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Persistence of Human Parvovirus B19 in Multipotent Mesenchymal Stromal Cells Expressing the Erythrocyte P Antigen: Implications for Transplantation

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Multipotent mesenchymal stromal cells (MSCs) are used to improve the outcome of hematopoietic stem cell transplantation (HCST) and in regenerative medicine. MSCs may harbor persistent viruses that may compromise their clinical benefit, however. Retrospectively screened, 1 of 20 MSCs from healthy donors contained parvovirus B19 (B19) DNA. MSCs express the B19 receptor (P antigen/globoside) and a co-receptor (Ku 80) and can transmit B19 to bone marrow cells in vitro, suggesting that the virus can persist in the marrow stroma of healthy individuals. Two patients undergoing HSCT received the B19-positive MSCs as treatment for graft-versus-host disease; neither developed viremia nor symptomatic B19 infection. These findings demonstrate for the first time that persistent B19 in MSCs can infect hematopoietic stem cells and underscore the importance of monitoring B19 transmission by MSC products.

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INTRODUCTION

Multipotent mesenchymal stromal cells (MSCs) are nonhematopoietic cells present in the human bone marrow (BM) [1]. MSCs can be readily cultured, have extensive proliferative capacity, and can differentiate into more mature lineages, such as fat, cartilage, and bone, prompting clinical trials using MSCs for tissue engineering [2-5]. These cells seem to have immunomodulatory properties; MSC infusions have been found to reverse therapy-resistant grade IV acute graft-versus-host disease (GVHD) of the skin, gut, and liver [6,7]. The risk of transmitting viruses from *ex vivo* expanded MSCs is of particular concern in hematopoietic stem cell transplantation (HSCT) recipients with severe GVHD, because these individuals, in whom GVHD-associated immunodeficiency is compounding the effect of multiple immunosuppressive agents, are at particular risk for viral infection [8-10]. We have found that MSCs are permissive for herpes simplex virus and cytomegalovirus (CMV) infections, but not for Epstein-Barr virus (EBV) infection. Herpes viruses have not been found in MSCs derived from healthy donors, however [11].

Parvovirus B19 (B19) is a nonenveloped, single-stranded DNA virus with pronounced tropism for erythroid precursors and megakaryocytes that express the erythrocyte P antigen (globoside), known as the B19 receptor [12]. The $\alpha 5\beta 1$ integrin (fibronectin) and the Ku80 autoantigen have been described as B19 co-receptors [13,14]. The replication of B19 is restricted. Only a few permissive cell lines have been described, including erythroleukemic and megakaryoblastoid cell lines [15,16]. B19 is common worldwide, and the seroprevalence increases with age, affecting 15% of preschool children, 50% of young adults, and

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approximately 85% of elderly persons. Primary infection in children can manifest as erythema infectiosum (so-called "fifth disease"), which is normally self-limiting with mild symptoms and results in lifelong immunity [17]. Clearance of the virus is slow and largely mediated by neutralizing antibodies [18]. Primary infection with B19 can cause aplastic crises in individuals with high red cell turnover and chronic red cell aplasia, or even severe pancytopenia in immunocompromised individuals [17,19]. The virus is resistant to inactivation used in the procurement of blood and hematopoietic stem cell (HSC) products, leading to a known risk of B19 transmission [20]. Thus, patients naïve to B19 with hypogammaglobulinemia after HSCT are at risk for severe cytopenias [17].

Endothelial cells and fetal myocytes have been reported to host B19 [21,22]. The virus can persist in the BM, and recently has been detected in MSCs from healthy donors [23-25]. Consequently, in the present work, we screened clinical-grade MSC products used to treat patients with GVHD for the presence of B19 and found persistent B19 in MSCs that could infect HSCs cells *in vitro*. We found no clinical consequences of MSC infusions containing B19 in immunocompromised recipients, however.

SUBJECTS AND METHODS

Donors and Recipients

The MSC donors (n = 20) were selected randomly and considered healthy after assessment of medical history, physical examination, and serologic screening for human immunodeficiency virus (n = 20), hepatitis viruses (n = 20), and herpes viruses (n = 15). The recipients underwent HSCT at the Center for Allogeneic Stem Cell Transplantation, Karolinska University Hospital Huddinge, between 2002 and 2006. MSCs were used for prophylaxis or treatment of GVHD. Second- and/or third-passage MSCs were given in dosages of ~1 to 2 $\times 10^6$/kg. Patients who received the B19-contaminated cells were assessed retrospectively for persistent anemia, leukopenia, thrombocytopenia, rash, or arthritis, possibly related to B19 infection. Donors and recipients gave informed consent, and the study design was approved by the Regional Ethics Review Board.

Isolation and *Ex Vivo* Culture of MSCs

To isolate MSCs, BM aspirates (mean, 50 ml; range, 16 to 80 mL) were obtained from the iliac crest of healthy donors. The donors were 9 males and 11 females, with a median age of 40 years (range, 24 to 66 years). The clinical-grade MSC expansion was performed according to the guidelines of the MSC Consortium of the European Blood and Marrow Transplantation Group and with the approval of the Swedish Medical Products Agency, as described in detail previously [7,26].

To assess the growth of the MSCs, time to first passage and doubling time in the first and second passages were recorded. The doubling time was calculated using the following equation: doubling time = $t/(\log_2(y/x))$, where t is the time in culture, y is the cell count at confluence, and x is the cell count at start. Adipogenic and osteogenic differentiation after induction were evaluated as described previously [27].

Characterized by flow cytometry, the MSCs uniformly fulfilled MSC criteria [1]. The MSC suspensions were culture-negative for bacteria and fungi, and polymerase chain reaction (PCR)-negative for *Mycoplasma pneumonia* [7,26].

PCR Detection of B19 DNA and Anti-B19 IgG Serology

For quantification of parvovirus B19 DNA, a human parvovirus genotype 1-, 2-, and 3-specific TaqMan real-time PCR assay was used, as described previously [18]. DNA from 10^5 MSCs and DNA from 200 μL of serum samples were extracted using an automated MagnaPure extractor (Roche Diagnostics Scandinavia, Stockholm, Sweden) using the LC Total Nucleic Acid Isolation Kit (Roche Diagnostics Scandinavia). Serum samples were analyzed for anti-B19 IgG using a commercial EIA (Biotrin International, Dublin, Ireland).

Isolation of Peripheral Blood Lymphocytes and Mixed Lymphocyte Cultures

Peripheral blood lymphocytes were isolated from the HSCT recipients who received the B19-positive MSCs and were used in mixed lymphocyte reactions (MLRs) to evaluate the responsiveness to allogeneic cells, as described elsewhere [11,28].

Determination of P Antigen, Ku80, and Glycophorin A Expression

The expression of P antigen by MSCs was determined by flow cytometry. MSCs were incubated with plasma from an individual with anti-P antigen antibodies (optimized to 1/20; anti-PP1Pk [ie, anti-Tja], blood group AB Rh-positive without anti-HLA antibodies; Department of Transfusion Medicine, Karolinska University Hospital Huddinge) and polyclonal rabbit anti-globoside antibody (optimized to 1/10; Matreya, Pleasant Gap, PA), respectively. Thereafter, the MSCs were incubated with monoclonal fluoresceinated anti-human immunoglobulin antibodies (optimized to 200 μg/mL; Jackson ImmunoResearch Laboratories, West Grove, PA) and anti-rabbit immunoglobulin (optimized to 100 μg/mL; Sigma-Aldrich, St Louis, MO), respectively. The plasma from the individual with anti-P antigen antibodies also was used after

absorption, with erythrocytes expressing these antigens as a specificity control. Ku80 expression was investigated using a monoclonal mouse anti-Ku80 antibody (optimized to 20 μg/mL; Abcam, Cambridge, UK) and a monoclonal anti-mouse antibody (optimized to 200 μg/mL; Jackson ImmunoResearch Laboratories). Glycophorin A (CD235a) expression was determined by incubation with a monoclonal phycoerythrin-labeled anti-CD235a antibody (optimized to 2 μg/mL; BD Biosciences, San Jose, CA). In all experiments, 6 MSCs were tested, and 3 BM cells were used as control cells. Finally, labeled cells were assayed in a flow cytometer (BD Biosciences). Fluorescence signals from 5 $\times 10^4$ cells were counted, and the percentage of fluorescence-positive cells was determined.

Evaluation of B19 Transmission

To evaluate possible transmission of B19, the B19-positive adherent MSCs were co-cultured in RPMI 1640 medium supplemented with 2 mM HEPES, 100 U/mL of penicillin, 100 μg/mL of streptomycin, 2 mM L-glutamine (Gibco-BRL, Life Technologies, Paisley, UK), and 10% heat-inactivated pooled human AB serum, with P antigen–positive BM cells (n = 3) in suspension for 3 days. BM cells incubated with B19-rich plasma (lot BPL9, with 10 genomic equivalents [geq]/target cell, kindly provided by Dr Kerr, Biotrin International) were used as controls. Adherent B19-negative MSCs (n = 3) also were co-cultured with the B19-positive MSCs in transwell inserts (1:1) and, as a control, the MSCs were exposed to B19-rich plasma (10 geq/cell). In an additional assay, blocking of possible B19 transmission was investigated using anti-B19 antibodies (optimized to 4 μg/mL; Santa Cruz Biotechnology, Santa Cruz, CA) in a culture medium of B19-positive MSCs/BM cell co-cultures (n = 3) for 3 days. BM cells were dried on microscopy slides, and adherent MSCs were cultured on slides. Both types of slides were fixed in a methanol-acetone solution at −20° C. Slides were stained with a mouse anti-B19 antibody (optimized to 2 μg/mL; Santa Cruz Biotechnology) and a secondary fluoresceinated anti-mouse immunoglobulin antibody (optimized to 100 μg/mL; Jackson ImmunoResearch Laboratories). After the slides were mounted with medium containing 4',6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA) to visualize the nuclei, they were read independently by 2 of the authors. For slides with BM cells and MSCs, more than 500 and 150 cells were counted, respectively, and the percentage of fluorescein-positive cells was determined. The analysis was performed using a DMRXA fluorescence microscope (Leica Microsystems, Wetzlar, Germany) at 22°C with a 40× oil objective (PL Fluotar Oil; Leica Microsystems) and filters for fluorescein and DAPI (Chroma Technology, Rockingham, VT). Images were captured using a camera (S/N 370 KL 0565; Cooke Corp, Romulus, MI) and SlideBook 2.1.5 software (Intelligent Imaging Innovations, Denver, CO). Finally, images were postprocessed and mounted using Photoshop 3.0 (Adobe Systems, San Jose, CA).

RESULTS

Expected Serologic Results of the Donors and Presence of B19 DNA in MSCs From a Donor

Serologic investigation of anti-B19 IgG was possible in 75% (15/20) of the donors included in this study who demonstrated a seroprevalence of 47%. DNA was successfully extracted from donor serum and MSCs (data not shown). None of the donors had B19 DNA present in their serum; however, in 1 of the seropositive donor MSCs (K11), B19 DNA was present in 1 $\times$ 10^3 geq/10^5 cells.

No Differences in MSC Growth and Phenotype

The B19 DNA–positive MSCs required 14 days in culture to first passage. This was within the range of 8 to 18 days found for the other 19 MSCs. Similarly, cell doubling time (mean ± standard deviation) was comparable in the first and second passages (3.1 and 4.1 days, respectively, for the B19 DNA–positive MSCs, compared with 3.7 ± 1.7 and 3.6 ± 0.8 days, respectively, for the B19-negative MSCs). Cells from seronegative and seropositive donors exhibited doubling times of 4.4 ± 1.9 and 3.0 ± 1.0 days, respectively, in the first passage and 3.7 ± 0.7 and 3.6 ± 0.9 days, respectively, in the second passage.

All expanded MSCs exhibited the same phenotype assessed by flow cytometry of surface markers. No differences in differentiation between the B19-positive MSCs and the other MSCs were demonstrated by morphological examination and spectrophotometry. This was also true of MSCs from seronegative donors and MSCs from seropositive donors (data not shown).

MSCs Express P Antigen and Ku80, but not Glycophorin A

We used flow cytometry to detect P antigen on MSCs, using plasma from a subject with anti-P antibodies and a commercial anti-globoside antibody. P antigen was expressed by all MSCs tested (n = 6), but less strongly than on BM cell controls (n = 3). MSCs displayed low expression using the anti-P antibody plasma or the commercial anti-globoside antibody, whereas BM cells displayed high expression. After absorption (with erythrocytes) of the anti-P antibody plasma, MSC expression was reduced to background (secondary antibody). The B19 PCR-positive MSC K11 displayed low expression using the anti-globoside

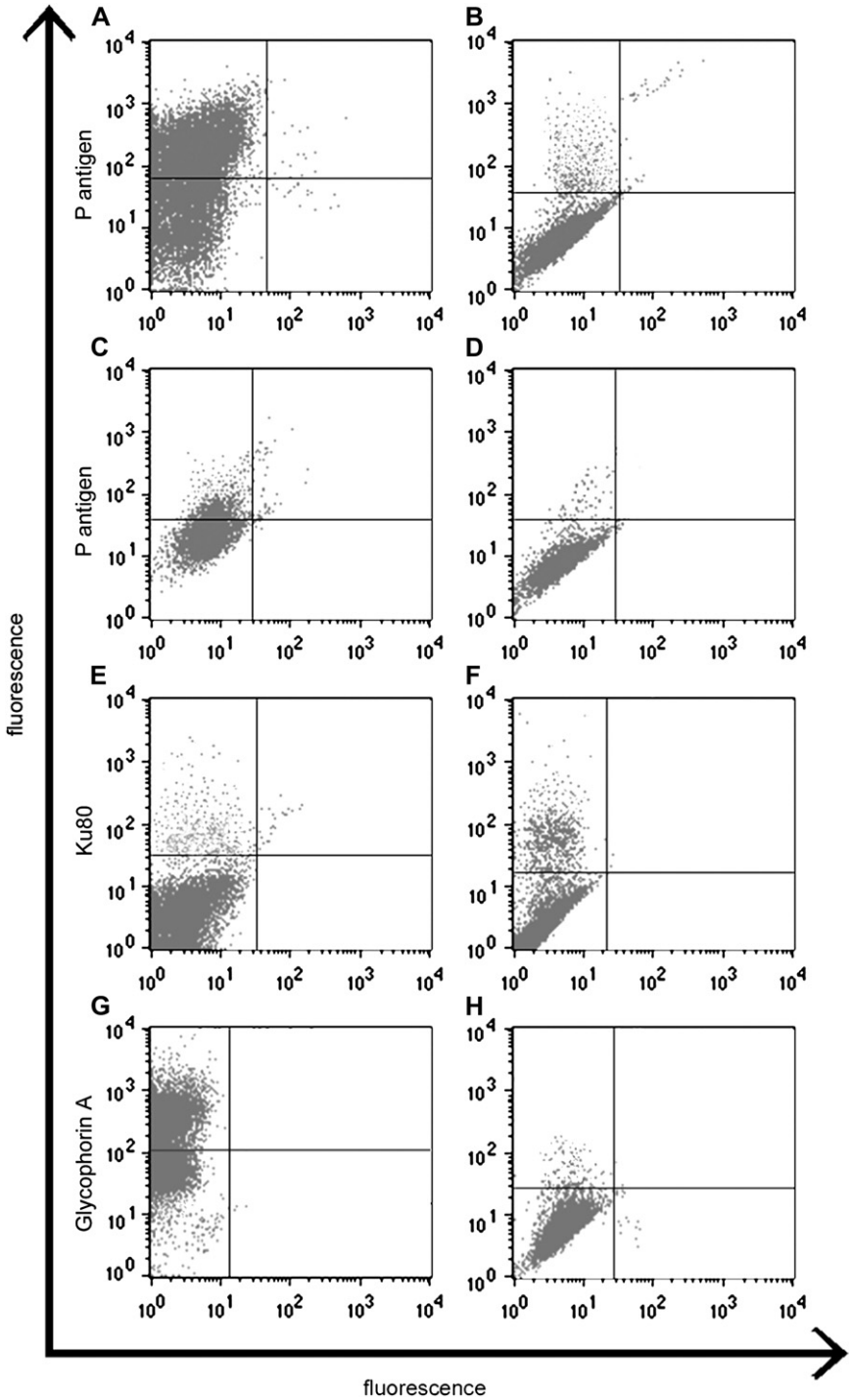

Figure 1. P antigen, Ku80, and glycophorin A expression in MSCs determined by flow cytometry. Unfractioned BM cells (A) and MSCs (B) expressed P antigen as investigated by a commercial anti-globoside antibody; however, the MSCs were less positive than the BM cells. Expression of P antigen on MSCs also could be demonstrated using patient plasma with anti-P antigen antibodies (C). The specificity of the plasma was determined by reduction of positivity after absorption with erythrocytes (D). Unfractioned BM (E) and MSCs (F) expressed Ku80, although MSCs at slightly higher levels. Unfractioned BM cells expressed glycophorin A (G), whereas MSCs exhibited only weak positivity (H). Matched isotype controls defined the quadrants. Values on the *y*-axis indicate intensity log. Representative dot plots of cells from 6 different donors are shown.

antibody. All MSCs expressed Ku80 at higher levels than found in BM cells. In contrast to BM cells, which displayed high expression of glycophorin A using the anti-glycophorin A antibody, MSCs displayed minimal levels (Figure 1).

MSCs Can Transmit B19 to BM Cells In Vitro

BM cells from 3 individuals exposed to B19-rich plasma for 3 days were 10%, 11%, and 12% positive, respectively, for intracellular B19 antigen by immunofluorescence. In comparison, nonexposed cells

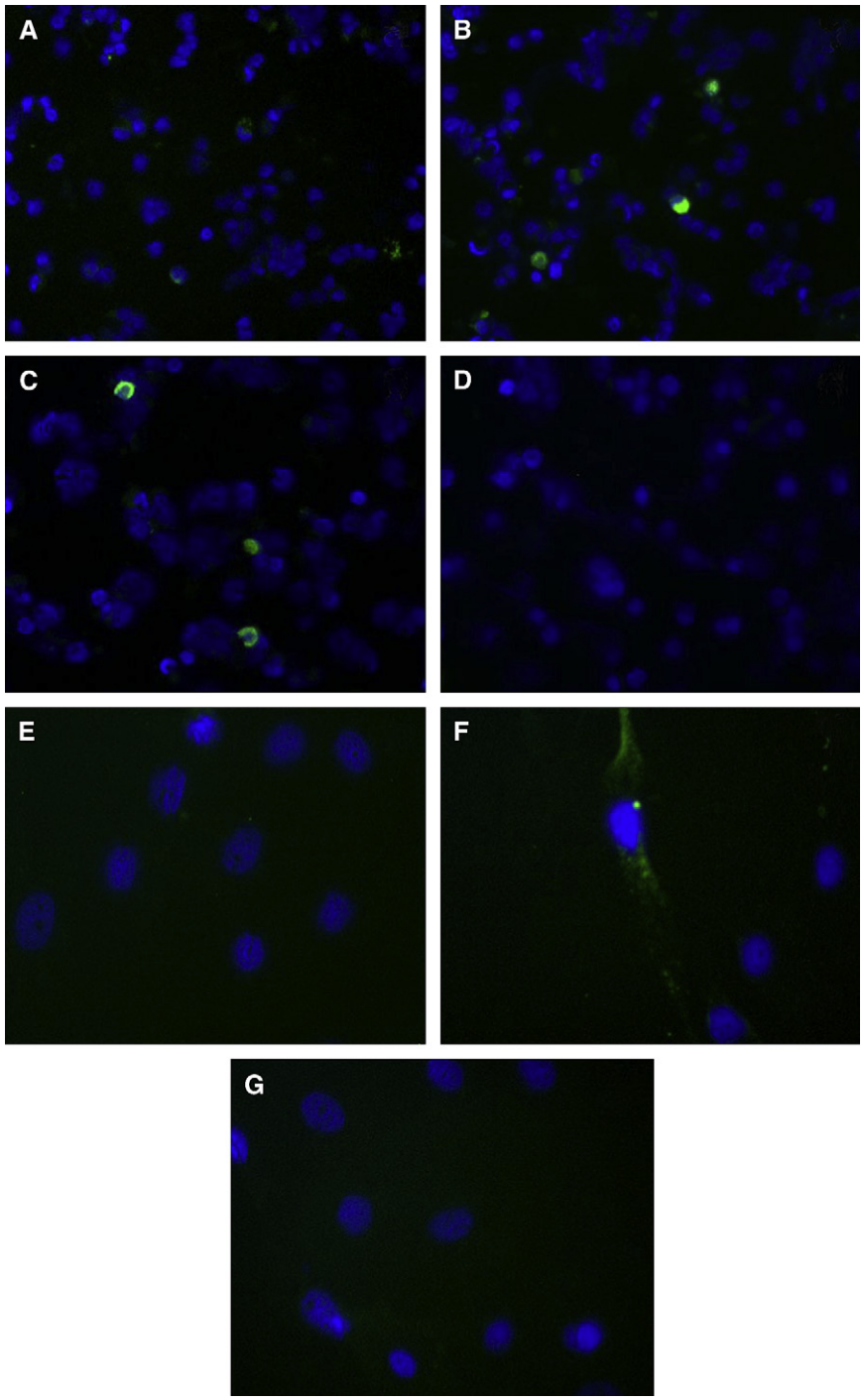

Figure 2. Parvovirus B19 immunostaining to investigate transmission from MSCs. (A) Unexposed BM cells were negative for B19. (B) BM cells incubated with B19-rich plasma were positive (*green*) for B19 fluorescence. (C) Transmission of B19 was demonstrated, because positive cells could be detected after B19-negative BM cells were co-cultured with B19-positive MSCs. (D) The transmission of B19 could be inhibited by adding anti-B19 antibodies to the co-culture medium. (E) Unexposed MSCs were negative for B19. (F) MSCs incubated with B19-rich plasma were positive for B19 green grainy fluorescence. (G) No transmission of B19 from the B19-positive MSCs to B19-negative MSCs was detected after co-culture. Representative photos are shown.

were < 1% positive for the antigen (Figure 2). In parallel, when the BM cells from these subjects were co-cultured with the B19-positive MSC K11, 6%, 6%, and 7% of the cells, respectively, were positive for B19 by immunofluorescence. In contrast, co-cultures to which anti-B19 antibodies was added showed only background (< 1%) fluorescence. B19-exposed MSCs demonstrated reduced viability (ie, low number of cells) compared with the unexposed or antibody-treated controls.

Immunofluorescence analysis of the B19 PCR-positive MSCs resulted in 3% positive cells. Exposure

Table 1. HSCT Patients Who Received Parvovirus B19-Positive MSC K11 as Treatment for GVHD

Patient		
Unique patient number	1044	1082
Sex/age, years	Male/60	Female/65
Diagnosis	Myeloma	Chronic lymphocytic leukemia
HSCT		
HLA match	HLA-identical sibling	HLA-identical sibling
Conditioning	RIC: Flu + TBI	RIC: Flu + Cy
Cell source	PBSC	PBSC
GVHD prophylaxis	CsA + MMF	CsA + MTX
Day of GVHD diagnosis	51	25
Grade of acute GVHD	III	III
MSC transplantation		
Indication	GVHD	GVHD
HLA match	MM	MM
Day of first MSC infusion	77	32
Number of MSC infusions	1	3*
MSC passage	2	2+3/3/2+3
MSC dose, $\times 10^6$/kg	0.9	1.7/1.7/0.9
GVDH response to MSC infusion	CR	PR
B19 investigations and symptoms		
HSC donor anti-parvovirus B19 IgG serology	+	-
HSC donor serum load of parvovirus B19 DNA, $\times 10^3$ geq/mL	-	-
Recipient anti-parvovirus B19 IgG serology, pretreatment	-	+
Recipient anti-parvovirus B19 IgG serology, 1 month posttreatment	-	-
Recipient anti-parvovirus B19 IgG serology, 3 months posttreatment	-	-
Recipient serum load of parvovirus B19 DNA, $\times 10^3$ geq/mL, pretreatment	-	-
Recipient serum load of parvovirus B19 DNA, $\times 10^3$ geq/mL, 1 month posttreatment	-	-
Recipient serum load of parvovirus B19 DNA, $\times 10^3$ geq/mL, 3 months posttreatment	-	-
Clinical symptoms of parvovirus B19 disease	None	None
Recipient immunocompetence at MSC transplantation		
Lymphocyte proliferation in allogeneic MLC, % of positive control	22%	14%
IgG level, g/L (reference interval, 6.1 to 14.9)	3.4	5.1
IgA level, g/L (reference interval, 0.88 to 4.5)	0.41	0.91
IgM level, g/L (reference interval, 0.27 to 2.10)	0.49	0.34
White blood cell count, $\times 10^9$/L (reference interval, 3.5 to 8.8)	8.7	1.8
Absolute neutrophil count, $\times 10^9$/L (reference interval, 1.6 to 7.5)	7.6	0.8
Blood parameters†		
Hemoglobin, g/L (reference interval: females, 117 to 153; males, 134 to 170)	95/90/85	114/117/124
White blood cell count, $\times 10^9$/L (reference interval, 3.5 to 8.8)	8.7/5.4/5.0	1.8/9.1/9.9
Platelet count, $\times 10^9$/L (reference interval: females, 165 to 387; males, 145 to 348)	104/119/78	55/96/92
Outcome	Deceased, relapse,day 773	Deceased, septicemia, fungi, day 120

Flu indicates fludarabine; TBI, total body irradiation; Cy, cyclophosphamide; PBSC, HSCs mobilized to peripheral blood; CsA, cyclosporine A; MMF, mycofenolate mofetil; MTX, methotrexate; MM, HLA-mismatched unrelated; CR, complete response; PR, partial response.
*Patient 1082 received 3 MSC infusions. The first infusion was MSC K11, which later was found to be contaminated with parvovirus B19.
†Measured at the time of MSC infusion and approximately 1 and 2 weeks after MSC infusion.

of MSCs from 3 donors to B19-rich plasma resulted in 41%, 48%, and 58% B19-positive MSCs, respectively, compared with background levels (<2%) in unexposed controls, and could be blocked by anti-B19 antibodies. We did not observe transmission of B19 from the B19-positive MSCs to 3 B19-negative MSCs in transwell co-cultures.

Clinical Outcome of Recipients of Parvovirus-Contaminated MSCs

MSC K11 (B19-positive by PCR) was administered to 2 subjects in a clinical trial to treat severe refractory acute GVHD (aGVHD). Characteristics of the subjects, HSCT, and MSC therapy are given in Table 1. One of the donor–recipient pairs was mismatched in terms of B19 serology. B19 could not be detected by PCR in either donors or recipients before HSCT. Both recipients were immunocompromised due to leukopenia, subnormal immunoglobulin levels, and poor responses in MLRs; however, neither had any clinical signs of B19 infection. Their blood counts remained stable, no B19 seroconversion occurred, and no B19 DNA was detected in the blood after MSC transplantation. The patients died, one due to relapse and the other due to septicemia, neither of which was likely related to infusion of B19-positive MSCs.

DISCUSSION

Plentz el al. [20] showed that 1% of all blood products are positive for B19 DNA, with the highest incidence (17.6%) found in HSCs mobilized to the periphery and in BM. Therefore, many manufacturers of blood and plasma derivates screen their products for B19 by quantitative PCR [29]. B19 infection could pose a risk for severe cytopenia in immunosuppressed HSCT recipients [30]. Because B19 has been reported

to occur in BM–derived MSCs [23], we retrospectively screened for B19 in MSCs used to treat GVHD in 20 HSCT recipients. Anti-B19 IgG was detected in about 50% of the healthy MSC donors, which correlates with the estimated seroprevalence in the adult population [17]. None of the MSC donors was viremic; they all tested negative for B19 DNA in serum. B19 DNA was found in MSCs from 1 donor, however. Hematopoietic contamination was not the likely source of B19, because the presence of such cells is excluded by flow cytometry before clinical MSC batches are released. Cassinotti et al. [24] demonstrated that B19 can persist in the BM of healthy and asymptomatic individuals. Cells expressing the P antigen are permissive for B19 entry [12,31]. In the absence of P antigen, the $\alpha5\beta1$ integrin and the Ku80 can serve as B19 co-receptors [13,14]. $\alpha5\beta1$ integrin expression on MSCs was reported recently [32]. Consequently, we tested MSCs from 6 donors, including the B19-positive cells, for surface expression of P antigen and Ku80. All MSCs expressed the antigens/B19 receptors. To clarify whether the MSCs share other features of erythroid cells, glycophorin A expression was measured, but was found to be minimal.

The B19-positive MSCs infected BM cells in a manner similar to B19-rich plasma. MSCs also could be infected by the B19-rich plasma, but they exhibited poor survival, suggesting a cytopathic effect. The B19 DNA–positive MSCs could not infect other MSCs, however. This may be explained by low virus concentrations, because the B19-rich plasma could infect the MSCs, along with weaker expression of P antigen on MSCs than on BM cells. Furthermore, B19- positive and -negative MSCs were separated in a transwell system, preventing cell-to-cell contact, which may be required for viral transmision. Compared with BM cells, MSCs exposed to B19-rich plasma showed stronger positivity for B19 antigens by immunofluorescence. This may be explained by the relatively higher proliferative and metabolic activity of *ex vivo*–cultured MSCs. We have shown that MSCs are permissive to some extent. B19 is known to have an extreme tropism for human erythroid cells, and only a few cell lines support semipermissive infection *in vitro* [15,16]. Recently, Wong et al. [33] reported that *ex vivo*–generated $CD36^+$ erythroid progenitors may be used to study B19 replication. The unavailability of *in vitro* culture systems for productive growth of B19 has limited this research thus far. The inhibition of B19 transmission by anti-B19 antibodies suggests that endogenous B19-neutralizing antibodies or intravenous administration of pooled immunoglobulins may be protective *in vivo*. Thus, MSCs may be a natural reservoir for persistent B19. This is in analogy with the findings of Scadden et al. [34], who described BM stromal cells as an HIV reservoir, passing the virus to both myelogenous and lymphoid cells. Thus, MSCs and the BM stroma might be sites of persistent viruses. In the absence of reliable long-term culture systems to generate B19 *in vitro*, MSCs, which can be readily infected by B19, could be used to propagate the virus long-term. Our findings do not explain why only a small minority of seropositive individuals demonstrate viral persistence in the marrow, however.

The viral load in the B19-positive MSCs was 1×10^3 geq/10^5 cells, roughly comparable to the median viral load of 0.7×10^3 geq/mL in blood components reported by Plentz et al. [20]. Therefore, MSCs and blood products should have comparable infectivity. In the study by Plentz et al. [20], 12% of the patients in a hematology ward (most of whom were undergoing HSCT) were exposed to B19-contaminated products. No patients developed clinical disease, but 1 patient developed viremia [20]. Here we report 2 heavily immunocompromised patients with severe GVHD who were exposed to B19-contaminated MSCs. Both patients were within 3 months of HSCT with severe GVHD. They were leukopenic and immunoglobulin-deficient and demonstrated functional T lymphocyte deficiency by MLRs at the time of MSC infusion. Neither patient developed clinical signs of infection or had detectable B19 DNA or anti-B19 IgG after MSC infusion, indicating the absence of infection. Possibly, the viral transmission was below the threshold for established infection. In one case in which the HSCT recipient developed B19 infection, the donor was found to have asymptomatic viremia, suggesting that much higher viral loads are required [35]. Quantitative information relating viral load to infectivity is lacking, however, and the risk of B19 infection in immunodeficient HSCT patients remains unclear. The low immunoglobulin levels encountered after transplantation might increase the risk of new infection or of reactivation in asymptomatic carriers; however, B19 infections do not constitute a major clinical problem in HSCT recipients (or perhaps they are underdiagnosed). Cytopenias and viral illness due to primary or reactivated B19 infection have been described previously [19]. B19-infected MSCs display low viability, which also may explain the absence of infection in our patients.

Our data support the possibility that B19 has low pathogenicity even in highly immunosuppressed individuals receiving MSCs, which themselves may further reduce immunity against viruses [11]. Nevertheless, given the lack of data, it seems prudent to screen MSC products from seropositive donors for B19 DNA and to administer only virus-negative products, or, at a minimum, to infuse MSCs containing B19 only in patients with a measurable titer of antibodies to B19. Further investigation of the relationship between MSCs and viral persistence and screening of MSC products for contaminating viruses are warranted.

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