The potential for apparently PCR-negative donors to harbor active infections raises additional issues applicable to donors, donor deferrals, and potential blood screening. In some instances, a PCR-negative result may indeed indicate that an active infection has cleared. This observation may be supported by an ensuing decrease in antibody titers to baseline levels. Persistence of antibody titers, however, may suggest an active, ongoing infection that cannot be detected by PCR. Although the PCR may be sensitive, the level of circulating parasites may be so low that they cannot be detected or the parasites may be present only in sequestered locations.[25] In either case, a later immunologic event (e.g., immunosuppression associated with aging) may result in an infection that recrudesces and again become measurable by PCR.[23,26] This scenario would make it difficult to consider reentering a deferred donor unless they had completely cleared the infection as determined by both serology and PCR. Similarly, if donor screening for *B. microti* is ever implemented, PCR or nucleic acid testing would be needed to detect early acute or window-period infections, although serology would be needed to detect ongoing, parasitologically silent infections.[27]

For this study, we used a two-step serologic screening approach to identify potential carriers of babesiosis, in which an experimental recombinant immunoassay was used in an initial screening step before performing a more labor-intensive, but highly sensitive and specific IFA procedure.[15,16] This approach allowed us to screen efficiently a large number of donations (n = 3490) and to then focus our confirmatory efforts on the 203 (5.8%) donations that were positive in the initial screening assay. This effort, however, also showed that the experimental immunoassay lacked specificity for *B. microti* infection, because the vast majority of EIA-reactive donations were shown to be IFA-negative. Poor specificity of a recombinant immunoassay can be explained by a variety factors, including low-level contamination by proteins from the *Escherichia coli* host strain or cross-reactive epitopes within the full-length recombinant proteins used in the assay. Since this study was initiated, a peptide-based immunoassay has been developed in which the immunodominant epitopes of the two recombinant proteins used in this study are used as an EIA substrate.[28] The peptide EIA shows significantly higher specificity than the assays used in the study described here and correlates very well with IFA and PCR results. Indeed, in a study of patients being evaluated for babesiosis, virtually all PCR-positive donors were detected with the peptide EIA.[28] The peptide EIA, however, has yet to be evaluated in the setting of blood donor screening to determine if it can indeed serve as an efficient replacement for IFA testing.

B. microti has been known to pose a blood safety risk for close to 20 years.[7] As the parasite's endemic range has expanded and closely related emergent agents have arisen,[29-33] the potential for transfusion-transmitted infections has continued to increase. For example, WA-1 was first described less than 10 years ago, but already this *Babesia*-like agent has been implicated in two transfusion cases.[34,35] With the knowledge that a large percentage of blood donors seropositive for *B. microti* are also parasitemic, some apparently for months, appropriate interventions to prevent continued transmission of this parasite to blood recipients should be considered.

ACKNOWLEDGMENTS

We thank Laura Triano who provided critical donor follow-up and Stephanie Johnson who developed maps identifying the relative locations of seropositive donors.

REFERENCES

1. Dodd RY, Leiby DA. Emerging infectious threats to the blood supply. Annu Rev Med 2004;55:191-207.
2. Scholtens RG, Braff EH, Healy GR, Gleason N. A case of babesiosis in man in the United States. Am J Trop Med Hyg 1968;17:810-3.
3. White DJ, Talarico J, Chang H, et al. Human babesiosis in New York state: review of 139 hospitalized cases and analysis of prognostic factors. Arch Intern Med 1998;158:2149-54.
4. McQuiston JH, Childs JE, Chamberland ME, et al. Transmission of tick-borne agents of disease by blood transfusion: a review of known and potential risks in the United States. Transfusion 2000;40:274-84.
5. Homer MJ, Aguilar-Delfin I, Telford SR, Krause PJ, Persing DH. Babesiosis. J Clin Microbiol Rev 2000;13:451-69.
6. Hatcher JC, Greenberg PD, Antique J, Jimenez-Lucho VE. Severe babesiosis in Long Island: review of 34 cases and their complications. Clin Infect Dis 2001;32:1117-25.
7. Leiby DA. Babesia and other parasites. In: Brecher ME, editor. Bacterial and parasitic contamination of blood components. Bethesda (MD): American Association of Blood Banks; 2003. p. 179-200.
8. Popovsky MA, Lindberg LE, Syrek AL, Page PL. Prevalence of Babesia antibody in a selected blood donor population. Transfusion 1988;28:59-61.
9. Linden JV, Wong SJ, Chu FK, Schmidt GB, Bianco C. Transfusion-associated transmission of babesiosis in New York State. Transfusion 2000;40:285-9.
10. Leiby DA, Chung APS, Cable RG, et al. Relationship between tick bites and the seroprevalence of Babesia microti and Anaplasma phagocytophila (previously Ehrlichia sp.) in blood donors. Transfusion 2002;42:1585-91.
11. Krause PJ, Telford SR, Ryan R, et al. Geographical and temporal distribution of babeisal infection in Connecticut. J Clin Microbiol 1991;29:1-4.
12. Anderson JF, Mintz ED, Gadbaw JJ, Magnarelli LA. Babesia microti, human babesiosis, and Borrelia burgdorferi in Connecticut. J Clin Microbiol 1991;29:2779-83.

13. Lodes MJ, Houghton RL, Bruinsma ES, et al. Serologic expression cloning of novel immunoreactive antigens of Babesia microti. Infect Immun 2000;68:2783-90.
14. Persing DH, Mathiesen D, Marshall WF, et al. Detection of Babesia microti by polymerase chain reaction. J Clin Microbiol 1992;30:2097-103.
15. Chisholm ES, Ruebush TK, Sulzer AJ, Healy GR. Babesia microti infection in man: evalulation of an indirect immunofluorescent antibody test. Am J Trop Med Hyg 1978;27:14-9.
16. Krause PJ, Telford SR, Ryan R, et al. Diagnosis of babesiosis: evaluation of a serologic test for the detection of Babesia microti antibody. J Infect Dis 1994;169:923-6.
17. Leiby DA, Gill JE. Transfusion-transmitted tick-borne infections: a cornucopia of threats. Transfus Med Rev 2004;18:293-306.
18. Centers for Disease Control and Prevention (CDC). Lyme disease United States, 2001-2002. MMWR Morb Mortal Wkly Rep 2004;53:365-9.
19. Herwaldt BH, McGovern PC, Gerwel MP, et al. Endemic babesiosis in another eastern state: New Jersey. Emerg Inf Dis 2003;9:184-8.
20. Summary of notifiable diseases, United States, 2000. MMWR Morb Wkly Rep 2000;53:49, 30-31.
21. Eskow ES, Krause PJ, Spielman A, et al. Southern extension of the range of human babesiosis in the eastern United States. J Clin Microbiol 1999;37:2051-2.
22. Jassoum BS, Fong IW, Hannach B, et al. Transfusion-transmitted babesiosis in Ontario: first reported case in Canada. Can Commun Dis Rep 2000;26:9-13.
23. Krause PJ, Spielman A, Telford SR, et al. Persistent parasitemia after acute babesiosis. N Engl J Med 1998;339:160-5.
24. Pantanowitz L, Telford SR, Cannon ME. The impact of babesiosis on transfusion medicine. Transfus Med Rev 2002;16:131-43.
25. Krause PJ, Telford S, Spielman A, et al. Comparison of PCR with blood smear and inoculation of small animals for diagnosis of Babesia microti parasitemia. J Clin Microbiol 1996;34:2971-4.
26. Vannier E, Borggraefe I, Telford SR, et al. Age-associated decline in resistance to Babesia microti is genetically determined. J Infect Dis 2004;189:1721-8.
27. Leiby D, Gill J, Johnson ST, et al. Lessons learned from a natural history study of Babesia microti infection in Connecticut blood donors. Transfusion 2002;42(Suppl):30S.
28. Houghton RL, Homer MJ, Reynolds LD, et al. Identification of Babesia microti-specific immunodominant epitopes and development of a peptide EIA for detection of antibodies in serum. Transfusion 2002;42:1488-96.
29. Quick RE, Herwaldt BL, Thomford JW, et al. Babesiosis in Washington State: a new species of Babesia? Ann Intern Med 1993;119:284-90.
30. Herwaldt BL, Persing DH, Précigout EA, et al. A fatal case of babesiosis in Missouri: identification of another piroplasm that infects humans. Ann Intern Med 1996;124:643-50.
31. Herwaldt BL, Cacciò S, Gherlinzoni F, et al. Molecular characterization of a non-Babesia divergens organism causing zoonotic babesiosis in Europe. Emerg Infect Dis 2003;9:942-8.
32. Herwaldt BL, Bruyn G, Pieniazek NJ, et al. Babesia divergens-like infection, Washington State. Emerg Infect Dis 2004;10:622-9.
33. Beattie JF, Michelson ML, Holman PJ. Acute babesiosis caused by Babesia divergens in a resident of Kentucky [letter]. N Engl J Med 2002;347:697-8.
34. Herwaldt BL, Kjemtrup AM, Conrad PA, et al. Transfusion-transmitted babesiosis in Washington state: first reported case caused by a WA1-type parasite. J Infect Dis 1997;175: 1259-62.
35. Kjemtrup AM, Lee B, Fritz CL, et al. Investigation of transfusion transmission of a WA1-type babesial parasite to a premature infant in California. Transfusion 2002;42:1482-7.

別紙様式第2-1

No. 8

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2005. 10. 24	該当なし	

一般的名称	人全血液	研究報告の公表状況	Lindblom A, Isa A, Norbeck O, Wolf S, Johansson B, Broliden K, Tolfvenstam T. Clin Infect Dis. 2005 Oct 15;41(8):1201-3. Epub 2005 Sep 2.	公表国 スウェーデン
販売名(企業名)	人全血液CPD「日赤」(日本赤十字社) 照射人全血液CPD「日赤」(日本赤十字社)			

研究報告の概要

○急性感染後のヒトパルボウイルスB19ウイルス血症の緩徐なクリアランス
パルボウイルスB19は、よく見られる臨床的に重要な病原体である。急性感染後のウイルス動態の再評価により、症状が早期に消失したにもかかわらず、このウイルスは健常宿主から急速に除去されないことが示された。この結果により、現在我々の考えているパルボウイルスB19の病態に疑問が持たれ、感染管理に影響が生じる。

報告企業の意見	今後の対応
パルボウイルスB19急性感染後のウイルス動態の再評価により、症状が早期に消失したにもかかわらず、健常宿主から急速に除去されないことが示されたとの報告である。	今後も引き続き、ヒトパルボウイルスB19に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

人全血液CPD「日赤」
照射人全血液CPD「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

Slow Clearance of Human Parvovirus B19 Viremia following Acute Infection

Anna Lindblom,[1] Adiba Isa,[1] Oscar Norbeck,[1] Susanne Wolf,[2] Bo Johansson,[2] Kristina Broliden,[1] and Thomas Tolfvenstam[1,2]

[1]Department of Medicine, Infectious Disease Unit, Center for Molecular Medicine, and [2]Department of Laboratory Medicine, Division of Clinical Virology, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

Parvovirus B19 is a common, clinically significant pathogen. Reassessment of the viral kinetics after acute infection showed that the virus is not rapidly cleared from healthy hosts, despite early resolution of symptoms. These findings challenge our current conception of the virus' pathogenesis and have implications for the management of the infection.

Human parvovirus B19 (B19) is ubiquitous throughout the world and causes a variety of symptoms, ranging from mild febrile illness to life-threatening anemia and fetal death. The infection is primarily thought to be controlled by humoral immune responses, because peripheral viremia decreases concurrent with the development of virus-specific antibodies, and the virus has been shown to be cleared in healthy hosts weeks to months after infection. Establishment of persistent infection is well characterized in immunocompromised individuals, primarily in association with congenital, iatrogenic, or infectious causes. However, cases of immunocompetent, symptomatic individuals with detectable B19 DNA in bone marrow and peripheral blood specimens for long periods of time have also been described [1, 2]. Recently, investigations of the cellular immune responses to B19 have shown a surprisingly large pool of circulating B19-specific $CD8^+$ T lymphocytes remaining for >2 years after infection, with maintained effector function in healthy subjects [3]. Because this would indicate that viral antigen is present for a much longer time than has previously been shown, we reassessed the viral kinetics after primary B19 infection with a newly developed real-time quantitative PCR.

Materials and methods. Five individuals were identified prospectively after their serum samples had been referred to the clinical virology laboratory at the Karolinska University Hospital and were found to be positive for B19 IgM. The patients had presented their general practitioners with symptoms of fever, arthralgia, fatigue, and rash. None of the patients had received immunosuppressive treatment or had showed clinical symptoms of any other underlying chronic infection. Furthermore, they did not have any medical history of increased frequency of reactivation of latent herpes virus infection, recurrent respiratory infection, or mucocutaneous infection and did not recall having previous episodes of symptoms that resembled those of B19 infection. During the subsequent 128 weeks after inclusion of the first individual in the study, samples of serum and PBMCs were collected at intervals from all individuals, together with medical history and data regarding clinical symptoms. In addition, 15 B19 IgG–positive and IgM-negative healthy laboratory workers who did not recollect having parvovirus-related symptoms were included as control subjects.

Serum samples were analyzed for B19 IgG and IgM using a commercial EIA (Biotrin International). For assessment of B19 DNA levels, a novel, parvovirus genotype 1–3–specific TaqMan real-time PCR assay was developed. In brief, 200 μL of serum was extracted with use of an automated MagnaPure extractor (Roche Diagnostics) using the LC Total Nucleic Acid Isolation Kit (Roche). The assay was performed in a ABI 7700 sequence detection system (Applied Biosystems) in a 50-μL reaction mixture containing 25 μL of TaqMan Universal PCR Master Mix (Applied Biosystems), 5 μL of template DNA, 3 μmol/L of each primer, and 1.5 μmol/L probe for 40 cycles consisting of 15 s at 95°C and 20 s at 60°C. The following primers were used in the amplification: sense, 5′-ACAAGCCTGGGCAAGTTAGC-3′, and antisense, 5′-GGCCCAGCTTGTAGCTCATT-3′, positioned at B19 genomic nucleotide positions 854–873 and 910–928, respectively (numbers refer to GenBank AY083239). Detection was provided by an FAM-TAMRA–labeled probe (Applied Biosystems) with the sequence 5′-CAACTACCCGGTACTAACT-ATGTTGGGCCTGG-3′ at B19 genomic nucleotide positions 877–908. A B19 viremic plasma, determined to contain 1.4×10^{11} genome equivalents (geq)/mL, lot BPL9 (kindly provided by Dr. Kerr, Biotrin International), was used as standard. The sensitivity of the assay was 2 geq/reaction, as determined by repeated testing of serial dilutions of the BPL9 standard. Negative controls were extracted and analyzed between every 5 patient samples throughout the procedure. Extraction, preparation of the master mix, and template and standard addition were performed in separate laboratories. Samples that had pos-

Received 29 March 2005; accepted 16 June 2005; electronically published 7 September 2005

Reprints or correspondence: Dr. Thomas Tolfvenstam, Div. of Clinical Virology, F60 Karolinska University Hospital, SE 14186 Stockholm, Sweden (Thomas.tolfvenstam@labmed.ki.se)

Clinical Infectious Diseases 2005; 41:1201–3

1058-4838/2005/4108-0020$15.00

itive results of quantitative PCR were partially sequenced to assess viral genotype using a separate assay. Outer primers in this assay were as follows: sense, 5′-GTGGTGAAAGCTCTGAA-GAACTCA- 3′, and antisense 5′-GCCCAGGCTTGTGTAAGT-CTTC- 3′, at B19 genomic nucleotide positions 37–60 and 844–865, respectively. The inner primers were as follows: sense, 5′-CGGGACCAGTTCAGGAGAATCA-3′, and antisense, 5′-GGGGTGGTCAGATAACTGTCCATG-3′, at B19 genomic nucleotide positions 137–158 and 757–780, respectively (numbers refer to GenBank AY083237). Amplification was performed in a volume of 50 μL in 1× buffer II (Applied Biosystems) and 25 mmol/L MgCl and 10 pmol/L primer at an annealing temperature of 55°C and for 40 cycles. The amplified product was sequenced using the Big Dye Termination Kit (Applied Biosystems) in an ABI 3100 sequencer (Applied Biosystems).

$CD4^+$ and $CD8^+$ T lymphocyte counts were determined by direct staining of PBMCs isolated by Ficoll-Paque (Amersham Biosciences) by fluorochrome-labelled monoclonal antibodies (BD), and subsequent analysis was performed by fluorescence-activated cell sorting (FACS). IFN-γ responses to phytohemagglutinin (Sigma-Aldrich) were assessed by enzyme-linked immunospot (ELISpot), which was performed as described elsewhere [4], using nitrocellulose plates (Millipore) and IFN-γ antibody (Mabtech AB). Approval for the study was obtained from the local ethics committee at the Karolinska University Hospital (Stockholm, Sweden).

Results. Serum and PBMC samples were obtained from patients for the first time 5 days (at the earliest) to 10 days (at the latest) after the onset of symptoms. FACS analysis revealed normal distribution of $CD4^+$ and $CD8^+$ T lymphocytes, as well as normal IFN-γ response to phytohemagglutinin in PBMCs obtained from all patients (data not shown). Symptoms present in all patients were arthralgia and erythematic eruptions. Additional symptoms, such as fever, malaise, pronounced myalgia, and peripheral edema, were present in some patients. All patients reported cessation of acute clinical symptoms (i.e., fever, exanthema, myalgia, and peripheral edema) 4–6 weeks after the onset of disease. The patient group was observed for a mean duration of 105 weeks (range, 77–128 weeks).

At the first point at which samples were obtained, serum samples contained a mean of 1.2×10^7 B19 geq/mL serum (range, 1.7×10^6–4.1×10^7 geq/mL) (figure 1) and all isolates were shown to cluster in genotype 1 (B19) [5]. At that point, all patients tested positive for both B19 IgM and IgG. The viral load peaked at the time that the first sample was obtained or earlier, after which the virus levels stabilized in the range 10^5–10^6 geq/mL. Patient 3 exhibited an increase in viral load after week 80, but no epidemiological or clinical information correlated with this observation. During the study period, only 1 patient (patient 1) had clearance of the peripheral viremia (in the interval between weeks 85 and 106). All other patients had

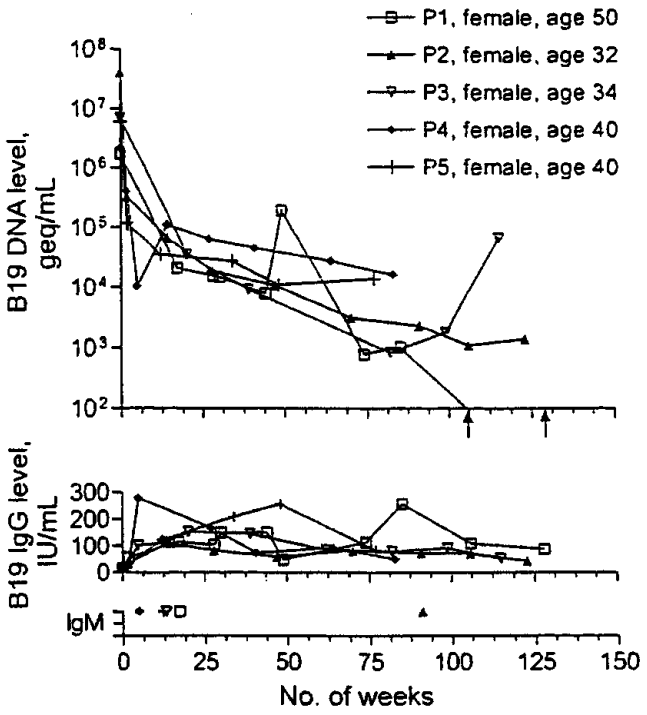

Figure 1. Kinetics of human parvovirus B19 (B19) DNA and antibody responses against B19 in serum after acute infection in patients 1–5 (P1–P5, respectively). The lower panel shows the last time point at which each patient tested positive for serum IgM. Arrows indicate negative sample time points for patient 1. The figure refers to the number of weeks after the first sample was taken.

persistently detectable B19 DNA levels during the entire follow-up period, whereas all control subjects were found to be B19 DNA negative (data not shown). B19 IgM was detected for 5–17 weeks in all patients, except for patient 2, in whom B19 IgM was detectable for 91 weeks.

Discussion. We assessed the kinetics of acute B19 infection by quantitative PCR in 5 immunocompetent individuals who presented with classic symptoms of parvoviral infection. The average initial virus level was in line with what was earlier published [6]. A rapid decrease in the viral load was observed to be inverted to the development of B19 IgG and coincidental with resolution of acute clinical symptoms. By week 17, B19 IgM cleared in all patients, except for patient 2, who continued to have positive results for 90 weeks; this could have been the result of cross-reacting antibodies. Detectable DNA levels were maintained after development of B19 IgG and symptom resolution in all patients. Only 1 patient had clearance of peripheral viremia during the study period. If we assume that these 5 individuals are representative of the general population, we can conclude that B19 exhibits delayed clearance after acute infection. Similarly, B19 DNA has been detected in specimens of skin, synovia, and testis obtained from healthy, IgG-positive individuals [7]. In contrast, dot-blot and nested-PCR assays

have shown that peripheral viremia clears weeks to months after acute infection [8, 9]. No comparable, quantitative data are available, because previous studies have described patients with long-term symptoms, documented persistent infections, and severe presentations when the immune status was not characterized [10–12]. Recent investigations of the cellular immune responses against B19 have revealed that these responses increase during the first year after infection, despite resolution of clinical symptoms [3].

B19-specific $CD8^+$ T cells were shown to possess strong effector function and proliferative capacity and to maintain an activated $CD38^+$ phenotype, with strong expression of perforin and CD57 and down-regulation of CD28 and CD27. The likely explanation for these observations, which supports the present findings, is low-level antigen persistence. The facts that none of the healthy control subjects included in this study had any detectable B19 DNA in serum samples and that the smaller populations of antigen-specific $CD8^+$ T cells detected in individuals who had been infected in the past indicate that the virus is eventually cleared from peripheral blood [13].

The emerging evidence that B19 exhibits slower clearance of peripheral viremia after acute infection than previously thought challenges our current understanding of the virus pathogenesis and suggests a new entity of viral persistence. Furthermore, this evidence has practical implications on the means of diagnosing B19 infection, the means of preventing nosocomial transmission of infection, and vaccine development—areas of research that are all currently evolving. Additional studies that use novel and sensitive techniques are warranted to elucidate the relationship between B19 and the host, to readdress the same questions asked when the pathogen was discovered >25 years ago.

Acknowledgments

We thank the patients and volunteers who repeatedly donated blood samples for the study.

Financial support. The Tobias Foundation, Swedish Cancer Foundation and Swedish Medical Research Council, and Commission of the European Communities (specific research and technological development program "Quality of Life and Management of Living Resources, Human Parvovirus Infection: Towards Improved Understanding Diagnosis and Therapy" [QLK2-CT-2001-00877]; however, this article does not necessarily reflect its views and in no way anticipates the European Commission's future policy in this area).

Potential conflicts of interests. All authors: no conflicts.

References

1. Heegaard ED, Brown KE. Human parvovirus B19. Clin Microbiol Rev 2002; 15:485–505.
2. Lundqvist A, Tolfvenstam T, Bostic J, Soderlund M, Broliden K. Clinical and laboratory findings in immunocompetent patients with persistent parvovirus B19 DNA in bone marrow. Scand J Infect Dis 1999; 31: 11–6.
3. Isa A, Kasprowicz V, Norbeck O, et al. Prolonged activation of virus-specific $CD8^+$ cells after acute B19 infection. PLOS Medicine (in press).
4. Lalvani A, Brookes R, Hambleton S, Britton WJ, Hill AV, McMichael AJ. Rapid effector function in $CD8^+$ memory T cells. J Exp Med 1997; 186:859–65.
5. Servant A, Laperche S, Lallemand F, et al. Genetic diversity within human erythroviruses: identification of three genotypes. J Virol 2002; 76:9124–34.
6. Manaresi E, Gallinella G, Zuffi E, Bonvicini F, Zerbini M, Musiani M. Diagnosis and quantitative evaluation of parvovirus B19 infections by real-time PCR in the clinical laboratory. J Med Virol 2002; 67:275–81.
7. Soderlund-Venermo M, Hokynar K, Nieminen J, Rautakorpi H, Hedman K. Persistence of human parvovirus B19 in human tissues. Pathol Biol (Paris) 2002; 50:307–16.
8. Anderson MJ, Higgins PG, Davis LR, et al. Experimental parvoviral infection in humans. J Infect Dis 1985; 152:257–65.
9. Musiani M, Zerbini M, Gentilomi G, Plazzi M, Gallinella G, Venturoli S. Parvovirus B19 clearance from peripheral blood after acute infection. J Infect Dis 1995; 172:1360–3.
10. Gallinella G, Bonvicini F, Filippone C, et al. Calibrated real-time PCR for evaluation of parvovirus b19 viral load. Clin Chem 2004; 50:759–62.
11. Buller RS, Storch G. Evaluation of a real-time PCR assay using the LightCycler system for detection of parvovirus B19 DNA. J Clin Microbiol 2004; 42:3326–8.
12. Cassinotti P, Siegl G. Quantitative evidence for persistence of human parvovirus B19 DNA in an immunocompetent individual. Eur J Clin Microbiol Infect Dis 2000; 19:886–7.
13. Tolfvenstam T, Oxenius A, Price DA, et al. Direct ex vivo measurement of CD8(+) T-lymphocyte responses to human parvovirus B19. J Virol 2001; 75:540–3.

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 2005年6月15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	乾燥濃縮人アンチトロンビンⅢ		研究報告の公表状況	Transfusion;45,1003-1010, 2005-07-27 Parvovirus B19 transmission by a high-purity factor VIII concentrate	公表国 米国	
販売名（企業名）	アンスロビンP-ベーリング（ZLBベーリング株式会社）					

研究報告の概要

問題点（ヒトパルボウイルスB19のNATスクリーニング検査未実施による感染）

47歳の血友病A患者が結腸内視術施行前に予防のため、S/D処理血液凝固第VIII因子製剤の同一ロット3Vと他のロット1Vで、総量約3800IUの投与を受けた。

患者は投与10分前に採血していた。その後2週間、インフルエンザ様の微熱、疲労感、断続的な腹痛と重度の関節痛があった。術後4週目に検査したところ、抗B19 IgMとIgGが陽性であることが判った。

血液凝固第VIII因子製剤投与前の検体を測定すると、抗B19 IgMとIgG、B19DNAが陰性、投与後の検体はすべて陽性であった。患者は結腸内視術前の少なくとも12年間は、血液凝固第VIII因子製剤や血液製剤の投与は受けていなかった。患者はELISA法で血清学的検査をしたが、HCV陽性、HAV、HBV、HIVは陰性であった。

その他3人の患者が同じ病院で、感染疑いのあるロットの製剤の投与を受けていた。その患者は頻繁に血液凝固第VIII因子製剤の投与を受けているが、B19に関連した症状は発現していない。

感染疑いのあるロットの製剤と感染の因果関係を決定するため、患者の投与後の検体、感染疑いのあるロットの製剤とそのロットのプール血漿の遺伝子配列を調べたところ、極めて類似していたが、WHOなどの標準B19の塩基配列とは異なっていた。

感染疑いのあるロットの製剤のB19DNAは$1.3x10^3$ geq/mLあり、米国でB19のNATのスクリーニング検査を実施していない血漿から製造された製剤のB19DNAレベルと同じレベルであった。

血漿分画製剤による伝播の潜在的危険性を低減するため、製造業者はハイタイターのミニプールNATスクリーニングを導入している。B19のNATスクリーニングを実施していたならば、今回報告した感染は起こらなかったかもしれない。B19 DNAのレベルの高いドナーが同定できれば、感染疑いのあるロットのミニプールは存在していなかった。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
ZLBベーリングでは、B19のNAT検査を実施しており、ウイルス値が高値なものを除外しているので、感染のリスクは極めて低いと考えられる。	今後とも感染症に関する情報収集に努める所存である。

Parvovirus B19 transmission by a high-purity factor VIII concentrate

Chuan-ging Wu, Bobby Mason, Julia Jong, Dean Erdman, Laurel McKernan, Meredith Oakley, Mike Soucie, Bruce Evatt, and Mei-ying W. Yu

BACKGROUND: Parvovirus B19 (B19) is known to cause a variety of human diseases in susceptible individuals by close contact via the respiratory route or by transfusion of contaminated blood or blood products. In this study, whether a case of B19 transmission was causally related to the infusion of implicated lots of a solvent/detergent (S/D)-treated, immunoaffinity-purified factor VIII concentrate (antihemophilic factor [human] [AHF]) was investigated.

STUDY DESIGN AND METHODS: Anti-B19 (both immunoglobulin M [IgM] and immunoglobulin G [IgG]) and B19 DNA (by a nucleic acid testing [NAT] procedure) were assayed in two implicated product lots, a plasma pool, and a recipient's serum sample. Analysis of the partial B19 sequences obtained from sequencing clones or direct sequencing of the samples was performed.

RESULTS: Only one of the two implicated lots was B19 DNA–positive. It contained 1.3×10^3 genome equivalents (geq or international units [IU]) per mL. The negative lot was derived from plasma screened for B19 DNA by NAT in a minipool format to exclude high-titer donations, whereas the positive lot was mostly from unscreened plasma. This high-purity AHF product had no detectable anti-B19 IgG. A 4-week postinfusion serum sample from a recipient, who received both lots and became ill, was positive for the presence of B19 antibodies (both IgM and IgG) as well as B19 DNA. The B19 sequences from the positive lot, its plasma pool, and the recipient's serum sample were closely related.

CONCLUSION: These findings and the recipient's clinical history support a causal relationship between the implicated AHF product and B19 infection in this recipient. The seronegative patient became infected after receiving 2×10^4 IU (or geq) of B19 DNA, which was present in this S/D-treated, high-purity AHF product.

Parvovirus B19 (B19) is a small, nonenveloped, single-stranded DNA virus that is known to resist viral inactivation procedures commonly used in manufacturing plasma-derived products. The virus is widespread, and manifestations of infection vary depending on the immunologic and hematologic status of the host (there are numerous reviews; see Young and Brown[1] for a recent review). B19 infection in immunocompetent persons is often asymptomatic or benign, or results in mild illness, including erythema infectiosum (fifth disease) in children and arthropathy in adults. B19, however, can cause more severe diseases in vulnerable individuals, such as transient aplastic crisis in persons with hematologic disorders, hydrops fetalis and subsequent congenital anemia or fetal death in pregnant women, and pure red cell aplasia and chronic anemia in those who are immune compromised.[1]

Diagnosis of B19 infection is based on detection of specific immunoglobulin M (IgM) and immunoglobulin M (IgG) antibodies by enzyme-linked immunosorbent assays (ELISA) or of viral DNA by nucleic acid testing (NAT) procedures. Serologic studies have shown that at least 50 percent of adults have circulating B19 IgG, which is evidence of past infection.[2,3] The prevalence of B19 viremia in blood and plasma donors has been reported to

ABBREVIATIONS: AHF = antihemophilic factor; Human B19 = parvovirus B19; HTC = hemophilia treatment center.

From the Division of Hematology, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland; the Centers for Disease Control and Prevention, Atlanta, Georgia; and the Dartmouth Hitchcock Hemophilia Program, Lebanon, New Hampshire.

Address reprint requests to: Mei-ying W. Yu, PhD, Division of Hematology, HFM-345, Center for Biologics Evaluation and Research, Food and Drug Administration, Room 303, 29 Lincoln Drive, Bethesda, MD 20852; e-mail: yu@cber.fda.gov.

Received for publication September 30, 2004; accepted November 24, 2004.

doi: 10.1111/j.1537-2995.2005.04387.x

TRANSFUSION 2005;45:1003-1010.

range from 0.003 to 0.6 percent, depending on whether the testing is performed at the time of an epidemic and the sensitivity of NAT methods.[4-6] Although B19 is normally transmitted via the respiratory route or transplacentally from mother to fetus, parenteral transmission can occur through the infusion of contaminated blood products. These latter transmissions are mainly due to the extremely high viremic levels in plasma, for example, 10^{13} genome equivalents (geq) of B19 DNA per mL, found at an early phase of the infection in acutely infected but asymptomatic donors.[7] Thus, the risk of B19 transmission via single-donor blood components is rare but is substantially greater via products from pooled plasma.[8]

B19 DNA has been detected in high proportions and high levels in plasma pools and their resulting plasma derivatives, especially the coagulation products.[9-11] Consistent with these findings, almost all persons with hemophilia in cross-sectional studies from 1982 to 1997 were found to be anti-B19 IgG–positive, in contrast to approximately 50 percent of the untransfused, untreated control population.[12] Reports of transmissions attributed to infused factor VIII (FVIII) concentrates (antihemophilic factor [human] [AHF]), subjected to solvent/detergent (S/D) or heat treatment or both, have been numerous.[12-17]

In most transmission cases, however, the causality assessment[18] between the product and the recipient's infection was not well established. The level of B19 DNA in the product that resulted in infection has rarely been determined, and the minimum viral exposure needed to produce an infection is not clearly defined. In this study, we investigated a case report and established a causal relationship between an implicated AHF product and a recipient. Infection occurred when the seronegative recipient received 2×10^4 geq (equivalent to 2×10^4 international units [IU] based upon our NAT method[19]) of B19 DNA, in a S/D-treated, high-purity AHF product that contained no detectable anti-B19 IgG. Two product lots were initially implicated. One lot, which was negative for B19 DNA, was derived solely from plasma screened for B19 DNA by NAT in a minipool format to exclude high-titer donations.

CASE REPORT

The Food and Drug Administration (FDA) received the following MedWatch report (an adverse event report) from a hemophilia treatment center (HTC) in 2001. A 47-year-old man with mild hemophilia A received a total of approximately 3800 IU of FVIII clotting activity in the form of three vials of one lot of AHF and one vial of another lot, as prophylaxis before undergoing colonoscopy. Both lots were the same AHF product. The patient had a blood specimen drawn 10 minutes before the product infusion for participation in an HTC-based blood safety monitoring program sponsored by the Centers for Disease Control and Prevention (CDC).[20] Beginning 1 week after the colonoscopy, he experienced 2 weeks of symptoms, including flu-like low-grade fever, extreme fatigue, intermittent abdominal pain, and significant joint pain. He visited his primary care physician 4 weeks after the procedure and a blood specimen was taken. It was found to be positive for the presence of anti-B19 IgM and IgG. The HTC notified the manufacturer and CDC, and the pre- and postinfusion specimens were tested at the CDC. The CDC found that the preinfusion sample was negative for the presence of anti-B19 IgM and IgG and for B19 DNA, whereas the postinfusion sample was positive for all three markers. Before the colonoscopy, the patient had not used any AHF or blood products for at least 12 years. He was positive serologically for the presence of hepatitis C virus but negative for the presence of hepatitis A virus, hepatitis B virus, or human immunodeficiency virus, all of which were performed by ELISA tests. He had no children and denied any association with children during the weeks before and following the colonoscopy.

The CDC informed the FDA of the incident and both agencies jointly investigated the case. Three other patients also received the implicated lots from the same treatment center. All three of these patients were retrospectively found to be frequent users of AHF products and did not experience any discomfort or B19-associated symptoms during the same time period.

MATERIALS AND METHODS

Patient specimens, AHF, and plasma pool samples

Serum specimens were collected from the patient before and 4 weeks after the infusion. Lot-release samples of the two implicated AHF lots (designated A and B) submitted by the manufacturer to the FDA were available. Several plasma pools used as starting material for manufacturing lot A were kindly provided by the manufacturer.

Detection and quantitation of B19 DNA by polymerase chain reaction

One vial of each implicated AHF lot was reconstituted with 5 mL of diluent, that is, half of the volume specified by the manufacturer, and 0.2-mL aliquots were used for DNA extraction. For serum specimens or plasma pools, 0.1-mL aliquots were used. For a plasma pool having a high titer of DNA, a 10^3-fold diluted sample was used. DNA was extracted by use of an isolation kit and procedures (NucliSens, Organon Teknika, Durham, NC), and the DNA from each sample was recovered with 100 µL of elution buffer. The following in-house nested B19 NAT procedure was used. A 25-µL aliquot of undiluted or $10^{0.5}$-fold serially diluted DNA extract was added to a 25-µL master mix so that the final reaction mixture for the first-round amplification contained 10 mmol per L Tris-HCl, pH 8.3,