and blood products has been frequently documented (Azzi et al., 1999). Attempting to reduce the risk of B19 infection has become a major concern for blood and blood product suppliers in Europe and America (Brown et al., 2001), with many countries now advocating screening of plasma pools to remove samples containing high B19 viral titres.

However, B19 DNA appears to be very stable and low levels of B19 DNA can persist in serum and a range of tissues for months following acute infection (Soderlund-Venermo et al., 2002), and detection of viral DNA does not equate necessarily with active viral infection. In addition, assays for detecting infectious virus are extremely limited. Although B19 has been shown to replicate in vitro in primary erythroid progenitor cells from bone marrow (Mortimer et al., 1983a), peripheral blood (Ozawa et al., 1986) and fetal liver (Brown et al., 1991; Yaegashi et al., 1989), very few cell lines have been found to be permissive for B19 infection, and even in these, viral replication is inefficient. The first cell line described to be permissive for B19 infection was an erythropoietin (Epo)-dependent subclone of UT7, a megakaryoblastoid cell line (Shimomura et al., 1992). Since then, a limited number of additional cell lines have been described, including KU812Ep6, an erythroleukaemic cell line (Miyagawa et al., 1999), and JK-1 cells (Takahashi et al., 1993). To date no comparative studies of the differences in susceptibility/sensitivity and permissivity amongst these cell lines have been published.

A number of different methods have been suggested for detecting parvovirus B19. Currently, methods for identifying active clinical B19 infections include detection of B19 nucleic acid testing by direct dot blot hybridization (Anderson et al., 1985) or PCR (Cassinotti et al., 1993; Clewley, 1992), and more recently RT-PCR for RNA transcripts (Wong et al., 2003). Similar methods have been used for detecting infection in cells or cell lines, but little has been published on the relative sensitivity of the different methods.

In this study we compared the susceptibility/sensitivity of various cell lines to B19 infection and evaluated different methods to detect viral infection. In addition, we established a high-throughput method for detection of B19 infection and validated the assay by using it to detect infectious virus in plasma pools and neutralizing antibodies in serum samples.

2. Materials and methods

2.1. Cell lines

Cell lines were obtained from American Tissue Type Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco's modified Eagle medium (DMEM) plus 10% fetal calf serum (FCS), penicillin, streptomycin and glutamine (P/S/G; Gibco/Invitrogen, Carlsbad, CA, USA) at 37 °C with 5% CO_2 unless otherwise stated. UT7/Epo (Shimomura et al., 1992) were maintained in RPMI 1640 with 10% FCS and 5 U/mL of Epo (Amgen, Thousand Oaks, CA, USA). UT7/Epo-S1, a subclone of UT7/Epo and a gift from Dr Sagamura (Morita et al., 2001) were maintained in Iscove's modified Dulbecco's media (IMDM) plus 10% FCS, and 2 U Epo/mL. KU812Ep6, a gift from Dr Miyagawa (Miyagawa et al., 1999) were maintained in RPMI 1640, 10% FCS and 6 U Epo/mL K-562 and JK-1 cells (Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany) were maintained in RPMI 1640, with 10% FCS.

2.2. B19, serum and plasma sources

Plasma and serum samples containing high-titre infectious B19 was obtained from several sources. J35 was obtained from a sickle-cell patient in aplastic crisis, and previously determined to be negative for both B19 IgM and IgG (data not shown). Additional plasma samples were obtained from normal donors at the time of blood donation, and provided by Mei-Ying Yu at CBER, FDA and Aris Lazo at V.I. Technologies, Inc. The WHO standard sample (Saldanha et al., 2002) was obtained from National Institutes of Biological Standards and Control (NIBSC), South Mimms, UK.

Plasma pools containing high-titre B19 were a kind gift of Matthias Gessner of Baxter. Serum samples from laboratory donors and healthy blood donors obtained as part of the National Heart, Lung, and Blood Institute (NHLBI) Retrovirus Epidemiology Blood Donor Study, NHLBI repository, and previously tested for antibody to parvovirus B19 (Brown et al., 2004) were used in the neutralization assay.

2.3. Comparison of infectivity of different cell types

The infection assay was as previously described (Nguyen et al., 2002). Briefly, on the day before infection, cells were split 1:2 with appropriate media to induce cell division. On the day of infection, cells were seeded at 2×10^5 cells/100 μL, an equal volume of virus dilution was added and the infection was allowed to incubate at 4 °C for 2 h. After the incubation, 800 μL of appropriate media was added to the infection bringing the volume to 1 mL. The cells were transferred to a 24-well plate and incubated at 37 °C and harvested for analysis on Days 1, 2, 3, or 6.

2.4. Immunofluorescence (IF) assay for B19 capsid proteins

Cells were evaluated for B19 protein production by IF staining. Approximately 5×10^4 cells were collected onto glass slides by cytocentrifugation at 1500 rpm for 8 min and fixed in methanol–acetone (1:1) at −20 °C. Viral capsid proteins were detected by mouse anti-B19 monoclonal antibody, 521-5D (a gift from Larry Anderson, CDC, Atlanta, GA, USA) followed by goat anti-mouse IgG FITC (Zhi et al., 2004).

2.5. B19 DNA

DNA direct hybridization was used to quantitate the B19 copy number as previously described (Brown et al., 1994). Briefly, plasmid pYT103 was diluted to give a range of DNA concentrations (0, 0.1, 1, 10, and 100 pg/μl). Plasmid dilutions, serum samples or extracted DNA (10 μl) were added to 200 μl 0.333 M NaOH and incubated at room temperature to denature the DNA. The samples were then applied to a nylon membrane (0.45 μm pore, Nytran Plus, Schleicher and Schuell, Keene, NH, USA) using a dot blot manifold apparatus (96-well, Schleicher and Schuell), the membrane washed in 6× SSC, and the membrane baked at 80 °C for an hour in a vacuum oven. The membranes were hybridized with a ^{32}P random-primed probe of the complete B19 coding region (*Eco*RI digest of pYT103) in Hybrisol (Serologicals Corporation; 42 °C overnight). The membranes were washed with 2× SSC; 0.1% SDS at room temperature and 0.1× SSC; 0.1% SDS for 20 min at 55 °C, and then exposed to either a "phosphor" screen (Molecular Dynamics, GE Healthcare) or X-ray film.

2.6. Quantitative PCR

DNA was extracted from cells and supernatant using the QIAmp DNA mini Kit (Qiagen, Valencia, CA, USA), and quantitated by qPCR using the QuantiTect Probe PCR kit (Qiagen), using primers in the capsid region of the virus. Specifically the primers and probe used were B19-Cap-F (5′-TACCTGTCTGGATTGCAAAGC-3′; 0.4 μM) and B19-Cap-R (5′-GATGGGTTTTCTAGGGGATTATC-3′; 0.4 μM) and 0.2:M B19-Cap-Probe probe (6-FAM-ATG GTG GGA AAG TGA TGA TGA ATT TGC TA-Black Hole Quencher). Quantitation of the number of genome copies was estimated by comparison to a standard curve obtained from serial dilutions of the pYT103, and confirmed by testing the NIBSC standard (Saldanha et al., 2002).

2.7. RT-PCR

As previously described, an RT-PCR assay was used to look for spliced viral transcripts as a marker for infection (Nguyen et al., 2002). Briefly, cells were harvested and total RNA extracted using 200 μL RNA STAT-60. Contaminating DNA was removed using RQ1 DNAse (Promega) incubation for 15 min at room temperature and RNA was reverse transcribed by initially incubating RNA with random primers and reverse transcriptase, Superscript II (Invitrogen), prior to PCR amplication with primers B19-9 and B19-1. To increase detection of spliced products from the RT-PCR reactions, products were resolved on a 2.5% NuSieve agarose gel and southern hybridization was performed using probe labeled with alkaline phosphatase (CDP-Star AlkPhos labeling kit, Amersham).

2.8. Quantitative RT-PCR

RNA transcripts were quantitated by real-time RT-PCR designed to amplify products in the capsid and NS regions using the QuantiTect Probe RT-PCR kit (Qiagen). The QuantiTect Probe RT-PCR master mix and QuantiTect RT mix was combined with 0.4 μM of the amplification primers (NS primers 5′-GTTTTATGGGCCGCCAAGTA-3′ and 5′-ATCCCAGACCACCAAGCTTTT-3′; capsid primers 5′-CCTGGGCAAGTTAGCGTAC-3′ and 5′-ATGAATCCTTGCAGCACTGTCA-3′), and 0.2 μM probe (NS probe FAM 6′-CCATTGCTAAAAGTGTTCCA-BHQ1; capsid probe FAM-TATGTTGGGCCTGGCAA-TAMRA). After an initial activation step of 15 min at 95 °C, 45 cycles of 15 s at 94 °C and 60 s at 60 °C were performed. Quantitation of the number of transcripts was by estimating the cDNA copy number by comparison of a standard curve of serial dilutions of pYT103 as described for the quantitative PCR.

To confirm extraction of RNA, and to normalize the number of transcripts per cell, quantitative RT-PCR (qRT-PCR) was performed using the same amplification conditions, but with primers β-actin F (5′-GGCACCC-AGCACAATGAAG-3′), β-actin R (5′-GCCGATCCACA-CGGAGTACT-3′) and actin probe (5′JOE-TCAAGA-TCATTGCTCCTCCTGAGCGC-3′BHQ). An actin standard curve was obtained from serial dilutions of a plasmid containing an extended region of the actin coding sequence.

2.9. High-throughput qRT-PCR

To develop a high-throughput method for detecting RNA transcripts, RNA was extracted from cells using GeneStrips™ (RNAture, Irvine, CA, USA) according to the manufacturer's protocols. mRNA extracted using this method was converted to cDNA using MMLV-RT (Invitrogen, Carlsbad, CA, USA) following manufacturer's protocol scaled up to a 50 μL reaction volume. B19 RNA transcript production was determined by qRT-PCR as described above.

In some experiments the infection volume was scaled down to 100 μL and incubated in 96-well plates.

2.10. Detection of infectious virus in plasma pools

Plasma pools of 2000 donors that contained B19 DNA by PCR B19 were tested for their ability to infect UT7/Epo-S1 cells. Infection was as described (100 μL infection volume) and RNA was extracted with RNAture Genestrips and analyzed by qRT-PCR. The number of B19 DNA copies in the original plasma pools was determined by quantitative PCR.

2.11. Neutralizing antibody detection

Detection of neutralizing antibodies was assayed by qRT-PCR. Serum or plasma from donors was incubated with serial dilutions of high-titre B19 containing serum for 1 h prior to

infection with UT7/Epo-S1 cells as described for infections (100 μL in a 96-well plate). RNA was extracted from cells using the RNAture Genestrips and analyzed by qRT-PCR.

3. Results

3.1. Comparison of sensitivity and permissiveness in haematopoietic cell lines

The majority of the haematopoietic lines tested (HL-60, HEL, KG-1, KG-1a, K-562, U-937) were negative by both IF for capsid protein and detection of spliced transcripts indicating that the cells were non-permissive. Only UT7/Epo cells, KU812Ep6, the UT7/Epo-S1 subclone and JK-1 cells showed evidence of B19 infection with the UT7/Epo-S1 cells having the greatest sensitivity. However, by IF, the number of positive cells was always low, with <1% positive staining for KU812Ep6, UT7/Epo and JK-1 cells, but approximately 15% positive staining for UT7/Epo-S1 cells. This greater sensitivity was confirmed by detection of transcripts in UT7/Epo-S1 cells at 10^4 ge/infection of the high-titre serum, 3 logs lower than that detected in the other cell lines. Subsequent studies were all done with the UT7-Epo-S1 cells.

3.2. Comparison of sensitivity of different methods to detect infectious B19

IF staining for B19 capsid protein production detected infected cells consistently at 10^{-2} to 10^{-3} dilutions of high-titre serum (>10^{12} ge/mL), but the number of positive cells was low (15–1%). Determining viral DNA production by direct hybridization of viral DNA by dot blot was limited to detection above 10^9 ge/infection, in part because only 10 μL of sample was analyzed from a 1 mL infection. In time course experiments, when infecting with high concentrations of virus (10^9 ge/mL) spliced transcripts could be detected on the first day, rising on the third day. When samples were tested on Day 3, the most sensitive methods were those detecting RNA transcripts, either by conventional or qRT-PCR assay or detecting the increase in viral DNA production by qPCR (Table 1).

Table 1
Comparison of the different sensitivities of assays used to determine B19 infectivity

Detection method	Sensitivity, genome equivalents (ge)
Protein: IF	~10^8
DNA: dot blot hybridization	10^9
DNA: quantitative PCR	10^4
RNA: RT-PCR	10^4
RNA: quantitative RT-PCR	10^4

UT7/Epo-S1 cells were infected with dilutions of B19, and cells assayed on Day 3. Results are the minimum amount of virus added to a 1 mL cell culture to detect infectivity.

Table 2
Comparison of the number of infectious units of B19 to the viral DNA in different serum or plasma samples

Viral stock	Genome equivalents ((ge)/mL × 10^{12})	Infectious (units/mL)	ge/infectious units
J35	33.2 ± 2.0	10^8	3.3×10^5
VS2	10.2 ± 2.9	10^9	1.0×10^4
V1	2.0 ± 0.3	10^8	2.0×10^4
V2	2.9 ± 0.2	10^8	2.9×10^4
V3	0.1 ± 0.1	2×10^7	5×10^3
CBER STD	1.0	10^8	1.0×10^4

Viral DNA measured by qPCR. UT7/Epo-S1 cells were infected with serial dilutions of virus, on Day 3 RNA was extracted with RNA STAT60 and B19 transcripts detected by RT-PCR.

3.3. Comparison of the infectivity of different serum samples with viral copy number

Serial dilutions of different viral sera stock were used to infect UT7/Epo-S1 cells, and the infectious titre determined by the endpoint of detection using RT-PCR (Table 2). The viral genome equivalents in each sample was determined for each sample by qPCR and confirmed by dot blot analysis and the ratio of viral DNA (ge) were compared to infectious units. The ratio of genome equivalents were relatively high compared to infectious units, with ratios ranging from 2×10^5 ge in the J35 stock to 5×10^3 ge per infectious unit in the CBER standard.

3.4. Comparison of RNA extraction methods

Cell lysates were directly incubated in RNAture "Genestrips", washed off, and cDNA synthesized in situ, prior to qPCR. In direct comparion tests RNA extracted from scaled down 0.1 mL cultures with RNAture Genestrips showed comparable sensitivity to 1 mL cultures extracted with RNA STAT-60 (Table 3).

Table 3
Equivalent sensitivity of detection of infectious virus using two different methods of RNA extraction and B19 transcript detection

Virus dilution	Standard method		High-throughput method	
	Day 0	Day 3	Day 0	Day 3
10^{-5}	–	+++	0	27,345
10^{-6}	–	+++	0	1328
10^{-7}	–	+++	0	231
10^{-8}	–	–	0	1
10^{-9}	–	–	0	0

A plasma stock (V1) was serially diluted in culture medium and used to infect UT7/Epo-S1 cells. Standard method, RNA was extracted from a 1 mL culture using RNA STAT-60 and transcripts detected by RT-PCR. +++, bands easily detected by ethidium bromide staining and with an alkaline phosphatase-labeled specifc probe; –, bands not detected; and high-throughput method, RNA was extracted from a 0.1 mL culture using RNAtureGenestrips and transcripts detected by NS qRT-PCR. Quantitations are given as ge/μL of RT reaction volume.

Table 4
Detection of infectious B19 in plasma pools

Sample #	Stock (ge)/mL × 10^{12}	RT-PCR result	VP qRT-PCR (ge/infection) × 10^8	NS qRT-PCR (ge/infection) × 10^8
Pool #1	1.8 ± 0.4	+++	2.1 ± 0.9	2.2 ± 1.4
Pool #2	3.2 ± 0.1	+++	4.6 ± 0.8	3.7 ± 0.9
Pool #3	0.5 ± 0.1	+++	2.8 ± 0.3	2.1 ± 0.4
Pool #4	0.5 ± 0.04	+++	2.6 ± 0.9	2.4 ± 0.5
Pool #5	0.005 ± 0.0005	+	0.0023 ± 0.001	0.0036 ± 0.002
Pool #6	1.1 ± 0.3	+++	3.0 ± 0.2	1.4 ± 0.05
No virus	0	–	0	0

The amount of B19 in six plasma pools (previously known to contain B19) was determined by qPCR and the presence of infectious virus was determined by infection of UT7/Epo-S1 cells and detection of RNA transcripts after by either RT-PCR or using RNAture Genestrips and qRT-PCR amplifying the capsid (VP) and nonstructural (NS) regions.

3.5. Detection of infectious virus in pooled blood products

To test the sensitivity of our high-throughput assay and to test its potential application for screening plasma pools for infectious virus we examined six plasma pools previously identified as containing high-titre B19 DNA. qPCR analysis confirmed that all samples contained B19 DNA, with five of the six samples containing high B19 titres with >10^{11} ge/mL. The sixth sample had ~5 × 10^9 ge/mL. However, after infection of UT7/Epo-S1 cells, RNA transcripts could be detected by both RT-PCR and qRT-PCR, indicating that all the pools contained infectious B19 (Table 4).

3.6. Neutralization

As further validation of the high-throughput assay the method was used to detect B19 neutralizing antibodies in six sera of known B19 antibody status. After incubation with serial dilutions of each serum with virus for 2 h, the antibody/virus complex was allowed to infect UT7/Epo-S1 cells in a microtitre plate, RNA extracted at Day 3 RNAture Genestrips, and qRT-PCR performed. No block of infection was detected in the two sera that were B19 IgG negative (Table 5). In contrast, at the highest concentration of virus, there was a marked reduction in the number of viral transcripts with all four sera. If a reduction of viral transcripts by >90% is considered the endpoint, then in two sera the neutralizing titre was >10^5, and in the other 10^3 and 10^4, respectively.

4. Discussion

Although the erythroid tropism and inhibition of erythroid colony formation was demonstrated in 1983 (Mortimer et al., 1983a) and replication in vitro infection of bone marrow was demonstrated in 1986 (Ozawa et al., 1986), there is still no readily available method for culturing parvovirus B19 in the laboratory, limiting both the virus availability and the ability to develop assays to determine B19 infectivity. Similarly there are no readily available methods for detecting neutralizing antibodies in patient samples, or for testing viral inactivation procedures for blood and blood products.

A number of cell lines have been described that support B19 infection, and a number of infectivity assays have been described, based on these cell lines. Specifically, infection and neutralization assays based on UT7/Epo cells (Bostic et al., 1999), KU812Ep6 (Blumel et al., 2002; Bonvicini et al., 2004; Miyagawa et al., 1999; Saito et al., 2003) and UT7/Epo-S1 (Prikhod'ko et al., 2005) cells have all been described. More recently, cells that are not fully permissive for B19 infection have also been evaluated (Caillet-Fauquet et al., 2004) However, there have been no attempts to compare the different cell types or sensitivity of the different methods. In addition, many of the methodologies are very labour intensive, and/or require reading of IF, and are therefore not readily automated or applicable to testing large numbers of samples.

In our study, IF was the least sensitive of the methods, with apart from UT7/Epo-S1 cells, generally less than 1% of cells being positive even after inoculation with high-titre virus.

Table 5
Neutralizing antibody assay using quantitative RT-PCR in the B19 NS region

Serum sample	4	5	44	45	A	B
IgG	+	+	–	+	+	–
Serum alone	0	0	9	0	0	8
B19 + 10^{-3} serum	189	0	5244	0	0	7393
B19 + 10^{-4} serum	30,215	3	14,423	50	34	5801
B19 + 10^{-5} serum	40,657	64	68,165	423	12,674	79,557
B19 alone	30,027	20,709	24,234	19,666	11,448	20,676
Cells only	0	0	0	0	1	2

Normal donor serum was preincubated with dilutions of high-titre B19 plasma (>10^{12} ge/mL) and used to infect UT7/Epo-S1 cells. A representation of quantitative data obtained is shown and given in ge/μL of RT reaction volume and normalized against the qPCR obtained for β-actin.

This percentage of positive cells was lower than published in the literature: Miyagawa reported KU812Ep6 cells as having about 30% of the cells positive for B19 infection (Miyagawa et al., 1999), and Morita reported that 40% of the UT7/Epo-S1 stained positive for B19 infection (Morita et al., 2001). Some of these discrepancies may be due to the amount of virus used for the infection (at higher titre we can observe that 30% of cells are positive), differences in culture techniques, and the specificity of the antibody used (Mab 521-5d used in these studies is specific for capsid conformational epitopes). However, as reading IF slides is not readily automated we did not spend more time optimizing the method.

Assays based on RT-PCR were the most sensitive assays, and in contrast to DNA-based assays were not confounded by input viral genomes. As with the IF assay, they also confirmed that the UT7/Epo-S1 cells were the most sensitive cell line, and using these cells, we could detect infectious virus with inoculums of $\sim 10^4$ genome copies. This is also in keeping with other published results that suggest the ratio of infectious virus:genome copies is 1:10,000 (Bonvicini et al., 2004; Miyagawa et al., 1999), not dissimilar to that of other *Parvoviridae* (Tattersall and Cotmore, 1988).

Due to the concerns of B19 contamination in blood and blood product, there is currently great interest in not only developing methods to detect infectious virus, but also to evaluate methods of viral inactivation/removal. Due to the difficulty in working with B19, many inactivation studies have been undertaken using a surrogate parvovirus, normally porcine parvovirus. However, when comparisons have been undertaken, porcine parvovirus and B19 have different properties as far as heat inactivation (Blumel, 2004) and pH stability (Boschetti et al., 2004), suggesting where possible the model virus should be studied. Although we, with colleagues, have previously described infections assays based on UT7/Epo cells by detection of spliced transcripts (Bostic et al., 1999; Lazo et al., 2002) or quantitative PCR (Prikhod'ko et al., 2005), we believe that the combination or RNA extraction process with quantitative RT-PCR is the easiest method for detecting B19 infection under a wide range of different clinical and experimental conditions.

Finally, the detection of neutralizing antibodies for parvovirus B19 continues to be challenging. Although methylcellulose-based assays were originally described, they are insensitive, and require large amounts of infectious virus, and are quite labour-intensive both to set up and to read. In contrast, the assay described here, requires small amount of B19 virus, and can be readily set up in a microtitre plate format. In some patients, especially immunocompromised patients, who have low levels of DNA in serum or tissues in the presence of B19 IgG, the decision as to whether treatment with IVIG would be beneficial can be difficult. Measurement of neutralizing antibodies in these circumstances would be helpful. In addition, such assays will be critical in determining the response to B19 immunization when the B19 vaccine becomes available.

5. Conclusions

This assay can be used to determine the infectious titre of parvovirus B19 in a number of different settings. In addition, the ability to automate many of the steps in the assay may allow this assay to be used more widely than is currently available.

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医薬品 研究報告 調査報告書

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研究報告の概要

○パルボウイルスB19暴露に対する抗体陰性および陽性の人における免疫反応
ウイルスが混入した血液製剤を投与された後のパルボウイルスB19に対する免疫反応に関する情報はほとんど得られていない。最近の研究で、B19DNAを含む(1.6x10^{6}IU/mL)プール血漿の輸血後、B19抗体陽性の患者のB19IgG抗体のレベルが19-39 IU/mLから50-100 IU/mLまで上昇して再感染を防いだことを発見した。B19抗体陰性の患者における1.6-2.2 x 10^{8}IU/mLのB19DNAの存在は、プール血漿のIgGレベル59.5 IU/mLではB19の伝播とそれに続くセロコンバージョンを防ぐのは不十分であることがわかった。これらのデータは血液製剤の安全性評価の進歩につながるだろう。

報告企業の意見	今後の対応
B19DNAを含む(1.6x10^{6}IU/mL)プール血漿の輸血後、B19抗体陽性の患者では抗体価が上昇して再感染を防いだが、陰性の患者における1.6-2.2 x 10^{8}IU/mLのB19DNAの存在は、プール血漿のIgGレベル59.5IU/mLではB19の伝播とセロコンバージョンを防ぐのは不十分であることがわかったとの報告である。	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。日本赤十字社では、以前よりRHA法によるB19抗原検査を導入、ウイルス量の多い血液を排除している。今後は検査方法の改善によりさらなる感度向上を目指すこととしている。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「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

BRIEF REPORT

The Immune Response to Parvovirus B19 Exposure in Previously Seronegative and Seropositive Individuals

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Little information is available on the immune response to parvovirus B19 after the administration of contaminated blood products. In the present study, we found that levels of B19 IgG in B19-seropositive recipients protect against re-infection and, after transfusion with pooled plasma containing B19 DNA (1.6×10^8 IU/mL), increase from 19–39 IU/mL to 50–100 IU/mL. We found that, in the presence of 1.6–2.2 $\times 10^8$ IU of B19 DNA/mL in B19-seronegative recipients, a pooled-plasma B19 IgG level of 59.5 IU/mL is insufficient to prevent B19 transmission and subsequent seroconversion. These data should lead to improvements in the assessment of blood-product safety.

Parvovirus B19 can cause severe disease in immunocompromised individuals, and B19 infection during pregnancy can lead to fetal mortality. B19 infection is transmitted either via respiratory secretions or via administration of contaminated blood or blood products. The latter mode of transmission is especially problematic because of the high resilience of B19 to many of the treatments used in plasma processing, such as solvent-detergent treatment, lyophilization, and high temperatures [1], and also because of the extremely high levels of viremia in acutely infected, and often asymptomatic, individuals (>10^{12} B19 DNA genome equivalents [GE]/mL or IU/mL) [2].

Significant efforts to minimize the B19 viral load in blood products commenced in the late 1990s because of the advent of robust DNA extraction and B19 polymerase chain reaction methodologies in addition to cases of B19 seroconversion in healthy volunteers who received contaminated plasma as part of a postmarketing surveillance study [3]. Most manufacturers now undertake minipool B19 nucleic acid testing to reduce plasma-pool levels of B19 DNA to <10^4 IU/mL, to conform with US Food and Drug Administration (FDA) proposals (available at: http://www.fda.gov/). Standardization of B19 DNA and IgG quantitation, as well as the establishment of validated serological assay systems, has also contributed to improvements in blood-product screening paradigms. The regulatory requirement that levels of B19 DNA in anti-D antibody preparations be <10^4 IU/mL [4] further illustrates the actions taken by regulatory agencies to effectively improve blood-product safety.

In the future, because of enhanced screening protocols, B19 transmission after the administration of blood products should become a less frequent event. However, heightened awareness of B19 has resulted in the emergence of relevant information regarding the infectious dose of B19 and the role played by B19 IgG in attenuating transmission. Koenigbauer et al. [5] reported a case of B19 infection in a 36-year-old woman that resulted from administration of a solvent/detergent-treated pooled plasma that was subsequently recalled by the American Red Cross after high levels (10^7–10^8 GE/mL B19 DNA) of B19 DNA were detected by the manufacturer. Blumel et al. [6] detailed 2 cases of B19 infection resulting from the administration of B19 IgG^- plasma protein–complex concentrates: 1 individual received 180 mL of heat-treated concentrate containing 8.6×10^6 GE of B19 DNA/mL (1.5×10^9 GE total), and the other received 996 mL of material containing 4×10^3 GE of B19 DNA/mL (3.9×10^6 GE total). The transmission of B19 by a factor VIII concentrate (free of B19 IgG) has been documented in a case in which seroconversion occurred as a result of infusion of 2×10^4 IU of B19 DNA (1.3×10^3 IU/mL) [7]. Solvent/detergent-treated plasma (Plas+SD) has also been identified, subsequent to a postmarketing surveillance study of this product, as the source of B19 infection that occurred in 18 individuals [3, 8]. It was concluded that B19 IgG in pooled plasma (64.7 ± 17.5 IU of B19 IgG/mL; [9]) was not protective in the presence of high B19 viral titers (10^7–10^8 GE/mL) and that plasma lots containing low viral titers ($10^{0.5}$–$10^{3.5}$ GE/mL) did not cause B19 infection in plasma recipients. However, detailed serological analysis of this event has not been forthcoming, and the significance that the data have for wider issues of

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