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Citation for published version:

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Journal of Clinical Microbiology

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Detection of Parvovirus B19 in Donated Blood: a Model System for Screening by Polymerase Chain Reaction

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Received 4 August 1992/Accepted 3 November 1992

A highly sensitive and rapid method for routinely screening large numbers of donated blood units for parvovirus B19 by the polymerase chain reaction (PCR) was developed. Over a 3-month trial period in Edinburgh, B19 DNA was detected in 6 of 20,000 consecutive units of blood (0.03%), in concentrations ranging from $2.4 \times 10^4$ to $5 \times 10^6$ copies of viral DNA per ml. Seroconversion for B19-specific immunoglobulin M and immunoglobulin G and disappearance of circulating B19 DNA occurred in the interval between donation and recall in four of the five implicated donors who could be recalled. B19 DNA was detected in 18 of 27 separate batches of non-heated-treated factor VIII and IX concentrate manufactured from donated plasma unscreened for B19 DNA. Dry-heat treatment at $80^\circ C$ for 72 h reduced but did not always eliminate detectable B19 from factor VIII concentrates, consistent with recent observations that current methods for virus inactivation during blood product manufacture are insufficient to entirely eliminate B19 infectivity. The methods developed in this study for PCR screening could be applied routinely to prevent transfusion of B19 in blood and blood products and could play an important role in the prevention of iatrogenic transmission of infection. PCR screening could also be used for detection and exclusion of other related transmission-associated viruses for which current serological detection methods are only partially effective.

**MATERIALS AND METHODS**

**Samples.** We collected 20,000 units of blood from volunteer donors in the Edinburgh area in the period from May to July 1991. Aliquots (200 µl) of plasma from each were taken and stored at $-20^\circ C$ prior to pooling and testing by PCR. Following identification of viremic donors, plasma from previous and follow-up donations or samples obtained on recall of donors were tested by PCR and by B19 serology (see below). Blood products were obtained from the U.K. National Institute of Biological Standards and Controls (NIBSC) and the Protein Fractionation Centre, Scottish National Blood Transfusion Service (SNBTS), Edinburgh. Freeze-dried preparations of factor VIII and factor IX were stored at $4^\circ C$ prior to reconstitution, and intravenous immunoglobulin (IgG) was stored as a reconstituted aqueous solution (50 g/liter) at $-20^\circ C$. Lyophilized vials were reconstituted as specified by the manufacturer at 11 and 36 U/ml for factor VIII and factor IX, respectively, immediately before use.

To investigate the kinetics of B19 inactivation during heat treatment, intermediate-purity factor VIII was spiked 1:100 with B19-positive plasma, freeze-dried, and heated using model SNBTS production cycles. Such levels of additional proteins in intermediate-purity factor VIII have been shown previously to have no effect on the freeze-drying or heating characteristics of this product.

**Primers.** Primers PV1 to PV4 corresponded to the sequences of the published W1 and Au variants of parvovirus B19 (5, 30). This region showed substantial nucleotide and amino acid sequence homology with adeno-associated virus (33), minute virus of mice (2), H1 (24), and feline parvovirus (7). Sense PV1 (GGTAAAGAAAAATACACTGT) and antisense PV2 (TGCCGCCCTAATAAGGGCTTT) primers (5' bases at positions 1390 and 1608, respectively, numbered as
McOMISH

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B19-infected

Sequences

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infectious

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numbers.

Blood donor screening. A total of 20,000 blood units collected from volunteer donors over a period of approximately 3 months in southeastern Scotland were screened by PCR in 40 separate pools of 500 units. The limit of sensitivity of this screening method was 5 virions per ml in a pool, and therefore 2,500 virions per ml in each component unit. On initial screening with the primers PV1 to PV4, 6 of the 40 pools were found to contain detectable amounts of B19 DNA. All six samples were also positive on testing with primers PV5 to PV8. To identify the viremic donors, each original positive pool was divided into five pools of 100 units, which were rescreened. Each set of five pools divided in this way yielded a single PCR-positive pool of 100. The six positive pools of 100 units were each in turn reconstituted as 10 pools of 10 donations, and each set of 10 pools produced a single PCR-positive sample. Finally, each unit used to make up the six positive pools of 10 was tested to identify the six positive blood donors (p1 to p6). An example of the steps used to identify a viremic blood unit is shown in Fig. 1. The six units of plasma corresponding to the positive samples were recalled from the Protein Fractionation Centre, and all were found to be PCR positive. With one exception, nucleotide sequences of DNA amplified with primers PV1-PV2 and PV5-PV6 from the six donors differed from published sequences of B19 (5, 30) and from each other by 1 to 6 substitutions (Table 1). In the one instance in which two donors yielded the same viral sequences, recall showed that the two infections were epidemiologically linked (husband and wife; see below). Finding different sequences in each donor rules out the possibility that the positive results obtained on screening were the result of inadvertent contamination of samples or buffers.

The concentration of parvovirus DNA ranged from 2 × 10^4 to 5 × 10^10 copies per ml (Table 2), the latter concentration corresponding to approximately 0.5 μg of circulating viral antigen per ml (35). Virus particles of diameter 22 to 25 nm, with a density of 1.41 g/cm³ in cesium chloride, were clearly visualized by electron microscopy in the sample from donor p5 (data not shown).

Clinical and virological studies of infected blood donors. Previously donated blood and follow-up samples from each of the PCR-positive blood donors were tested for B19-specific IgG and IgM and for B19 by PCR (Fig. 2). Five of the six individuals (p1 to p5) were negative for both IgM and IgG in all samples prior to donation, and four of the five were still negative at the time when viremia was detected. All four of
TABLE 1. Variation in nucleotide sequence of B19 DNA amplified from viremic blood donors

<table>
<thead>
<tr>
<th>Nucleotide position&lt;sup&gt;a&lt;/sup&gt;</th>
<th>B19-AU</th>
<th>Nucleotide change&lt;sup&gt;b&lt;/sup&gt; in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p1</td>
<td>p2</td>
</tr>
<tr>
<td>Region 1 (&lt;nt 1415–1584&gt;)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>A</td>
<td>G (–)</td>
</tr>
<tr>
<td>1503</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1530</td>
<td>A</td>
<td>G (–)</td>
</tr>
<tr>
<td>1576</td>
<td>G (V)</td>
<td>T (L)</td>
</tr>
<tr>
<td>Region 2 (&lt;nt 3118–3444&gt;)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>3172</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3182</td>
<td>T (S)</td>
<td>C (L)</td>
</tr>
<tr>
<td>3214</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3223</td>
<td>T</td>
<td>A (–)</td>
</tr>
<tr>
<td>3307</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>3355</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>3394</td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Positions of variable nucleotides between blood donor samples and with the B190-AU variant, numbered as in reference 30.

<sup>b</sup> Standard single-letter codes used in parentheses to indicate variable amino acids: V, valine; L, leucine; S, serine. Minus signs in parentheses indicate silent nucleotide substitutions.

<sup>c</sup> Region amplified by primers PV1 and PV2 (see Materials and Methods).

<sup>d</sup> Region amplified by primers PV5 and PV6 (see Materials and Methods).

Those recalled subsequently became IgG positive and IgM positive 3 to 6 months after donation. Samples from p6 were IgG positive before, during, and after donation. No B19-specific IgM was detected in follow-up samples from this individual. B19 DNA was undetectable in all samples collected from donors on recall (60 to 100 days after donation); blood units donated previously by all six donors were uniformly PCR negative.

Rash, fever, and arthropathy were reported by the two female donors (p1 and p2), whereas the male donors were generally asymptomatic, including p5, who had extremely high levels of circulating virus (Table 2). Two of the donors were husband and wife (p2 and p3), and they reported parvovirus-like symptoms of infection in other members of their immediate family.

**Detection of parvovirus B19 in blood products.** The frequency of PCR-positive units detected in the above study (1/3,300) indicates that B19 might frequently contaminate blood products in which pools of 3,000 to 10,000 plasma units are used as raw material in the manufacturing process. We therefore tested several blood products for B19 DNA and investigated the effect of heat treatment on its survival in factor VIII preparations. Of 18 non-heat-treated batches of factor VIII and 9 non-heat-treated batches of factor IX, 13 and 7, respectively, were PCR positive with primers PV1 to PV4 (Table 3). None of the intravenous Ig preparations contained detectable B19 DNA.

Five PCR-positive batches of factor VIII were heat treated by a standard manufacturing process (80°C for 72 h) and retested. Two of the five batches remained PCR positive. Determination of the B19 DNA titer before and after heat treatment in the two batches that remained positive showed at least 90% reduction in the amount of detectable viral DNA (Table 4). To investigate the kinetics of virus inactivation, we spiked a batch of factor VIII 1:100 with plasma from donor p5. During lyophilization, the viral DNA titer declined from $4.5 \times 10^8$ to $7 \times 10^7$ copies per ml. The lyophilized material was then subjected to dry-heat treatment for 24, 48, and 72 h at 80°C; a reduction to the final titer of $8 \times 10^6$

![FIG. 2. Time course of the appearance of anti-B19-specific IgM and IgG in donors identified as viremic on screening by PCR.](image)

**TABLE 2. Clinical symptoms and B19 DNA titers in viremic blood donors**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Age (&lt;yr)</th>
<th>Sex&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Symptoms</th>
<th>No. of copies of B19 DNA/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>p1</td>
<td>19</td>
<td>F</td>
<td>Rash, fever, arthropathy 14 days postdonation</td>
<td>$4 \times 10^5$</td>
</tr>
<tr>
<td>p2</td>
<td>35</td>
<td>F</td>
<td>Rash, fever, arthropathy 5–6 days postdonation; rash recurred after 1 mo</td>
<td>$2.5 \times 10^4$</td>
</tr>
<tr>
<td>p3</td>
<td>36</td>
<td>M</td>
<td>Asymptomatic</td>
<td>$4 \times 10^4$</td>
</tr>
<tr>
<td>p4</td>
<td>30</td>
<td>M</td>
<td>Asymptomatic</td>
<td>$2 \times 10^7$</td>
</tr>
<tr>
<td>p5</td>
<td>36</td>
<td>M</td>
<td>Asymptomatic</td>
<td>$5 \times 10^{10}$</td>
</tr>
<tr>
<td>p6</td>
<td>35</td>
<td>M</td>
<td>Tired with occasional joint pains over several months before and after donation</td>
<td>$5 \times 10^4$</td>
</tr>
</tbody>
</table>

<sup>a</sup> F, female; M, male.
copies per ml occurred during this process (Table 5). The 98% overall reduction in viral DNA titer is comparable to that observed on heat treatment of nonspiked samples (Table 4).

**DISCUSSION**

**Detection of B19 infection in blood donors.** The incidence of B19 viremia in healthy blood donors in this study was approximately 1/3,300, considerably higher than that reported by previous surveys in which antigen detection methods were used (9, 22). This presumably reflects the greater sensitivity of the PCR. Blood units containing less than 10^6 copies, equivalent to approximately 5 pg of viral antigen (as occurred in four of the six positive samples identified in this study) would be unlikely to have been detected by the most sensitive enzyme-linked immunosorbent assay-based methods. Hybridization methods typically detect 0.1 to 1 pg of B19 DNA (corresponding to approximately 2 x 10^4 to 2 x 10^5 copies). A hybridization assay with a chemiluminescent probe showed a sensitivity of 20 fg of B19 DNA, or 4,000 copies (23), but even this method would not have been suitable for testing large pools because it is considerably less sensitive than the PCR (5 copies per ml of plasma). The PCR also enabled us to carry out direct sequence analysis of amplified DNA and thus to make a positive identification of B19 sequences and distinguish B19 from other parvoviruses. Minor variations in sequence between B19 in different blood units confirmed that we had selected five independent B19 infections in the six donors. Sequence variability in the VP2 region was comparable to that observed among other epidemiologically unrelated isolates of B19 (34).

All viremic individuals were asymptomatic at the time of donation, and three of four male donors remained asymptomatic. Symptoms of rash and arthropy similar to those reported in previous studies of adult infection (1, 37) were reported in both female donors in the following 5 to 14 days. A higher rate of symptomatic infection in women has been reported previously (37). Four of the five donors recalled showed a specific IgM and IgG response to B19 following donation, consistent with a primary infection (1, 10). Donor p6 was unusual in that he was anti-B19 IgG positive before donation and before the time of viremia and no IgM was detected at or after donation. Reinfection with parvovirus is one possible explanation; in a previous study, experimental inoculation of a healthy adult volunteer with a low level of anti-B19 IgG led to reinfection, manifested by a second IgM response and increased levels of anti-B19 IgG (1). However, neither serological response was observed in the blood donor in this study. Interestingly, this individual had complained of recurrent polyarthritis for several months both before and after donation. It is possible that he had a chronic, low-grade infection with subclinical reactivation of B19 at the time of donation.

**Parvovirus contamination of blood products.** B19 DNA was detected in two-thirds of the batches of noninactivated clotting-factor concentrates tested. This might be expected given the frequency of viremic donors, the size of the pools used for manufacture (3,000 to 10,000 plasma units), and the fractionation process used (28). Whether these blood products are infectious depends on the stability of the virus, the possible neutralization of infectivity by mixture with antibody from other units in the pool (17), and the partitioning of the virus during cryoprecipitation and cold ethanol precipitation during manufacture.

There is convincing serological evidence for transmission of B19 by non-heat-treated factor VIII and prothrombin complex concentrates (22, 25, 36). Clinical data on recipients concerning the infectivity of "virally inactivated" concentrates are contradictory and may reflect variation in the precise conditions used for virus removal. In one study, serological testing of hemophiliacs who had received factor VIII subjected to dry-heat treatment for 72 h at 80°C showed that they had no increased risk of infection over age-matched controls (36). However, elevated rates of infection were found in recipients of dry heat-treated or steam-treated factor VIII (4). Furthermore, previously nontransfused hemophiliacs have been observed to become acutely infected 7 to 14 days after the first infusion of detergent-treated (3, 20) or heat-treated (3, 4, 19) clotting factor. The persistence of B19 DNA in factor VIII concentrates found in this study after heat treatment for 72 h at 80°C does not necessarily indicate continued infectivity. However, these results contrast with the studies of other viruses, such as hepatitis C virus, in which the same treatment not only apparently eliminates infectivity but also completely destroys all traces of viral nucleic acid (14). More precise studies of B19 inactivation by different treatment regimens await infectivity studies with recently developed in vitro culture systems for B19 (6, 29).

**Feasibility of donor screening by PCR.** The identification of
a PCR-positive blood unit required four sequential separate DNA extraction and nested amplification reactions. However, plasma making up the initial pools of 500 found to be negative (34 of the 40 tested) could have been safely transfused after only one amplification reaction. An insignificant proportion of units (in this study, 54 of 20,000) would require four screens by PCR to rule out contamination. In this study, no attempt was made to optimize the time taken for DNA extraction and PCR. Despite this, we managed to retrieve all six units of infected plasma from the Protein Fractionation Centre before manufacture. As the DNA extraction and amplification steps could be carried out within a single working day, we could also have prevented transfusion of all contaminated units of erythrocytes and platelets with a minimal effect on the time taken from collection to blood issue. However, routine application of this method for virus detection in donor centers would be greatly facilitated by methods for rapid DNA extraction and automation of the amplification reactions; such methods are currently being developed.

As well as greatly reducing the risk of parvovirus B19 infection of susceptible individuals, with its attendant complications, this method of screening could be used to supplement serological screening for other transfusion-transmitted viruses, notably hepatitis B virus, for which continued transmission of infection occurs from hepatitis B surface antigen-negative blood.

ACKNOWLEDGMENTS

We are grateful to G. Kemball-Cook, National Institute of Biological Standards and Controls, for supplying archived non-heattreated factor VIII and IX concentrates. We are also grateful to the staff at the Blood Donor Centre, Royal Infirmary of Edinburgh, for help in preparing the plasma pools and for the retrieval of previously donated blood and to W. G. Hart and J. Crossley, SNBTS Protein Fractionation Centre, for carrying out the viral inactivation procedures on factor VIII. Thanks are also due to S. Moore for synthesis of parvovirus oligonucleotides. We are grateful to the family practitioners who were involved in recall and assessment of the viremic blood donors. Finally, thanks are due to J. D. Cash for initiating and encouraging this program of research.

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