Persistence of Parvovirus B19 (B19V) DNA and humoral immune response in B19V-infected blood donors

D. Juhl, S. Görg & H. Hennig
Institute of Transfusion Medicine, University Hospital of Schleswig-Holstein, Lübeck/Kiel, Germany

Background and Objectives Parvovirus B19 (B19V) DNA seems to persist in the plasma of B19V-infected blood donors. The relevance of this for recipients of single-donor blood components is yet unclear.

Material and Methods We studied serial archive and follow-up samples from 75 B19V-infected blood donors to obtain more data about the duration and degree of viraemia and the presence of IgG and IgM anti-B19V. IgG antibodies were further characterized by Western blot analysis in 29 donors.

Results In 411 B19V DNA-positive samples collected, we found high concentrations (>10^6 IU B19V DNA/ml plasma) in five. B19V DNA persisted for a mean of 21/5 months (range: 2–52.4; 95% confidence interval, 19.1–23.9 months) in all donors. Only 15 such samples had either no or low-titre IgG anti-B19V. IgG antibodies were predominantly directed against epitopes on the minor capsid protein VP1, thus probably of neutralizing type with high avidity. IgM anti-B19V was detectable in 9/13 samples with high DNA concentrations.

Conclusions The vast majority of single-donor blood components with detectable B19V DNA are probably not infectious for their recipients because DNA is at only low levels and the donors also have potentially neutralizing antibodies with high avidity. Anti-B19V IgM testing does not identify every donation with high B19V DNA concentrations, but, in addition to B19V NAT testing, donors with persistent IgG anti-B19V might be considered ‘B19V-safe’ for single-donor blood components.

Key words: B19V, blood donors, parvovirus b19 infection, transfusion-transmitted infections.

Introduction Parvovirus B19 (B19V), a small (approximately 25 nm diameter), non-enveloped and single-stranded DNA virus, is ubiquitous in the human population and naturally transmitted by aerosol via the upper respiratory tract. Common clinical manifestations of B19V infection are erythema infectiosum (‘fifth disease’) in children or an arthropathy in adults, but many are asymptomatic [1, 2].
several reports showed long-term persistence of B19V DNA in blood donors [7–9].

In addition to the natural mode of infection via aerosol, B19V is a bloodborne agent, by plasma derivatives [10] and single-donor blood components [11–14]. However, despite the spread and duration of B19V viraemia in blood donors and the general population, transmission of B19V by single-donor blood components seems to be rare. In Japan, the rate of transfusion-transmitted (TT) B19V infections is about one of 6 million donations [12], possibly due to the concurrent presence of neutralizing antibodies in the donors [5, 7]. The aims of our study were thus to obtain more data about (1) the duration and degree of B19V viraemia in blood donors, (2) the humoral immune response in blood donors with ongoing B19V infection, (3) further characterization of antibodies of the IgG class and (4) whether antibodies of the IgM class are associated with high B19V DNA concentrations and thus a suitable surrogate marker for at-risk donations.

Materials and methods

Blood donors and blood donations

In the institute of Transfusion Medicine of the University Hospital of Schleswig-Holstein in Northern Germany, approximately 55 000 donations (whole blood, and, to a considerably lesser extent, platelet apheresis and plasmapheresis) are taken annually at two sites in the cities of Lübeck and Kiel.

The 75 blood donors for this study were a subgroup of B19V DNA-positive donors who tested positive for the first time in the context of another study in 2011 [5]. All were whole blood donors. The study was approved by the local ethical committee.

Blood samples

To assess the period of viraemia in those donors, all of their archive samples since January 2008 were gently thawed and tested retrospectively for B19V DNA until a B19V DNA-negative sample was found.

These donors were then followed up either until no B19V DNA was detectable in two consecutive donations or until the end of the study period (1 July 2013). For this purpose, EDTA-anticoagulated blood samples were centrifuged and the plasma supernatant was removed and either investigated without delay or stored at < -30°C prior to further investigation.

Only in a minority of B19V-infected donors could the entire duration of the viraemia, from its beginning to its resolution, be estimated. In most donors, either the last DNA-negative samples before viraemia occurred or the first DNA-negative sample after resolution of viraemia, or both samples were not available. As shown in Fig. 1, we allocated the donors into different groups, according to the available samples. In five samples derived from five different donors, no B19V DNA was detected in the earlier samples, but in later samples, low concentrations of B19V DNA were detected again, indicating a very low level of the viraemia, close to the cut-off of the assay. These five samples were not considered to be negative in terms of a resolution of the viraemia.

Fig. 1 Allocation of the blood donors to the different groups according to whether B19V DNA-positive or B19V DNA-negative, respectively, archive and/or follow-up samples were available.

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Vox Sanguinis (2014) 107, 226–232
B19V DNA testing

Samples were tested for B19V DNA by the cobas TaqScreen DPX Test (Roche Diagnostics GmbH, Mannheim, Germany; 95% detection limit 11–5 IU/ml plasma, linear range: 75–3–0 × 10^9 IU/ml plasma) according to the manufacturer’s recommendations using the cobas AmpliPrep/cobas TaqMan 96 device (Roche Diagnostics GmbH).

B19V ELISA

The presence and titre of IgG antibodies against B19V were determined by ELISA (Anti-Parvovirus B19 ELISA [IgG], EUROIMMUN, Lübeck, Germany) in all samples that tested positive for B19V DNA. The assay was performed as recommended by the manufacturer. The optical density of each sample was compared with a standard curve, derived from the four calibrators which have been provided by the manufacturer. The results were then expressed quantitatively in IU/ml plasma.

In donors of groups A and B, at least in the first and second B19V DNA-positive donations and in donations with B19V DNA concentrations exceeding 10 000 IU/ml, additional anti-B19V IgM testing was performed by ELISA (Anti-Parvovirus B19 ELISA [IgM], EUROIMMUN).

Characterization of B19V IgG antibodies by Western blot analysis

Western blot analysis (recomLine Parvovirus B19 IgG, MIKROGEN GmbH, Neuried, Germany) was done in those donors with an available B19V DNA-negative sample before infection (groups A and B), starting with the first B19V DNA-positive sample until potentially neutralizing anti-B19V IgG antibodies became detectable. The assay detects antibodies against epitopes on the minor capsid protein VP1 (VP-N, VP-1S, VP-C) and on the major capsid protein VP2 (VP-2p, VP-2r, VP-C) and against the non-structural protein NS1. Band intensities on the strips were compared with a cut-off band, classified as −, −/+ , 1+, 2+ and 3+, and the result was interpreted as recommended by the manufacturer. The avidity testing of anti-B19V IgG antibodies was performed for all samples in parallel using the avidity reagent (MIKROGEN) according to the manufacturer’s instructions. In brief, one of the strips was washed with the avidity solution. In this way, low-avidity antibodies were removed from the strip, but highly avid ones remained bound. Band intensities between both strips, washed and unwashed with avidity solution, were compared. An antibody was considered to have low avidity if the band intensity was decreased by at least 50%.

Statistical analysis

We used Excel 2003 (Microsoft Corporation, Redmond, WA, USA) and SPSS version 19 (IBM GmbH, Ehningen, Germany) for statistical analysis. Statistical analysis was performed by the Wilcoxon rank-sum test for paired samples. A P-value < 0.05 was considered significant.

Results

We included 75 blood donors (41 men, 34 women) with a mean age of 34.8 years in the study. They provided 456 donations, which were considered for the study, with a mean interdonation interval of 135.8 days (95% confidence interval [CI], 126.2–145.4 days). Of those donations, 411 (90.1%) tested positive for B19V DNA. DNA concentrations in the 411 samples ranged between < 75 IU/ml plasma and 6.4 × 10^9 IU/ml plasma. In most samples, only low DNA concentrations between < 75 and 999 IU/ml plasma were detectable, while samples containing intermediate or high DNA concentrations were less frequently present (Fig. 2).

Duration of B19 viraemia

Only two donors provided two consecutive B19V DNA-negative donations (one in group A and one in group C). The others of groups A and C provided one negative donation before the study period expired.

The mean interval between the first DNA-positive sample and the last DNA-positive sample in all 67 donors (Fig. 1), who provided at least one further donation, was 21.5 months (range: 2.3–52.4; 95% CI, 19.1–23.9 months). The mean period between the first B19V DNA-positive sample and the last B19V DNA-positive sample in the donors of group A was 29.6 months (range: 28.5–34.5; 95% CI, 25.6–33.7 months), in group B...
Changes of B19V DNA concentration

In the donors of groups A and B, we measured the B19V DNA concentration in all of the first available positive donations of the 29 donors. We then compared these with the DNA concentrations in all the second donations obtained after a mean time of 135.8 days and with the further consecutive (third, fourth, etc., until more than eight were added) donations if such samples were available and if they were still B19V DNA-positive. Table 1 shows the results. In two of the first donations, only low B19V DNA concentrations, < 75 IU/ml, were detectable. One of those samples was presumably drawn during an acute infection in the early ramp-up phase, since the next positive sample had a value of 2548 IU B19V DNA/ml. In acute infection in the early ramp-up phase, since the next DNA-negative sample had been drawn was 425 days, and the other sample, the time period since the previous positive sample had a value of 2548 IU B19V DNA/ml. In

IgG antibodies against B19V

Six of 410 (1.5%, one not tested) B19V DNA-positive samples were negative for anti-B19V IgG (≤5 IU/ml). In nine samples (2.2%), we found low antibody titres (between 6 and 25 IU/ml); in 134 (32.7%), 26–100 IU/ml; in 223 (54.4%), 101–500 IU/ml; and in 38 (9.3%), > 500 IU B19V IgG/ml.

Figure 3 shows the anti-B19V IgG titres in correlation with the B19V DNA concentration of 410 DNA-positive samples. The IgG titre of the 5 samples with > 10⁴ IU B19V DNA/ml did not differ significantly from the three samples with DNA concentrations between 10⁵ and 10⁶ IU/ml and from those with < 10⁵ IU/ml (the latter probably due to the large numerical difference), but were lower than the other groups (P = 0.043 vs. 10⁴, and P = 0.043 vs. 10⁴–<10⁵).

The antibody titres in the 29 donors of groups A and B, like the DNA concentrations in their consecutive donations, were essentially the same, other than a slight difference between all the second and fourth donations (P = 0.041).

Characterization of IgG antibodies against B19V

In 24/29 samples from the first DNA-positive donation of groups A and B donors, we found IgG antibodies against epitopes on the minor capsid protein VP1 with high avidity. In 5/29, no anti-B19V IgG was detectable by ELISA (one sample, presumably in the early ramp-up phase, with

Table 1 Changes in the DNA concentration of 29 donors (groups A and B), assessed by the mean DNA concentration values of the first DNA-positive and the consecutive donations

<table>
<thead>
<tr>
<th>Donation no</th>
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<th>Rangea</th>
<th>Meana</th>
<th>95% CIa</th>
<th>Mediana</th>
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<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>&lt; 75–6394 177 000</td>
<td>223 935 075</td>
<td>0–648 376 572</td>
<td>2802</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>&lt; 75–4774</td>
<td>1598</td>
<td>1157–2039</td>
<td>1173</td>
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<tr>
<td>3</td>
<td>28</td>
<td>&lt; 75–1750</td>
<td>638</td>
<td>430–846</td>
<td>479</td>
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<tr>
<td>4</td>
<td>27</td>
<td>&lt; 75–1410</td>
<td>343</td>
<td>187–500</td>
<td>116</td>
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<tr>
<td>5</td>
<td>25b</td>
<td>&lt; 75–665</td>
<td>176</td>
<td>111–242</td>
<td>&lt; 75</td>
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<tr>
<td>6</td>
<td>20</td>
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<td>86</td>
<td>70–102</td>
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<tr>
<td>7</td>
<td>17</td>
<td>&lt; 75–251</td>
<td>97</td>
<td>72–122</td>
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<td>8</td>
<td>14</td>
<td>&lt; 75c</td>
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<td>&gt; 8</td>
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n. a., not applicable.

a In IU B19V DNA per ml plasma.
b One donor of group A tested negative at the fifth donation. In all other donors, who were omitted from analysis, no further donations were available.
c In all donations, < 75 IU B19V DNA/ml plasma was detectable.

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B19V DNA < 75 IU/ml, four with concentrations > 10^6 IU/ml. Four of these were also negative by Western blot (one not tested due to lack of further material). In the samples taken at the second donations 72, 84, 92, 124 and 140 days later, IgG antibodies have appeared with high avidity, showing a very strong reaction (3+) with epitopes on VP1 (VP-N and VP-1S). In all the 29 second samples, antibodies with high avidity against VP-N and VP-1S were detectable.

IgM antibodies against B19V

We were able to demonstrate IgM anti-B19V in 9/13 donations with > 10 000 IU B19V DNA/ml, also IgG anti-B19V in 7 of them. Four of 13 had no detectable IgM antibodies. Two of the latter had DNA concentrations > 10^6 IU/ml, and these two were also negative for IgG antibodies.

In 16/28 donors of groups A and B (one not tested), we could detect no IgM antibodies at any time after infection, while in 7/28, we found IgM only at the first donations with detectable B19V DNA. In four donors, more than one sample after infection tested positive and in one, in the second sample, IgM antibodies became detectable once.

Discussion

More than 10 years ago, the consensus was that long persistence of B19V is rare except in immunodeficient patients [15]. Since then, it has become evident, with the availability of more sensitive NAT tests [16–19], that B19V DNA might persist for a long time in non-immunodeficient patients [8] and even in otherwise healthy blood donors [7, 16, 20]. We recently reported the persistence of B19V DNA and hypothesized that viraemia in B19V infection may last considerably more than a year [5]. By the combined examination of both archive samples as well as follow-up samples from a donor subgroup of our previous study, we measured a mean duration of B19V viraemia of 21.5 months with a maximum of 52.4 months. However, only in a few of our blood donors (those of group A), did we have a sample from before viraemia occurred and one after that. Furthermore, not all our subjects provided donations during the entire study period between January 2008 and July 2013, so our data provide only an approximation of the duration of viraemia. The real duration of viraemia may well be longer in many cases. And we cannot rule out that, once B19V DNA has disappeared, it might reappear later, since either no further donations were obtained or the study period expired.

Others have provided different data on the decline of B19V DNA viraemia. Lefrère et al. reported that B19V DNA concentration decreases to 100 IU/ml in the first year after infection, remains at 50–100 IU/ml for two more years, then persists at around 50 IU/ml [8]. Matsukura et al. [9] found a decline to below 10^4 IU/ml after 1 year and to 10^3 IU/ml after 2 years. In our study, DNA concentration was between 10^2 and 10^3 IU/ml after 1 year (by about the third donation) and below 10^2 IU/ml after 2 years (by about the sixth). These differences may reflect different assays used in different studies, but the data also support the recent supposition that the long persistence of B19V DNA is not an exception, but rather the norm [21]. And it is independent of any distinct immunomodulatory effects like that related to repeated transfusions as proposed by Lefrère et al. [8], because our blood donors have not had transfusions.

However, the detectable DNA persistence is at an extremely low level, close to the cut-off of our assay (11.5 IU/ml), resulting in intermittently negative NAT results, as in five of the donors in our study.

This low-level persistence of B19V DNA seems not to affect otherwise healthy donors. Neither their blood cell counts nor their general well-being seems to be affected [5]. B19V DNA persists in several tissues (e.g. liver, heart, tonsils, synovia) [22–24], and release of virions or at least viral DNA from these tissues has been suggested [4, 23]. But due to its tropism, mostly depending on erythrocyte precursor cells, a release of mature virions from these tissues is not likely. The ongoing high titres of anti-B19V IgG in our donors are rather compatible with an ongoing productive infection with generation of mature virions, which is well controlled, although not resolved, by the immune system.

Fig. 3 Mean anti-B19V IgG titre in relation to the B19V DNA concentration of 410 samples (one not tested). The numbers in parentheses below the DNA concentration give the number of investigated samples in each group. The numbers at the top of the columns display the mean anti-B19V IgG titre and (in parenthesis) the 95% confidence intervals.

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The presence of anti-B19V IgG in pooled plasma has been reported to be protective for the recipients if the DNA concentration is relative low [25]. Thus, our data confirm the established approach to accept plasma for fractionation if the B19V DNA concentration is below $10^4$ IU/ml [26]. In the vast majority of our donors, intermediate to low B19V DNA concentrations accompany increasing titres of anti-B19V IgG (Fig. 3), higher than the mean titres found in plasma pools considered non-infectious [64-7 +/- 17-5 IU anti-B19V IgG/ml [27]], at least if the DNA concentration is below $10^3$ IU/ml [25]. Moreover, these antibodies have potentially neutralizing qualities, as they are directed against epitopes on VP1; a protective effect of such antibodies for erythrocyte precursor cells has been well demonstrated in vitro [28, 29]. Also, they have a high avidity. These facts, taken together, probably indicate a reduction or even prevention of virus transmission not only by plasma derivatives but also by single-donor blood components. Only 5 samples with high B19V DNA concentrations $>10^6$ had no anti-B19V IgG or low antibody titres, and it is reasonable to conclude by the currently available data [11, 13, 30] that single-donor blood components obtained from such donors are infectious.

Satake et al. [12] report one TT-B19V infection by a red blood cell concentrate taken from a donor with a relatively low B19V DNA concentration ($5.1 \times 10^3$ IU/ml). They detected IgG and IgM anti-B19V in the archive samples taken with the donation, but did not report antibody titres or characterization of the antibodies for neutralizing qualities. Therefore, transmissibility of B19V by blood products seems not to depend only on the viral burden, and the presence of IgG antibodies against B19V in the donor is not always protective. It is possible that in single cases, the antibody titre might not be sufficient for complete neutralization of B19V. We found nine donations with low titres of $<26$ IU/ml anti-B19V IgG, some of them with low B19V DNA concentrations, but possibly not completely neutralized virions.

Clinicians rarely suspect TT-B19V infection by single-donor blood components [12, 14], and it is mainly transfusion medical scientists who study it. Several reasons could exist for this. TT-B19V infection is rare (for the reasons discussed above) and not recognized or reported by clinicians, thus suggesting minor clinical relevance. For that reason, we should consider thoroughly whether to introduce general testing and, if so, what kind of testing. NAT testing of donors for single-donor blood components meets the requirements for adequate B19V DNA screening, even if done on mini-pools, and appropriate assays are already available. But we must also consider the cost-effectiveness of general NAT testing.

Satake et al. [12] found a strong correlation between TT-B19V infection and detection of IgM anti-B19V in the donors from whom the infectious blood components were obtained. These findings suggest that anti-B19V IgM testing might also be suitable to detect donations at risk of TT-B19V transmission. But in our study, only nine of 13 donations with at least intermediate B19V DNA concentrations $>10^6$ IU/ml had detectable anti-B19V IgM, and only 3 of 5 had B19V concentrations $>10^6$ IU/ml. Moreover, anti-B19V IgM was not regularly detectable in all of the acutely infected donors, indicating that screening for IgM anti-B19V is not suitable to prevent all TT-B19V infections.

However, selection of single-donor blood components obtained from long-term sero-positive donors offers a reasonable measure. In the Netherlands, they consider single-donor blood components as ‘B19V-safe’ if they come from donors, in whom anti-B19V IgG has been detected in two separate samples, taken after an interval of at least 6 months [31]. And although B19V DNA is usually detectable over a longer period than 6 months after seroconversion, the concentration is low and accompanied by protective antibodies in donors with ongoing B19V infection.

In conclusion, we can confirm the observation of a long persistence of low-level B19V DNA in blood donors. Due to rapid formation of neutralizing B19V IgG antibodies with high avidity, it is doubtful whether this phenomenon is of relevance for the recipients of single-donor blood components and could explain why TT-B19V infections seem to occur so rarely. In addition to NAT testing of blood donors, selection of long-term sero-positive donors offers an alternative measure for the supply of ‘B19V-safe’ single-donor blood components, if desired by clinicians.

Acknowledgements

The authors are indebted to Ursula Thiessen for her excellent technical assistance.

Conflict of interests

The authors declare no conflict of interests.
References


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*Vox Sanguinis* (2014) 107, 226–232