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研究報告の概要

米国においてヒトのバベシア症の症例数の増加に伴って、Babesia microti の輸血感染の報告がここ数年上昇している。献血者の B.microti 抗体測定の幾つかの研究が報告されているが、寄生虫血症と輸血による寄生体の感染リスクについてはよくわかっていない。コネチカット州の Babesia 流行地及び非流行地における献血者の 7～9 月の供血の B.microti 抗体を検査した。その後、抗体陽性者から追加で集められた検体の B.microti 遺伝子の PCR 検査を行った。
コネチカット州の Babesia 流行地及び Babesia 非流行地の血液ドナーそれぞれ 1,745 人、合計 3,490 人のうち、30 人(0.9%)が抗体陽性であり、7 月がピークであった。Babesia 流行地の血清学的陽性血液ドナーは 24 人（1.4%）で、Babesia 非流行地の血清学的陽性血液ドナーの 6 人（0.3%）より多かった。また、血清学的陽性の血液ドナー19 人のうち 10 人（53%）が PCR により陽性であった。コネチカット州の Babesia 流行地では B. microti 抗体陽性者が多く、B. microti 抗体陽性者の半数以上から原虫血症が証明されたことにより輸血による B. microti 感染リスクが高いと思われる。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1) 本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理(ナノフィルトレーション)を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
米国においてヒトのバベシア症の症例数の増加に平行して、B. microtiの輸血感染の報告がここ数年上昇しており、コネチカット州のBabesia流行地ではB. microti抗体陽性者が多く、B. microti抗体陽性者の半数以上から原虫血症が証明されたことにより輸血によるB. microti感染リスクが高いという報告である。 血漿分画製剤からのバベシア原虫伝播の事例は報告されていない。また、万一原料血漿にB. microtiが混入したとしても、除菌ろ過等の製造工程において十分に除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

(4)

Demonstrable parasitemia among Connecticut blood donors with antibodies to *Babesia microti*

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BACKGROUND: Reports of transfusion-transmitted *Babesia microti* have risen steadily during the past several years, reflecting a concurrent increase in US cases of human babesiosis. Although several studies have measured *B. microti* antibodies in blood donors, little is known about associated parasitemia and the inherent risk of transmitting the parasite by transfusion.

STUDY DESIGN AND METHODS: Donations from blood donors located in *Babesia*-endemic and nonendemic areas of Connecticut were tested for *B. microti* antibodies from July through September. Subsequently, an additional blood sample was collected from selected seropositive donors and tested by nested polymerase chain reaction (PCR) for *B. microti* nucleic acids.

RESULTS: A total of 3490 donations, 1745 each from endemic and nonendemic areas, were tested for *B. microti* antibodies; 30 (0.9%) were confirmed as positive and seroprevalence rates peaked in July. Significantly more seropositive donations were from endemic areas (24, 1.4%) than nonendemic areas (6, 0.3%). Ten (53%) of 19 seropositive donors subsequently tested by PCR were positive.

CONCLUSION: *B. microti* seroprevalence was highest in those areas of Connecticut where the parasite is endemic. More than half of seropositive donors tested had demonstrable parasitemia, indicating that many are at risk for transmitting *B. microti* by blood transfusion. Three donors were identified as parasitemic in October, suggesting that donors may be at risk for transmitting the parasite outside of the peak period of community-acquired infection.

For the past several years, blood safety concerns in the United States have focused primarily on a series of newly emerging agents and diseases.[1] In the late 1990s, a variant form of Creutzfeldt-Jakob disease was described in humans that appears now to be transmissible by transfusion. Thereafter, the first US case of West Nile virus appeared in humans during 1999 followed closely in 2002 by reports of 23 transfusion cases involving this agent. As demonstrated by the emergence of severe acute respiratory syndrome (SARS) in 2003, each new emerging agent is assessed for potential transmission by blood transfusion. Unfortunately, the ongoing preoccupation with newly emerging agents has allowed previously described agents, some of which pose significant blood safety threats, to be overshadowed. Among these agents is the intraerythrocytic protozoan parasite *Babesia microti*, the primary agent of human babesiosis in the United States.

B. microti is endemic to the northeastern and upper midwestern United States where it is transmitted naturally by exposure to black-legged ticks (*Ixodes scapularis*) infected with this parasite. Since the first US case of babesiosis was described in 1966,[2] hundreds of human cases

ABBREVIATIONS: IFA = indirect immunofluorescent antibody; PBST = phosphate-buffered saline containing 0.1 percent Tween 20; RT = room temperature.

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rinsed in distilled water, and air-dried. Samples were examined by fluorescence microscopy at 400× magnification. Positive samples were titered to endpoint. Appropriate negative and positive controls, as described above, were included as part of all IFA testing.

Detection of *B. microti* DNA through PCR

A portion of seropositive donors provided a subsequent whole-blood sample for analysis by PCR. All donors with IFA titers ≥ 1:256 and approximately 75 and 50 percent of randomly selected donors with titers of 1:128 and 1:64, respectively, were selected for PCR analysis. Briefly, donors selected were contacted via letter and notified of their initial test results indicating the presence of antibodies to *B. microti*. Donors received information regarding an expanded study investigating the relationship between antibodies to *B. microti* and the potential presence of the parasite and were invited to participate. Informed consent was obtained from all donors agreeing to participate, as well as, four 7 mL ethylenediaminetetraacetate tubes to be used for serologic testing and PCR analysis. Test results were reported to participants via letter. All PCR-positive donors were considered *B. microti*–infected, contacted for counseling, asked to complete a brief questionnaire regarding risk factors for babesiosis, referred to their physician for evaluation and possible treatment, and indefinitely deferred from future American Red Cross blood and tissue donations. Later, based on the results in this article, all donors seropositive by IFA for *B. microti* were deferred.

All blood samples were analyzed with a nested PCR protocol, modified from the original, designed to amplify the 18S ribosomal RNA gene of *B. microti*.[14] Parasite DNA was extracted from whole blood with a DNA blood kit (QIAamp DNA blood mini kit, Qiagen, Inc., Valencia, CA) as per the manufacturer's instructions and resuspended in 200 µL final volume. The initial PCR was performed by adding 10 µL of extracted DNA to 40 µL of PCR master mix containing 12.5 pmol per µL of primers Babl (5′-CTTAG TATAAGCTTTTATACAGC-3′) and Bab4 (5′-ATAGGTCA GAAACTTGAATGATACA-3′), 1.25 U of AmpliTaq Gold *Taq* polymerase, GeneAmp 10× Buffer I (10 mmol/L Tris, pH 8.3, 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, and 0.001 percent gelatin), GeneAmp dNTP blend (250 µmol/L each dNTP; all from Perkin-Elmer), and sterile water. An additional 1 mol per L $MgCl_2$ (Sigma-Aldrich Corp.) was added to a final concentration of 2.5 mmol per L. Amplification was performed in a thermal cycler (GeneAmp 9700, Applied Biosystems, Foster City, CA) with the following settings: 95°C for 15 minutes, 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 1.5 minutes, and then final extension at 72°C for 10 minutes. The nested PCR was performed with 5 µL of the initial Babl-Bab4 amplification product (238 bp) diluted 1:10, 45 µL of PCR master mix (described above) containing 5 pmol per µL primers Bab2 (5′-GTTATAGTTTATTTGATGTTCGTTT-3′) and Bab3 (5′-AAGCCATGCGATTCGCTAAT-3′), and sterile water for a total reaction volume of 50 µL. No additional $MgCl_2$ was added. The amplification settings were the same as described above; however, the annealing temperature was 55°C. The 155-bp products were visualized on a 2 percent agarose gel stained with ethidium bromide in 1× TAE buffer (Invitrogen Corp., Carlsbad, CA). Appropriate positive controls (again provided by P.J. Krause), negative controls, and extractions controls were included.

Statistical analyses

Statistical analyses were performed when appropriate with the chi-square test. A p value of less than 0.05 was considered significant in all cases.

RESULTS

Seroprevalence screening

From July through September 1999, a total of 3490 blood donations from *Babesia*-endemic (n = 1745) and nonendemic (n = 1745) areas of Connecticut were tested for antibodies to *B. microti*. Two-hundred and three (5.8%) were positive by EIA, and when tested by the supplemental IFA, 30 (0.9%) were confirmed positive (Table 1). When endemic and nonendemic areas were compared, there was no significant difference ($\chi^2 = 3.5$; $p = 0.06$) in the initial reactive rates as determined by EIA, but when those samples positive by EIA were tested by the supplemental IFA, a significantly greater number of donations ($\chi^2 = 9.7$; $p < 0.002$) from the endemic area (1.4%) were confirmed as positive compared to the nonendemic area (0.3%). The geographic distribution of seropositive donors (based on residence) in the endemic and nonendemic areas of Connecticut is detailed in Fig. 1.

The initial reactive rates for the combined *Babesia*-endemic and nonendemic areas were similar each month when measured by EIA (Fig. 2). When the monthly rates for endemic and nonendemic areas were compared, however, different trends were observed. For endemic areas, the EIA-reactive rate dropped from a high of 8.2 percent in July to 5.0 percent in September. In contrast, the non-

TABLE 1. Serologic testing of blood donors from endemic and nonendemic areas of Connecticut for antibodies to *B. microti*

Area	Number	EIA-positive (%)	IFA-positive (%)
Endemic	1745	115 (6.6)	24 (1.4)*
Nonendemic	1745	88 (5.0)	6 (0.3)
Totals	3490	203 (5.8)	30 (0.9)

* Seroprevalence in the endemic region was significantly greater than that of the nonendemic region ($\chi^2 = 9.7$; $p = 0.002$).

TABLE 2. Results of subsequent serologic and parasitemia testing of seropositive blood donors with nested PCR

Sample	Region	Initial IFA titer	Subsequent IFA titer	Days between draws	PCR result
1	Endemic	1:512	1:512	28	Positive
2	Nonendemic	1:256	<1:64	29	Positive
3	Endemic	1:64	<1:64	38	Negative
4	Endemic	1:512	1:256	32	Negative
5	Endemic	1:512	1:512	18	Positive
6	Endemic	1:256	1:128	35	Positive
7	Endemic	1:256	<1:64	30	Positive
8	Endemic	1:1024	1:1024	34	Positive
9	Endemic	1:512	1:256	29	Negative
10	Nonendemic	1:512	1:512	29	Negative
11	Endemic	1:1024	1:1024	33	Negative
12	Endemic	1:64	1:64	29	Negative
13	Endemic	1:256	1:64	34	Negative
14	Endemic	1:2048	1:1024	29	Negative
15	Endemic	1:4096	1:512	32	Negative
16	Endemic	1:512	1:512	17	Positive
17	Endemic	1:256	1:256	32	Positive
18	Nonendemic	1:512	1:512	30	Positive
19	Endemic	1:512	1:512	63	Positive

ulation, which serves as the main host for adult *I. scapularis* ticks, has increased in part owing to a lack of predators, but also owing to the reforestation of agricultural land and suburban neighborhoods that provide suitable habitat. Concurrently, humans have increased outdoor recreational activities and built homes that place them in close proximity to tick populations.[17] These interactions with ticks have led to a dramatic rise in reports of tick-borne diseases throughout the northeast, especially Lyme disease, babesiosis, and ehrlichiosis.[18-20] Although the increase in cases of Lyme disease is well documented, the steady increase in cases of babesiosis due to infections with *B. microti* has gone largely unnoticed. Previous studies have identified blood donors with antibodies to *B. microti* and this study indicates that many are also demonstrably parasitemic. Indeed, the high percentage (53%) of donors with demonstrable parasitemia mirrors the high numbers of transfusion cases reported in recent years. These observations suggest that blood donors seropositive for *B. microti* may pose a greater risk for blood safety than once thought.

Previously, we reported seroprevalence rates for *B. microti* in Connecticut blood donors to range from 0.3 to 0.6 percent.[10] These samples, however, were collected primarily during late fall and winter (i.e., October to March) when tick exposure is at a minimum and new infections are unlikely to be acquired. This study clearly demonstrates the influence of seasonality on overall seroprevalence rates because the rate of confirmed IFA positives peaked in July (1.2%), rapidly declining thereafter to 0.3 percent in September. Perhaps peak seroprevalence actually occurred earlier than July, but further studies bracketing the entire tick season would be needed to more precisely define this period. Seroprevalence rates are also dramatically influenced by geographic location with significantly more confirmed positive donors identified in *Babesia*-endemic versus nonendemic areas. The defined areas of *B. microti* endemicity are continually expanding,[21] however, and thus may now encompass areas previously designated as nonendemic. Alternatively, residents of nonendemic areas can acquire infections during visits to *Babesia*-endemic areas. This scenario led to the only case of transfusion-transmitted *B. microti* reported in Canada.[22] Similarly, infected donors from a *Babesia*-endemic area may donate blood in a nearby nonendemic area as part of a local or work-related blood drive. Although the defined areas of *Babesia* spp. endemicity are expanding in some parts of the United States, the parasite has not been identified in many states or regions. Therefore, selective geographic testing has been suggested as a possible intervention, but this paradigm has not been commonly used in operational blood banking.

As already noted, in addition to measurable antibody titers, a majority of seropositive donors identified in this study were also shown to be parasitemic based on PCR. The presence of parasite DNA is thought to be indicative of an active infection, because free DNA or dead parasites would be cleared rapidly from the peripheral blood.[23] It is unclear, however, how long our donors were demonstrably parasitemic, because only a single sample was tested by PCR. Of the three donors whose samples were drawn for PCR testing during October, all three were identified as PCR-positive, suggesting that parasitemia in some cases is persistent, occurring outside of the reported period during which tick-borne diseases are primarily transmitted. Indeed, past studies have suggested that *Babesia* infections may recrudesce after long periods of silence (i.e., 26 months),[23] but the possibility of reinfection must also be considered, particularly in *Babesia*-endemic areas. Persistent, perhaps year-round, infections suggest that proposed donor management policies that avoid collection of blood in endemic areas during the summer months when transmission is thought to be at its peak would be partially, though not completely effective. As stated above, it is not only difficult to define the peak transmission period, but ongoing persistent infections suggest that many donors may be at risk for transmitting *B. microti* throughout the year.[24] Thus, these observations suggest that seasonally based collection criteria, much like reported geographic areas of agent endemicity, only partially reduce the risk of transmission, while producing detrimental effects on blood availability.

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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	
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)			スウェーデン	

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

(57)

BRIEF REPORT

Slow Clearance of Human Parvovirus B19 Viremia following Acute Infection

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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-

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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 (Amersh am 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

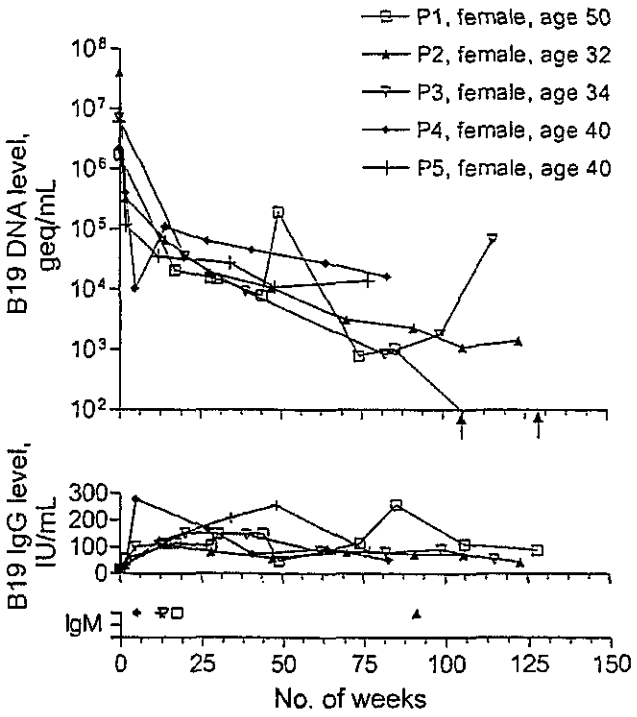

Figure 1. Kinetics of human parvovirus B19 (B19) DNA and antibody responses against B19 in serum after acute infection in patients 1–5 (P1–P5, respectively). The lower panel shows the last time point at which each patient tested positive for serum IgM. Arrows indicate negative sample time points for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

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Potential conflicts of interests. All authors: no conflicts.

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