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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