severe and viral clearance is delayed or missing.

STUDY DESIGN AND METHODS: A total of 2123 blood products given to all patients of a hematologic ward over a period of 6 months were retrospectively examined for the presence of parvovirus B19 DNA by an in-house real-time polymerase chain reaction (PCR; TaqMan). Patients who had received B19 DNA–positive blood products were further investigated serologically and by PCR for the presence of parvovirus B19 antibodies and DNA.

RESULTS: Twenty-one (1%) of 2123 blood products tested positive for the presence of B19 DNA (2% of pooled products, 0.7% of single-donor products, and 17.6% of allogeneic peripheral blood progenitor cells), the median viral load was 700 genome equivalents per mL. During the study period, 114 patients were treated on the ward, and 14 (12%) of them received B19 DNA–positive blood components. None of them developed symptoms of an acute B19 infection, although one had a short low-level viremia.

CONCLUSIONS: Although B19 DNA was detected in 1 percent of blood products given to hematologic patients, the exposure of 12 percent of patients did not result in symptomatic infections.

Parvovirus B19 is a nonenveloped single-stranded DNA virus. The infection is associated with a wide spectrum of diseases.[1] In the immunocompetent host, infection leads to lifelong immunity. In immunocompromised patients, the virus can persist for several months and years.[2,3] In general, these persistent infections are associated with viremia and may result in clinical manifestations such as severe chronic aplastic anemia or pancytopenia.[4-14]

Transmission of parvovirus B19 is usually airborne. Because high-level viremia regularly occurs during primary infection, iatrogenic transmission by blood or blood products is also possible. There are numerous reports on transmission by this route. In some of them, a temporal correlation has been documented between the donation of a pooled blood product[15-17] or a single-donor transfusion[18] and viremia or clinical signs of infection. In others, it was proven that the pooled blood products[19-22] or single-donor transfusions[23,24] were contaminated with the virus. In further studies it was shown that the prevalence for B19-specific antibodies in groups receiving clotting factors was much higher than in control groups.[25,26]

The aim of this study was to estimate the risk of symptomatic parvovirus infection for a hematologic patient—a highly immunosuppressed patient who usually receives many doses of blood cell preparations and blood products—due to donations of blood components. We screened retrospectively all blood cell preparations and blood products that had been applied to patients on a hematologic ward over a period of 6 months for the presence of parvovirus B19 DNA. We further investigated the outcome in the patients who received contaminated blood products.

MATERIALS, PATIENTS, AND METHODS

Investigated blood cell preparations and blood products

In a 6-month period from February to August 2002, a total of 2123 different blood cell preparations and blood products that were given to the patients of one hematologic ward were analyzed retrospectively: 966 units red blood cells (RBCs), 630 thrombocyte concentrates, 235 immuno-

globulin doses, 206 units of fresh-frozen plasma, 52 albumin doses, 17 allogeneic peripheral blood progenitor cell (PBPC) or marrow preparations, 12 antithrombin III doses, 4 granulocyte concentrates and 1 fibrinogen dose. Additionally 36 autologous PBPC preparations were investigated.

Patients

The adult patients on the hematologic ward all had hematologic malignancies and were hospitalized for leukemia treatment or PBPC or bone marrow transplantation (BMT). Those who had received contaminated blood cell preparations or blood products were further investigated. Charts were reviewed, nonblinded, retrospectively with regard to clinical symptoms such as persisting anemia, rash, or arthritis, possibly related to parvovirus B19 infection. Serum samples that had been sent to our laboratory for other diagnostic tests were retrospectively tested for B19-specific antibodies before and by B19 DNA polymerase chain reaction (PCR) one to three times three to 35 days after the transfusion.

Methods

PCR of all blood products was performed in duplicates by a quantitative parvovirus B19 DNA real-time PCR (System A) with TaqMan technology as described before.[27] DNA was extracted from 200 μL by the QIAamp blood kit (Qiagen, Hilden, Germany) and eluted in 100 μL of which 5 μL were used for each PCR. The samples were tested in pools of five thus using 40 μL of each sample for DNA extraction. In the case of a positive pool, single samples were retested and quantified. The sensitivity of this PCR is 100 percent for 600 to 800 genome equivalents (geq) per mL and 50 percent for 60 to 80 geq per mL. In each run a (DNA extraction and PCR), serum sample of a serologically and DNA-negative donor was used as a negative control. Positive samples were investigated for the presence of immune complexes with protein A-Sepharose beads as described before.[28] Parvovirus-specific immunoglobulin G (IgG) and IgM to the viral capsid protein VP2 were detected by the parvovirus B19 enzyme immunoassay by Biotrin (Sinsheim-Reihen, Germany).

RESULTS

Blood cell preparations and blood products

Of the 2123 blood components, 21 tested positive for the presence of parvovirus B19 DNA. The median viral load was 700 geq per mL. The highest concentration could be found in one RBC unit with 2.2×10^6 geq per mL. The highest rate of positive samples was found among allogeneic PBPCs or marrow (17.6%). In addition, 4 of 36 (11.1%)

autologous PBPC samples were positive. Thus the overall rate of B19 DNA detection in PBPCs and marrow was 13.2 percent. The distribution of the positive samples among all (allogeneic) products is shown in Table 1. The 235 immunoglobulin doses were from 13 different lots; the one that tested positive was the only one from one lot. The 52 albumin samples were from five different lots. The four positive samples were all from the same lot, but 17 additional samples from this lot tested negative.

In the 21 positive blood products and blood cell preparations, it was investigated if the viruses are part of immune complexes because this has an impact on the infectivity. Free viral genomes are either representing free virus or free DNA. DNA was present in both immune complexes and free in four plasma samples, the three whole blood samples, one albumin sample, the fibrinogen preparation, and two PBPC preparations. Free viral DNA only was found in two albumin samples, two plasma samples, and the two thrombocyte preparations. Virus DNA exclusively in immune complexes was found in one PBPC preparation. In the immunoglobulin sample, one plasma sample and one albumin sample, no DNA could be amplified in either the protein A fraction or in the supernatant owing to the low viral load in these samples.

Patients

During the observation period, 114 adult hematologic patients were on the ward. Fourteen (12%) received at least 1 of the 21 contaminated blood cells or blood products (Table 2). Ten of them had received allogeneic PBPCs or marrow and four of them were treated for leukemia. The serologic measures of these patients and the results of testing for viremia are shown in Table 2. No one developed clinical symptoms that could be associated with a parvovirus B19 infection. Only in one asymptomatic leukemia patient, a low level viremia could be detected 5 days after transfusion of the RBCs that were contaminated with 2.2×10^6 geq per mL. A consecutive serum sample was available only 30 days later; this sample tested negative.

TABLE 1. Distribution of blood products and blood cell preparations tested positive for parvovirus B19 DNA

Blood component	Number tested	Number positive	Percent
Multiple donor products	300	6	2.0
Immunoglobulins	235	1	0.4
Albumin	52	4	7.7
Antithrombin III	12	0	
Fibrinogen	1	1	
Single-donor products	1,806	12	0.7
RBCs	966	3	0.3
Thrombocytes	630	2	0.3
FFP	206	7	3.4
Granulocytes	4	0	
PBPCs (allogeneic), marrow	17	3	17.6

Patient	Sex, age (years)	Positive blood component	Viral load (geq/mL)	Day of transfusion*	Day of serum PCR	PCR result (geq/mL)	IgG before transfusion	IgG before SCT	IgG of donor
S1	Male, 48	PBPCs	600	0	16	–	+	+	+
		Fibrinogen	1,400	43	50, 54				
S2	Male, 46	FFP	<600	0	3, 5, 17	–	+	+	+
S3	Male, 58	FFP	600	0	6	–	+	+	+
S4	Female, 31	FFP	<600	0	3	–	+	+	+
		FFP	<600	0					
		FFP	18,000	4	7, 14	–			
S5	Female, 44	Marrow	18,000	0	4, 25	–	+	+	+
S6	Male, 56	Marrow	2,000	0	14, 28	–	–†	–	+
S7	Male, 37	Albumin	<600	0	4	–	+	+	+
		Albumin	700	7					
		Albumin	<600	11	37	–			
		Albumin	6,900	59	69	–			
S8	Female, 45	RBCs	<600	0	3, 11	–	+	–	–
S9	Male, 38	Thrombocytes	<600	0	4, 17	–	+	–	+
S10	Male, 25	Immunoglobulins	<600	0	6, 13	–	+	+	+
L1	Female, 65	FFP	1,300	0		ND	+		
L2	Male, 62	RBCs	2,200,000	0	5	<600	+		
		RBCs	<600	0	35	–			
L3	Female, 71	Thrombocytes	1,500	0	3, 18	–	+		
L4	Female, 64	FFP	6,700	0		ND	ND		

TABLE 2. Patients who received contaminated blood components with the respective viral loads

* Day of transfusion of first contaminated component is designated as 0; additional transfusions and dates of PCR from serum of the patients are related to this day.

† Last serum sample before BMT was available on Day –10, but patient received immunoglobulins thereafter.

Abbreviations: SCT = PBPC or BMT; S = PBPC or bone marrow recipients; L = patients treated for leukemia; ND = not done because no serum sample available; + = positive; – = negative.

He had tested positive for VP2-specific IgG before. In 11 patients, no viral DNA could be detected in serum samples 3 to 35 days after transfusions of contaminated products, and in 2 patients, no serum samples were available for testing.

DISCUSSION

Parvovirus B19 DNA could be detected in 1 percent of all blood cell preparations and blood products applied to the patients on a hematologic ward—in 17.6 percent of allogeneic PBPC or marrow preparations and in 0.9 percent of standard blood components (in 2.0 percent of pooled plasma products and in 0.7 percent of single donor products). These rates are within the wide range found by others, as in 0.006 percent of blood donations,[29] in 0.14 percent of single-donor blood products,[24] in 0.16 percent of plasma samples,[30] in 0.6 to 1.3 percent of blood donors,[31,32] in 12 percent of plasma pools with more than 10^4 geq per mL,[33] in 43 percent of clotting factor concentrates,[34] and in 56 percent of manufacturing plasma pools.[29] There are several reasons for the very different numbers found in diverse studies: first, the investigated collectives are different; second, the numbers are related to the sensitivity of the methods used; and third, there are seasonal variations in transmission and thus viremia.

The median viral load of the 21 samples was quite low and close to the detection limit of the assay. This explains that only 4 of 21 albumin samples from one lot tested positive.

In some products, the viral genomes were found to be partly complexed by immunoglobulins, both in cellular- and in plasma-derived products. Therefore, the presence of viral DNA neither correlates with infectivity, nor does it mean the reverse, that the presence of immunoglobulins would correlate with protection. Because the virus load in B19 infections can be 10^{12} geq per mL serum, neither immunoglobulins contained in a blood product nor the dilution in pooled products can be sufficient enough in preventing infectivity. Because free virus can be found in the presence of antibodies even at low viral loads below 10^3 geq per mL, it is presumed that the affinity may be low or escape mutants have evolved.[32] This explains the transmission of B19 even by immunoglobulin doses.[17,22]

Because the marrow is a site of viral persistence,[35] it is not surprising that viral DNA was detected in as many as 13.2 percent of all PBPC or bone marrow samples. It is, however, unclear if virions are produced and released from these cells in the immunocompetent donor. Because these donors had previously been infected with parvovirus B19 and were producing B19-specific antibodies, IgG-producing plasma cells are contained in the transplants. Thus, immunity to the transferred virus should be assumed, but antibody production is usually highly impaired in the first months after PBPC or BMT,[36] and symptomatic infection by this transmission route has

been described.[23,37] In both allogeneic and autologous PBPCs, transmission of parvovirus B19 cannot be avoided by donor selection. Therefore, recipients from IgG-positive PBPC donors should be monitored for B19 DNA in serum.

Although B19 DNA was detected in 1 percent of 2123 blood cell preparations and blood products used for treatment of hematologic patients (mostly transplant recipients), the exposure of 14 patients (12%) did not result in symptomatic infections. This is probably due to low virus concentrations or the presence of protective IgG—own, from the PBPC/marrow donor, or transfused. In addition overall numbers of exposed patients being studied were quite low, which might contribute to an error of small numbers. There are reports on severe infections in immunocompromised patients, for example, severe anemia over 7 months in a marrow recipient,[13] complicated erythrodermia, hepatitis, or myocarditis with a high lethality in marrow recipients,[36] recurrent severe anemia,[10,14] or pancytopenia[7] in renal transplant recipients or severe anemia over 7 years in a human immunodeficiency virus-positive patient.[8] Symptoms in all patients had to be treated with repeated transfusions of RBCs and resolved only after repeated treatment with high-dose immunoglobulins and induction of highly active antiretroviral therapy in the latter case. Reports on transmission of B19 via fibrin sealant,[19] clotting factor,[20] plasma,[21] immunoglobulins,[22] or blood[24] underline the potential risk of iatrogenic transmission. Because immunocompromised patients often receive blood products or blood cell preparations, these reports illustrate the basic hazard of a parvovirus B19 infection for these patients. Thus, despite the overall low risk for iatrogenic transmission, the severity of disease once it comes to infection should justify testing of blood donors for parvovirus B19 at least for risk groups.[39,40]

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医薬品
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一般的名称			研究報告の公表状況	Viral safety of Nanogam®, a new 15 nm-filtered liquid immunoglobulin product F. G. Terpstra, J. Parkkinen, H. Tolo A. H. L. Koenerderman, et al. Vox Sanguinis 2006; 90; 21-32	公表国 米国	
販売名（企業名）						

研究報告の概要	オランダ Sanquin 社の静注用免疫グロブリン製剤 Nanogam® において採用されている新規の各種ウイルスに対する除去能について検討した。本剤では特徴的な 2 段階のウイルス不活化工程を実施している。すなわち 15 nm フィルター濾過工程および pH4.4 でのペプシン処理と、SD 処理の組み合わせである。これらの 2 段階工程を含む全製造工程をスケールダウンした製造工程において、ウイルス除去能を検討した。除去能の測定実験用として、エンベロプをもつウイルスとして、HCV のモデルウイルス BVDV、ヘルペスウイルスのモデルウイルス PRV、HIV、HBV、EBV、CMV、エンベロプを持たないウイルスとして、パルボウイルス B19 のモデルウイルス CPV および EMC を使用した。 　この 2 段階のウイルス除去工程の除去能は、CPV を除く全てのウイルスについて 6log を上回るものであり、ウイルスは完全に除去されることが示された。また、全製造工程を通したパルボウイルス B19 ウイルスの除去能については、10log を上回る高い値が得られた。 　Nanogam® の製造工程では、エンベロプを持つウイルスに有効な 2 つの工程およびエンベロプをもたないウイルスに有効な 1 段階の工程が含まれている。さらに Chon 分画および中和抗体により高いウイルス除去能が得られる。全ての工程を経ることで、エンベロプを持たないパルボウイルス B19 に対しても $10log_{10}$ を上回る高い除去能が得られることが示された。	使用上の注意記載状況・その他参考事項等 BYL-2005-0208

報告企業の意見	今後の対応
弊社の静注用免疫グロブリン製剤のウイルス不活化工程（分画、透析・限外濾過、S/D 処理、低 pH インキュベーション）によるパルボウイルス B19 のモデルウイルスの除去率は 9.3log 以上と非常に高い。しかしながら現時点でもなお感染の可能性を完全には否定できない。本稿において示されたパルボウイルス B19 の不活化工程の実用化については引き続き注視して情報を収集する。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続きプリオン除去および検出技術に関する関連情報の収集に努める。

ORIGINAL PAPER

Viral safety of Nanogam®, a new 15 nm–filtered liquid immunoglobulin product

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Background and Objectives Producers of plasma derivatives continuously improve the viral safety of their products by, for example, introducing additional virus-reducing steps into the manufacturing process. Here we present virus-elimination studies undertaken for a number of steps employed in a new manufacturing process for liquid intravenous immunoglobulin (Nanogam®) that comprises two specific virus-reducing steps: a 15-nm filtration step combined with pepsin treatment at pH 4·4 (pH 4·4/ 15NF); and solvent-detergent (SD) treatment. The manufacturing process also includes precipitation of Cohn fraction III and viral neutralization, which contribute to the total virus-reducing capacity of the manufacturing process. In addition, the mechanism and robustness of the virus-reducing steps were studied.

Materials and Methods Selected process steps were studied with spiking experiments using a range of lipid enveloped (LE) and non-lipid-enveloped (NLE) viruses. The LE viruses used were bovine viral diarrhoea virus (BVDV), human immunodeficiency virus (HIV) and pseudorabies virus (PRV); the NLE viruses used were parvovirus B19 (B19), canine parvovirus (CPV) and encephalomyocarditis virus (EMC). After spiking, samples were collected and tested for residual infectivity, and the reduction factors were calculated. For B19, however, removal of B19 DNA was measured, not residual infectivity. To reveal the contribution of viral neutralization, bovine parvovirus (BPV) and hepatitis A virus (HAV) were used.

Results For the pH 4·4/15NF step, complete reduction ($> 6 \log_{10}$) was demonstrated for all viruses, including B19, but not for CPV ($> 3·4$ but $\leq 4·2 \log_{10}$). Robustness studies of the pH 4·4/15NF step with CPV showed that pH was the dominant process parameter. SD treatment for 10 min resulted in complete inactivation ($> 6 \log_{10}$) of all LE viruses tested. Precipitation of Cohn fraction III resulted in the significant removal ($3–4 \log_{10}$) of both LE and NLE viruses. Virus-neutralization assays of final product revealed significant reduction ($\geq 3 \log_{10}$) of both BPV and HAV.

Conclusions The manufacturing process of Nanogam® comprises two effective steps for the reduction of LE viruses and one for NLE viruses. In addition, the precipitation of Cohn fraction III and the presence of neutralizing antibodies contribute to the total virus-reducing capacity of Nanogam®. The overall virus-reducing capacity was $> 15 \log_{10}$ for LE viruses. For the NLE viruses B19, CPV and EMC, the overall virus-reducing capacities were > 10, > 7 and $> 9 \log_{10}$, respectively. Including the contribution of immune neutralization, the overall virus-reducing capacity for B19 and HAV is estimated to be $> 10 \log_{10}$.

Key words: B19, immunoglobulin, nanofiltration, neutralization, SD treatment, viral safety.

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