

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 reinfection 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 – 2.2×10^8 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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B19 infectivity and immunity merits consideration. In the present article, we describe the serological analysis of specimens obtained from 10 individuals who participated in the post-marketing study [3, 8]; this analysis extends our knowledge of the immune response to B19 exposure.

Materials and methods. As part of a postmarketing study, 100 adult volunteers, previously determined to be B19 IgG⁺ by use of an *Escherichia coli*-based EIA to detect B19 IgG, were each transfused with 1 unit (200 mL) of pooled plasma (Plas+SD) [3, 8, 10]. Paired plasma specimens (blinded) were obtained pretransfusion and 1 month posttransfusion from 10 of the volunteers.

The 20 plasma specimens were analyzed for both B19 IgM and B19 IgG reactivity against capsid (conformational) VP2 (VP2-N), by use of FDA-approved EIAs (Biotrin). B19 IgG levels were quantified using the World Health Organization B19 IgG International Standard (93/724) [11]. Furthermore, B19 IgG reactivity against conformational (N) and linear (D) epitopes on VP1 (VP1-N and VP1-D, respectively) and to linear VP2 (VP2-D) was analyzed as described elsewhere [12]. The subsequent classification of pooled-plasma recipients into groups I, II, and III, as well as the details of plasma pools used for transfusion, is shown in table 1.

Results. Figure 1A shows that specimens from groups I and II contained no VP2-specific IgM reactivity, whereas specimens from group III exhibited high levels of B19 IgM reactivity posttransfusion, thereby confirming acute B19 infection in this cohort. Analysis of the B19 IgG reactivity of individual plasma specimens was performed both pre- and posttransfusion, and 3 specimens (01002, 01052, and 01098) of 10 exhibited reactivity against VP2-N pretransfusion, with the range of B19 IgG in these specimens being 19–39 IU/mL (figure 1B, group I); there was a subsequent increase in the level of B19 IgG reactivity posttransfusion, which resulted in 2 of 3 specimens (both transfused with plasma pool PS3 [table 1]) exhibiting B19 IgG levels >100 IU/mL and the third specimen exhibiting an increase to 50 IU/mL.

A further 3 specimens (01023, 01053, and 01055), 2 of which were from individuals transfused with plasma pool PS2A (table 1), were seronegative for antibodies against VP2-N (B19 IgG <3 IU/mL), both pre- and posttransfusion (figure 1B, group II).

The remaining 4 paired pretransfusion specimens tested contained no detectable B19 IgG against VP2-N; however, the corresponding paired posttransfusion specimens exhibited evidence of B19 seroconversion and exhibited high levels of reactivity against VP2-N epitopes (figure 1B, group III); this reactivity corresponded to B19 IgG levels >100 IU/mL in 2 of 4 of the specimens, whereas the remaining 2 specimens contained lower levels of B19 IgG, equivalent to 50 and 78 IU/mL, respectively. For each specimen, the pattern of reactivity against VP1-N epitopes was identical to that exhibited against VP2-N, whereby

IgG specific for VP1-N was increased posttransfusion in group I and was also evident only posttransfusion in group III (figure 1C).

When specimens were analyzed for reactivity against VP1-D epitopes, 2 specimens (01002 and 01098) of 3 from group I did not exhibit significant pretransfusion IgG reactivity; however, these 2 specimens did display significant posttransfusion antibody reactivity (mean \pm SD IgG index value, 3.5 ± 1.7 [IgG index value >1.1 is reactive]) (figure 1D). The remaining specimen (01052) was seronegative for VP1-D IgG, both pre- and posttransfusion. Group II specimens were unreactive against VP1-D. All group III specimens were seronegative for B19 VP1-D IgG pretransfusion; posttransfusion, however, all had high levels of antibody reactivity against VP1-D epitopes (mean \pm SD IgG index value, 3.9 ± 0.96).

B19 IgG reactivity was observed only against VP2-D epitopes in group III specimens, with a mean \pm SD VP2-D IgG index value of 4.5 ± 1.8 (figure 1E). It should be noted that, although posttransfusion group I specimens exhibited an increase in levels of B19 IgG against VP2-N epitopes (figure 1B), they had no increase in antibody reactivity to VP2-D epitopes.

Discussion. The present study demonstrates that, in B19-seropositive recipients transfused with plasma containing high levels of B19 DNA (1.6×10^8 IU/mL), levels of parvovirus B19 IgG against VP1-N and VP2-N epitopes and against linear

Table 1. Classification of pooled-plasma recipients, according to B19 IgG reactivity against conformational epitopes on B19 VP2.

Group no., pooled-plasma- recipient code no.	Plasma pool transfused	B19 DNA level
Group I		
01002	PS3	1.6×10^8 IU/mL
01052	NA	NA
01098	PS3	1.6×10^8 IU/mL
Group II		
01023	PS2A	$10^{3.5}$ GE/mL
01053	PS2A	$10^{3.5}$ GE/mL
01055	NA	NA
Group III		
01005	PS1	2.2×10^8 IU/mL
01048	PS1	2.2×10^8 IU/mL
01057	PS1	2.2×10^8 IU/mL
01069	PS3	1.6×10^8 IU/mL

NOTE. Plasma pools PS1 and PS3 contained 59.5 and 72.0 IU of B19 IgG/mL, respectively [9]. The level of B19 DNA in plasma pool PS2A was provided by A. Lazo. Group I and II recipients remained symptom free, whereas group III recipients experienced mild fever and malaise, after transfusion. Group I, recipients who were seropositive before transfusion ($n = 3$); group II, recipients who were seronegative both before and after transfusion ($n = 3$); group III, recipients who were seronegative before transfusion and seropositive after transfusion ($n = 4$); GE, genome equivalent; NA, not available.

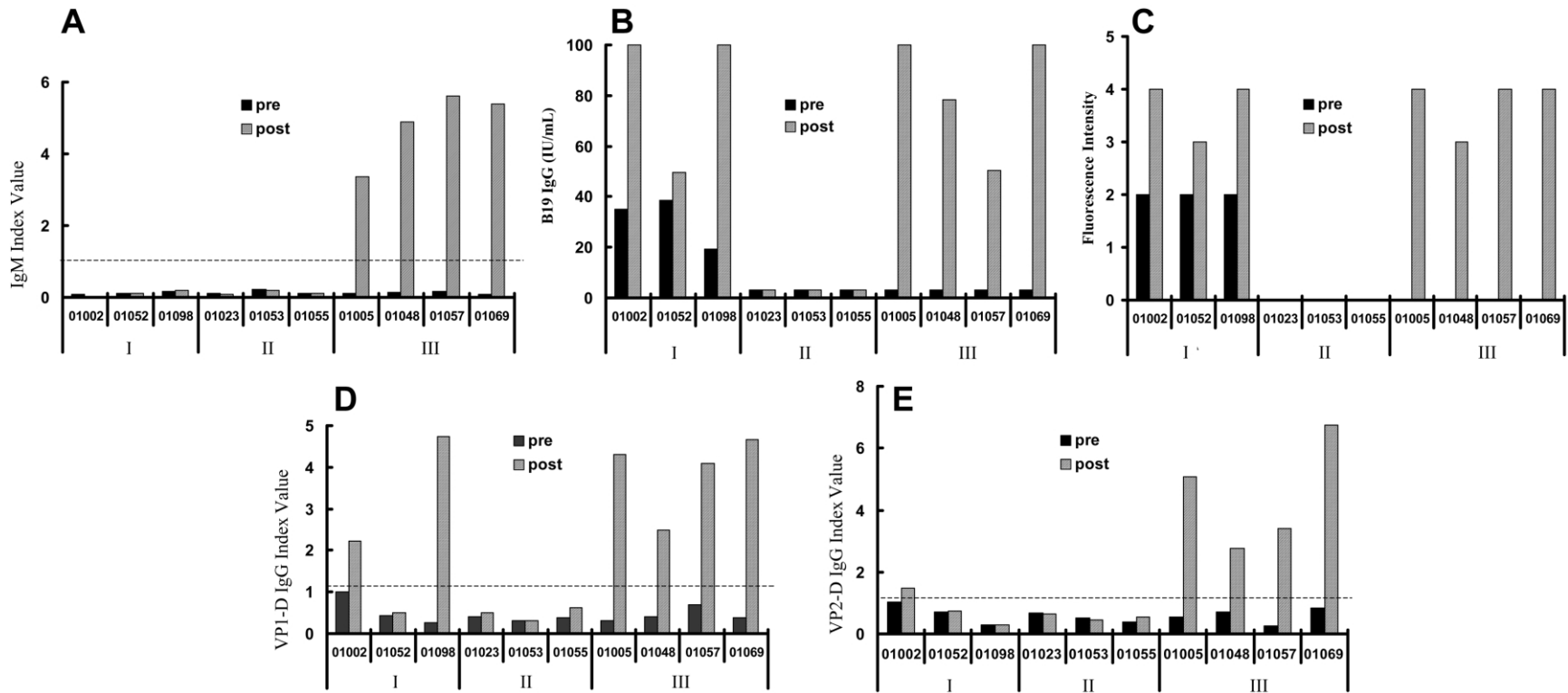


Figure 1. A, B19 IgM reactivity against conformational VP2, in pooled-plasma recipients (before and after transfusion). Reactivity is measured as IgM index value (reactivity >1.1 is reactive [dashed line]). B, B19 IgG reactivity against capsid (conformational) VP2 (VP2-N), as determined by EIA and expressed as IU/mL, in pooled-plasma recipients. C, B19 IgG reactivity against conformational epitopes on VP1 (VP1-N), in pooled-plasma recipients. Reactivity is assessed using an immunofluorescence assay and is graded, according to the manufacturer's instructions on a scale of 1–4, depending on the extent of fluorescence. D, B19 IgG reactivity against linear VP1 (VP1-D), in pooled-plasma recipients (before and after transfusion). E, B19 IgG reactivity against linear VP2 (VP2-D), in pooled-plasma recipients. D and E, Reactivity measured as IgG index value (index value is specimen:cutoff OD ratio; reactivity >1.1 is reactive [dashed line]).

epitopes on the unique region of VP1 increase dramatically. Moreover, it also proposes that the levels of B19 IgG in pooled-plasma products protect against infection in B19-seronegative recipients when only low levels of B19 DNA (i.e., $<10^{3.5}$ GE/mL) are present. Finally, we have shown that, in the presence of $1.6\text{--}2.2 \times 10^8$ IU of B19 DNA/mL, B19 IgG levels of 59.5 (plasma pool PS1) and 72.0 IU/mL (plasma pool PS3), respectively, are insufficient to prevent B19 transmission to B19-seronegative recipients (group III) and subsequent seroconversion.

Group I recipients were seropositive for B19 IgG before transfusion with pooled plasma. The level of IgG specific for VP2-N increased to >100 IU/mL in 2 recipients after transfusion with plasma pool PS3; however, the observed increase in the remaining recipient (01052) was lower (50 IU/mL). This subsequent increase in IgG response was mirrored by the increased reactivity against VP1-N that was observed posttransfusion, whereby the increase in fluorescence exhibited by the specimen from recipient 01052 was less than that for the others in group I. It is relevant that, because of the presence of high-titer B19 DNA, blood products lacking B19-specific antibodies were most at risk of transmitting B19 infection and that, despite high levels of B19 DNA, recipients with preexisting B19 IgG (or who were the administered blood products containing B19 IgG) were not infected [13]. Plentz et al. [14] have also confirmed that the presence of B19 IgG in either the recipients of the blood products or in the administered material offers protection against B19 DNA (at concentrations of $<6 \times 10^2\text{--}2.2 \times 10^6$ GE/mL) present in therapeutic products, to the extent that no individual ($n = 14$) receiving a B19-contaminated blood product showed symptoms of acute B19 infection. The results of the present study demonstrate that, in a healthy immunocompetent individual (recipient 01098), a B19 IgG level of 19 IU/mL confers protection against the development of symptoms of B19 infection when that individual is reexposed to the virus. To our knowledge, the present study is the first to demonstrate that there is a specific level of B19 IgG that protects against reinfection. The postexposure B19 IgG profile will also contribute to avoidance of reinfection.

Although all recipients in group I had either lost or never developed antibody reactivity against VP1 or VP2 epitopes before transfusion, 2 of them subsequently displayed strong IgG responses against linear epitopes on the VP1-unique region only and not against VP2-D. This observation is in accordance with the work of Soderlund et al. [15] and significantly strengthens our hypothesis that VP1-specific B-cell memory is maintained only with respect to linear epitopes of the unique region of VP1, as well as with respect to VP1-N/VP2-N epitopes [12]. Recipient 01052 in group 1 exhibited the lowest increase in B19 IgG reactivity after transfusion and was seronegative for antibody reactivity against VP1-D both before and after trans-

fusion, possibly as a result of infusion with plasma containing a B19 viral load lower than that required for reactivation of the memory response.

Group II recipients all remained seronegative after receipt of pooled plasma. Although information was not available on which plasma pool was transfused into recipient 01055, both recipient 01023 and recipient 01053 were transfused with plasma pool PS2A, which contained $10^{3.5}$ B19 GE/mL [3]. Given that the mean level of B19 IgG observed in pooled plasma is 64.7 ± 17.5 IU/mL [9], it is clear that B19 IgG within this range appears to be protective against infection of seronegative recipients when the B19 viral load is $\leq 10^{3.5}$ B19 GE/mL.

Group III recipients who underwent B19 seroconversion after transfusion exhibited both strong VP2-specific IgM reactivity and significant levels of B19 IgG against VP2-D epitopes. The latter result is in accordance with previously published findings that production of antibody directed against VP2-D epitopes occurs shortly after exposure to B19 [15].

Traditionally, plasma-product manufacturers have relied on the presence of high levels of B19 IgG in pooled-plasma products alone to indicate product safety [2]. The data presented in the present study reinforce the strategy of identifying and removing high-titer B19 plasma donations from plasma pools, given that 4 of 7 recipients seroconverted because of the presence of B19 DNA in solvent/detergent-treated pooled human plasma. Although many companies have introduced minipool screening to address this problem, such screening is not presently mandatory, despite the fact that it is usually high-risk populations (e.g., pregnant women and immunocompromised patients) who are administered such products. This regulatory ambiguity is likely to change in coming years, as improved product-safety profiles are demanded by consumers.

In summary, the present study has provided new data relevant to the B19 IgG level necessary to confer protection after reexposure to the virus, as well as to the B19 IgG level that, in pooled-plasma products, may prevent infection of seronegative recipients.

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