Impaired Endothelial Regeneration Through Human Parvovirus B19–Infected Circulating Angiogenic Cells in Patients With Cardiomyopathy

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Human parvovirus B19 (B19V) is a common pathogen in microvascular disease and cardiomyopathy, owing to infection of endothelial cells. B19V replication, however, is almost restricted to erythroid progenitor cells (ErPCs). Endothelial regeneration attributable to bone marrow–derived circulating angiogenic cells (CACs) is a prerequisite for organ function. Because of many similarities of ErPCs and CACs, we hypothesized that B19V is a perpetrator of impaired endogenous endothelial regeneration. B19V DNA and messenger RNA from endomyocardial biopsy specimens, bone marrow specimens, and circulating progenitor cells were quantified by polymerase chain reaction analysis. The highest B19V DNA concentrations were found in CD34+KDR+ cells from 17 patients with chronic B19V-associated cardiomyopathy. B19V replication intermediates could be detected in nearly half of the patients. Furthermore, chronic B19V infection was associated with impaired endothelial regenerative capacity. B19V infection of CACs in vitro resulted in expression of transcripts encoding B19V proteins. The capsid protein VP1 was identified as a novel inducer of apoptosis, as were nonstructural proteins. Inhibition studies identified so-called death receptor signaling with activation of caspase-8 and caspase-10 to be responsible for apoptosis induction. B19V causally impaired endothelial regeneration with spreading of B19V in CACs in an animal model in vivo. We thus conclude that B19V infection and damage to CACs result in dysfunctional endogenous vascular repair, supporting the emergence of primary bone marrow disease with secondary end-organ damage.

Keywords. apoptosis; endothelial progenitor cells; endothelial regeneration; reendothelialization; viruses.

The pattern of clinical disease caused by human parvovirus B19 (B19V), belonging to the genus Erythoparvovirus, is generally a self-limiting subclinical disorder [1]. However, on rare occasions, B19V is associated with chronic disorders in susceptible individuals, such as disseminated endothelial activation [2], transplant vasculopathy [3, 4], coronary microvascular disease with myocardial ischemia, and general or circumscribed left-ventricular dysfunction despite normal coronary arteries [5]. Thus, chronic B19V-associated cardiac disease [6] may be an illustrative model of the clinical findings associated with B19V infection.

B19V infection is restricted to cells expressing the receptor blood group P antigen [7], which is necessary for B19V binding but not sufficient for internalization [8]. The coreceptor β1-integrin is required for B19V
internalization [9], and Ku80 has been shown to be involved in B19V binding to different cell types [10]. The B19V genome encodes the major nonstructural protein NS1, the small 11-kDa nonstructural protein, and the capsids proteins VP1 and VP2 [11]. Efficient transcription of 11-kDa protein, VP1, and VP2 depends on B19V genome replication [12], limited to the semi-permissive erythroblastoid cell line UT7/Epo-S1 or permissive CD36+ erythroid progenitor cells (ErPCs) [13], allowing production of viral particles. In chronic B19V-associated disease, endothelial cells have been demonstrated as B19V-specific targets, whereas cardiomyocytes, which are devoid of B19V receptors, are precluded from infection. A replication block in endothelial cells, however, impedes B19V propagation and long-term persistence even after infection [14]. The widespread endothelial cell damage of different organs during chronic B19V infection may comprise indirect immune-mediated effects [15] or direct induction of apoptosis [16] through NS1 and 11-kDa protein, which may result in detachment of mature endothelial cells. However, persistently high copy numbers of B19V DNA [17] in the endothelial layer of endomyocardial biopsy specimens from symptomatic patients with chronic cardiomyopathy over a long period prompted our search for alternative target cells in B19V-associated chronic endothelial damage.

A heterogenous group of endogenous bone marrow–derived circulating angiogenic cells (CACs) has been shown to play a key role in cardiovascular regeneration [18]. CACs are identified or characterized by either surface antigen expression or culture and colony characteristics. CD34+KDR+ cells, carrying the hematopoietic marker CD34 and the endothelial marker kinase insert domain receptor (KDR), can be isolated from blood and bone marrow. They are responsible for an improvement in cardiac hemodynamics and reduced infarct size [19] and promote vascular regeneration [20] in vivo. Furthermore, they predict cardiovascular morbidity and mortality [21, 22]. Specific culture conditions are most commonly used to generate 3 distinct cell types that enhance neovascularization through different mechanisms. Colony-forming unit–Hill (CFU-Hill) colonies contribute to angiogenesis and endothelial function [23]. The so-called early outgrowth epithelial progenitor cells (eo-EPCs) have been shown to parallel disease progression in atherosclerosis and to functionally promote cardiovascular regeneration [24]. Clonally distinct endothelial colony-forming cells (ECFCs), which have high proliferative potential and express endothelial cell surface antigens, form robust vascular structures in vivo and in vitro [25, 26] and are associated with improved cardiac function [27]. As a first hint that B19V affects endogenous cellular endothelial regeneration, we have shown altered numbers of circulating CD34+KDR+ cells in chronic B19V–associated cardiomyopathy and endothelial dysfunction [28].

Hematopoietic cells and CACs are closely interrelated, as shown by genomic, transcriptome, proteomic, and functional analyses, as well as by cell surface marker findings [29, 30]. We thus set out to unveil whether B19V would be able to infect and damage bone marrow–derived CACs, to unravel the conundrum of persistent ubiquitous detection of B19V in patients with vascular disease.

**MATERIALS AND METHODS**

**Patients and Control Subjects**

Patients with clinical indication for endomyocardial biopsy were recruited, to evaluate B19V presence in the myocardium, blood, and bone marrow. Inclusion and exclusion criteria, indications for invasive procedures, and hemodynamic measures are described in the Supplementary Materials.

As a control for the study, 7 patients undergoing elective cardiac surgery with a previous unnoticed B19V infection (all tested negative for anti-B19V immunoglobulin M and positive for anti-B19V immunoglobulin G) were recruited. Five of these 7 patients had atherosclerotic disease. To analyze the function of endogenous endothelial cellular regeneration, we quantified eo-EPCs in specimens from patients with B19V-associated cardiomyopathy. Since it is well known that function of eo-EPC is restricted in atherosclerosis, we recruited 13 healthy age-matched and sex-matched individuals, to exclude atherosclerotic disease as confounder. These healthy subjects did not undergo bone marrow aspiration or endomyocardial biopsy. All study participants gave written informed consent, and the study was approved by the ethics committee of the Charité-Universitätsmedizin, Berlin.

**Cell Lines and Isolation of Primary Cells**

Cultivation of eo-EPCs and staining for functional assays was performed as described previously [24]. Eo-EPCs were counted in 3 randomly selected high-power fields with a DMI4000B microscope (Leica Microsystems, Wetzlar, Germany) as a surrogate measure for functional endothelial regenerative capacity [31].

CFU-Hill colonies were cultured using the EndoCult Liquid Medium Kit (StemCell Technologies, Vancouver, Canada) according to the manufacturer’s instructions. ECFCs were purchased from Lonza and cultivated as recommended by the manufacturer. UT7/Epo-S1 cells, an erythropoietin-dependent megakaryocytic leukemia cell line [32], were kindly provided by Susan Wong (Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland) and cultured as described elsewhere [14].

For specifically indicated experiments performed under hypoxic conditions, cells were incubated for indicated time points, using a gas mixture of 1% O2, 5% CO2, and 94% N2 (Air Liquide, Düsseldorf, Germany) in a modular incubator chamber (Billups-Rothenberg, Del Mar, California).

**Virus Stock and Infection**

B19V belonging to genotype 1 was purified from patient serum specimens as described previously [14]. Further details are available in the Supplementary Materials.
To directly assess the impaired functional regenerative capacity of eo-EPCs after B19V infection in vivo, a murine model of reendothelialization was chosen. eo-EPCs from 3 different healthy donors were injected with 10,000 B19V genome equivalents (8299 IU)/cell on day 2 after isolation or with equal amounts of control plasma and incubated for an additional 48 hours under normoxic conditions. To quantify reendothelialization, the left carotid artery was injured with an electric current of 2 W for 2 seconds over a length of 4 mm, using an ICC50 bipolar microregulator (ERBE-Elektromedizin, Tuebingen, Germany) in male SCID beige mice aged 7–8 weeks (Charles River, Wilmington, Massachusetts), as described previously [33]. Mice were injected with either phosphate-buffered saline (PBS; sham), 5 × 10^6 control-plasma-treated eo-EPCs in PBS (control), or 5 × 10^6 B19V-infected eo-EPCs in PBS 3 hours after injury. After 3 days, mice were injected with Evans blue (Sigma-Aldrich) to stain denudated areas of the endothelium and then euthanized. Mice were anesthetized by inhalation of isoflurane and subcutaneous receipt of buprenorphine and underwent artificial ventilation during all investigations. Areas of denudation were quantified by computer-assisted morphometric analysis.

Table 1. Baseline Demographic and Clinical Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients With B19V Infection (n = 17)</th>
<th>Subjects With Previously Unnoticed B19V Infection (n = 7)</th>
<th>Healthy Controls (n = 13)</th>
<th>P Value</th>
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<td>Demographic</td>
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<td>5 (39)</td>
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<td>13 (100)</td>
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<tr>
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<td>4 (57)</td>
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</tr>
<tr>
<td>IV</td>
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<td>1.16 ± 1.0</td>
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<td>7 (100)</td>
<td>11 (85)</td>
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<tr>
<td>Seronegative</td>
<td>0</td>
<td>2 (15)</td>
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Data are no. (%) of participants or mean value (±SD). See “Materials and Methods” section for descriptions of study groups.

Abbreviations: ACE, angiotensin-converting enzyme; B19V, human parvovirus B19; CABG, coronary artery bypass graft; CVD, cardiovascular disease; IgG, immunoglobulin G; IgM, immunoglobulin M; LVEF, left ventricular ejection fraction; NA, not applicable; NYHA, New York Heart Association.

a For comparison of patients with B19V infection to subjects with previously undetected B19V infection.

b For comparison of patients with B19V infection to healthy controls.

Animal Model for Endothelial Regeneration

To directly assess the impaired functional regenerative capacity of eo-EPCs after B19V infection in vivo, a murine model of reendothelialization was chosen. eo-EPCs from 3 different healthy donors were injected with 10,000 B19V genome equivalents (8299 IU)/cell on day 2 after isolation or with equal amounts of control plasma and incubated for an additional 48 hours under normoxic conditions. To quantify reendothelialization, the left carotid artery was injured with an electric current of 2 W for 2 seconds over a length of 4 mm, using an ICC50 bipolar microregulator (ERBE-Elektromedizin, Tuebingen, Germany) in male SCID beige mice aged 7–8 weeks (Charles River, Wilmington, Massachusetts), as described previously [33]. Mice were injected with either phosphate-buffered saline (PBS; sham), 5 × 10^6 control-plasma-treated eo-EPCs in PBS (control), or 5 × 10^6 B19V-infected eo-EPCs in PBS 3 hours after injury. After 3 days, mice were injected with Evans blue (Sigma-Aldrich) to stain denudated areas of the endothelium and then euthanized. Mice were anesthetized by inhalation of isoflurane and subcutaneous receipt of buprenorphine and underwent artificial ventilation during all investigations. Areas of denudation were quantified by computer-assisted morphometric analysis. To quantify the anticipated endogenous murine reaction to compensate for dysfunctional eo-EPCs, murine mononuclear cells from blood and bone marrow specimens were stained with anti-mouse CD117-APC (Miltenyi) and CD309-PE (ebioscience, San Diego, California) antibodies (both diluted 1:100) for fluorescence-activated cell-sorting analysis. Double-positive cells from the lymphocyte region were analyzed. To detect B19V in human eo-EPCs at the site of denudation, immunofluorescence staining of human eo-EPCs in the murine carotis was performed (Supplementary Materials).
RESULTS

Patient Characteristics
The baseline characteristics of patients with B19V-associated cardiomyopathy and the control subjects are summarized in Table 1. Patients with B19V-associated cardiomyopathy presented with symptoms of angina and cardiac-related limitations in physical ability and had a mean B19V DNA load (± standard deviation [SD]) of 847 ± 444 copies/µg genomic DNA (equivalent to 618 ± 324 IU) in endomyocardial biopsy specimens. Symptoms persisted for ≥ 6 months and were indications for cardiac invasive diagnostic procedures. No patients had a history of exposure to infectious diseases or traveling abroad. They were significantly younger, had less cardiovascular risk factors and hypertension, and were less likely to be receiving antithrombotic agents than subjects with serologic evidence of past B19V infection but no findings of current acute or chronic infection. A normal left ventricular injection fraction was confirmed by angiography or echocardiography in all healthy controls. Compared with healthy controls, a greater frequency of patients with B19V-associated cardiomyopathy had dyspnea, had angina, and were receiving medication (ie, β blockers and angiotensin-converting-enzyme inhibitors/AT1 blockers). No other differences were apparent.

CD34*KDR* Cells Have the Highest B19V DNA Copy Numbers During B19V-Associated Cardiomyopathy
To show a disseminated infection with B19V during cardiomyopathy, B19V DNA was quantified from magnetically sorted

![Diagram showing quantification of human parvovirus B19 (B19V) DNA from different cell fractions and impaired endothelial regeneration in patients with B19V-associated cardiomyopathy.](image)
cell fractions in blood and bone marrow specimens obtained from patients with chronic B19V-associated cardiomyopathy. The highest concentration of B19V DNA was found in CD34+ KDR+ cells (mean [±SD] in bone marrow, 5620 ± 12,551 copies B19V DNA/µg genomic DNA [4102 ± 9160 IU]; mean [±SD] in blood, 10,455 ± 19,845 copies B19V DNA/µg genomic DNA [7631 ± 14,484 IU]), compared with hematopoietic progenitor cells (CD34−KDR−; \( P < .05 \) and \( P < .1 \), compared with CD34+KDR+ cells in bone marrow and blood, respectively; Fig-ure 1A). Similarly, high copy numbers were found in the more immature CD133+KDR+ cells (mean [±SD] in bone marrow, 209 ± 442 copies B19V DNA/µg genomic DNA [152 ± 322 IU]; mean [±SD] in blood, 2173 ± 5329 copies B19V DNA/µg genomic DNA [1586 ± 3890 IU]; data not shown). This contrasts with findings for subjects with serologic evidence of past B19V infection but no findings of current acute or chronic infection, in
whom neither B19V DNA nor messenger RNA (mRNA) were found \((P < .05; \text{Figure 1A})\). However, eo-EPCs cultivated from bone marrow and blood specimens obtained from patients with B19V-associated cardiomyopathy did not show B19V DNA in any case. Highly varying B19V DNA copy numbers were detectable in different cell fractions in all patients with cardiomyopathy. There was no correlation in the number of B19V DNA copies between the different cell fractions or endomyocardial biopsy specimens. Because of the highly heterogenous cell population contained within an endomyocardial biopsy sample, we refrained from directly comparing the copy numbers of B19V DNA from endomyocardial biopsy specimens with magnetically separated homogeneous cell populations.

Detection of B19V mRNA in CD34\(^+\)KDR\(^+\) Cells

Since a block in mature endothelial cells precludes B19V replication, we questioned whether B19V replication alternatively might occur in CD34\(^+\)KDR\(^+\) cells. Strikingly, B19V VP1/2 mRNA was found in CD34\(^+\)KDR\(^+\) cells from bone marrow \((n = 6)\) and blood \((n = 2)\) specimens obtained from 8 of 17 patients, indicating viral replication. In these patients, B19V mRNA was found in only 2 endomyocardial biopsy samples (data not shown). Most likely, the absence of mRNA in these cells reflects a latent infection with likely undetectable copy numbers. Exploratory analysis of this small number of patients revealed no differences regarding demographic characteristics, clinical characteristics, or disease course among patients in whom B19V mRNA was detected in CD34\(^+\)KDR\(^+\) cells. Without DNA in eo-EPCs, we refrained from further analysis of eo-EPCs for B19V mRNA.

B19V-Positive Patients Show Dysfunctional Endothelial Regenerative Capacity

B19V-associated cardiomyopathy is associated with endothelial damage and abnormal numbers of endogenous bone marrow–derived CACs [28]. To assess the functional capacity of the admittedly heterogeneous group of CACs, the standardized test of eo-EPC differentiation was performed. Here, significantly lower mean numbers \((\pm \text{SD})\) of eo-EPCs \(256 \pm 174 \text{ vs } 370 \pm 128 \text{ cells/mm}^2; P = .016; \text{Figure 1B})\) were measured in patients with B19V-associated cardiomyopathy, compared with healthy controls, implying impaired endogenous endothelial regeneration. We subsequently sought to exclude demographic and clinical differences (Table 1) between patients with cardiomyopathy and healthy controls as confounders for reduced eo-EPC numbers. Clinical symptoms (ie, extent of dyspnea and atypical angina), medication, and presence of B19V-associated cardiomyopathy were included in a multivariate analysis as independent predictors of reduced numbers of eo-EPCs \(\beta = 0.46; T = 2.40; P = .026; \text{Supplementary Table 2})\).

Presentation of B19V Receptors and Coreceptors on CACs

Having shown the association of B19V with angiogenic cells from patients with B19V-associated cardiomyopathy, we investigated the in vitro susceptibility to infection among different types of CACs. Expression of the necessary cellular receptor blood group P antigen and the coreceptor \(\beta\)1-integrin on CACs was comparable to that among UT7/Epo-S1 cells (Figure 2A and 2B) and greater than that among mature endothelial cells [8]. As previously demonstrated [13, 34], presentation of the coreceptor Ku80 is infrequent among UT7/Epo-S1 cells but could assist B19V binding to CACs. In contrast, a significantly decreased expression of these proteins was found on CD34\(^+\)KDR\(^+\) cells \(\text{P antigen, } 12\% \text{ of cells}; \beta\)1-integrin, 89%; Ku80, 0%; \(P < .05\)) and lineage-committed cells \(\text{CD34}^\text{−} \text{cells; P antigen, } 33\% \text{ of cells; } \beta\)1-integrin, 85%; Ku80, 0%; data not shown).

In Vitro–Infected CACs Express B19V Genes

To prove the intracellular presence of B19V after in vitro infection of CACs, nucleic acids were quantified by in situ hybridization.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Human parvovirus B19 (B19V) infects circulating angiogenic cells (CACs) in vitro. A, Quantification of in situ hybridization analysis of B19V-infected CACs under normoxic conditions \((n = 3)\). Data are means \pm SDs. Abbreviations: CFU-Hill, colony-forming unit Hill; ECFC, endothelial colony-forming cell; eo-EPC, early outgrowth epithelial progenitor cell.
24 or 72 hours after infection (Supplementary Figure 1). CD34+ KDR+ cells had very poor survival under culture conditions and were thus precluded from further quantitative analysis. As positive controls, 65% of infected UT7/Epo-S1 cells stained positively (Figure 3A). Comparable B19V infection was proven for eo-EPCs, ECFCs, and to a lesser extent for CFU-Hill cells. HeLa cells, as negative control, showed no infection. The frequency of infection among Eo-EPCs was highest (68%) 24 hours after infection, with a further increase (93%) 72 hours after infection. ECFCs showed high susceptibility to infection, but considerably fewer infected ECFCs were present 72 hours after infection. Owing to low infection rates among CFU-Hill colonies and their cellular heterogeneity of primarily lymphocytic cells [26], further experiments were pursued only with eo-EPCs and ECFCs.

Figure 4. Expression of human parvovirus B19 (B19V) genes and detection of B19V capsid proteins in in vitro–infected circulating angiogenic cells (CACs). A–C, Detection of B19V NS1 (A), VP1/VP2 (B), and spliced 11-kDa messenger RNA (C) transcripts in in vitro–infected CACs. Total RNA was isolated from in vitro–infected endothelial colony-forming cells (ECFCs) 24 and 48 hours after infection under normoxic or hypoxic conditions for reverse transcription–polymerase chain reaction (PCR) analysis. PCR products were separated on a 3% agarose gel. H, hypoxic conditions; N, normoxic conditions; +, reverse-transcribed complementary DNA; −, RNA without reverse transcription. D and E, Detection of B19V capsid proteins VP1 and VP2 72 hours after infection under normoxic conditions, for early outgrowth epithelial progenitor cells (eo-EPCs; D), or 48 hours after infection under hypoxic conditions, for ECFCs (green; E). Nuclei were stained with DAPI (blue). All experiments were performed with the appropriate noninfected controls. eo-EPCs underwent immunofluorescence analysis (original magnification, 400×; scale bar, 20 µM; D) and ECFCs underwent confocal laser scanning analysis (original magnification, 600×; scale bar, 25 µM; E).
B19V induces cell damage through lysis when productively replicating or by expression of the cytotoxic nonstructural genes, even in the absence of virus propagation [12]. The level of B19V NS1 mRNA increased in in vitro–infected ECFCs from 24 to 48 hours after infection (Figure 4A). The expression of 11-kDa protein and VP1/VP2 transcripts (Figure 4B and 4C) in ECFCs is evidence that viral replication is occurring. The bone marrow microenvironment is hypoxic, which influences cell maturation and differentiation [35, 36]. As demonstrated for permissive ErPCs [37], incubation in hypoxic conditions yielded increased NS1 expression, as well as VP1/VP2 mRNA expression (Figure 4C). We therefore addressed the intriguing question of whether productive viral replication was underway. In ECFCs, B19V genome equivalents strongly decreased from 0 to 72 hours after infection and were undetectable up to 172 hours after infection (Supplementary Figure 3). These data thus indicate that ECFCs are nonpermissive for B19V DNA replication in vitro. As described for the early steps of infection in UT7/Epo-S1 cells [38], our data indicate a rapid degradation of viral particles during the first hours of infection.

Immunofluorescence microscopy detected B19V capsid proteins in the cytoplasm of a small percentage of infected eo-EPCs and ECFCs (Figure 4D and 4E and Supplementary Movies 1 and 2).

**B19V Induces Apoptosis in CACs Through the Death Receptor Signaling Pathway**

We next sought to dissect the mechanism of apoptosis induction in CAC after B19V infection. Both B19V-infected eo-EPCs and ECFCs showed strong activation of the effector caspase-3 (Figure 5A) and binding of annexin V (Figure 5B) 24 hours after infection, indicating early apoptosis. This finding was confirmed by a TUNEL assay that detected terminal apoptosis (Figure 5C). Stress due to hypoxic conditions increased numbers of TUNEL-positive cells by 3.5-fold (P < .01; data not shown), compared with normoxic conditions. Likewise, numbers of TUNEL-positive eo-EPCs increased in a time-dependent fashion by 2.5-fold between 24 and 72 hours after infection (P < .005; data not shown). In contrast, no apoptosis was induced by the culture conditions among nonadherent, nonangiogenic cells present in the same well as the adherent eo-EPCs.

**Figure 5.** Induction of apoptosis after human parvovirus B19 (B19V) infection or transfection with B19V genes. A and B, Active caspase-3 (A) or annexin V (B) fluorescence-activate cell-sorting analysis of B19V-infected or control early outgrowth epithelial progenitor cells (eo-EPCs) and endothelial colony-forming cells (ECFCs) 24 hours after infection under normoxic conditions. C, TUNEL assay of B19V-infected or control eo-EPCs under normoxic and hypoxic conditions at indicated time points after infection. Adherent and nonadherent cells were stained. Data are means ± SD.
indicating specificity of B19V infection (Figure 5C). We subsequently proved the individual contribution of NS1, 11-kDa protein, and VP1 to apoptosis induction (Supplementary Figure 4A and 4B) through activation of caspase-8 and caspase-10 (Supplementary Figure 4C and 4D).

**B19V Impairs Endothelial Regeneration In Vivo**

To prove B19V involvement in endothelial regeneration, B19V-infected human eo-EPCs were transplanted in a murine model of carotid injury. The significant improvement of reendothelialization ($P < .01$) in carotid injury after transplantation of human eo-EPCs in SCID beige mice was abolished after transplantation of B19V-infected eo-EPCs ($P < .001$, compared with control eo-EPCs; $P < .001$ by the test for trend, with adjustment for multiple comparisons; Figure 6A). The number of endogenous murine progenitors double stained with the stem cell markers CD117 (c-kit) and CD309 (flk-1) significantly increased in bone marrow and blood specimens obtained

![Figure 6](https://example.com/figure6.png)
from mice that received B19V-infected cells, compared with sham-treated mice and mice that received noninfected eo-EPCs ($P < 0.009$ by the test for trend, with adjustment for multiple comparisons; Figure 6B). The lowest number of murine progenitors in the bone marrow were found after transplantation of control eo-EPCs, indicating bottom proliferation. The stark increase in the number of endogenous murine progenitors reflects endogenous proliferation and mobilization in response to B19V-infected eo-EPCs. In contrast to sham-treated animals, human cells were found attached to the denuded vessels after transplantation of eo-EPCs. We then demonstrated B19V-infected human eo-EPCs in the murine carotis, indicating that B19V is carried to the damaged vessel walls (Figure 6C and Supplementary Movie 3).

**DISCUSSION**

Our results show that the vasculotropic B19V infects bone marrow–derived CACs and has an impact on endogenous endothelial regeneration capacity, thus providing a novel pathogenetic approach for B19V-associated cardiac damage and persistence. B19V-associated cardiomyopathy [6] may represent an illustrative example for the proposed mode of chronic B19V vascular disease.

Although there is an ongoing debate about a common precursor [39] of the populations of hematopoietic precursors and cells involved in endogenous endothelial regeneration, there is abundant evidence for a close physical association between these cell subsets [40]. Since B19V replicates and persists primarily in ErPCs of the bone marrow [13], we hypothesized that infection of cells belonging to the heterogeneous group of bone marrow–derived CACs represents a link to explain B19V-associated vascular disease.

Our data showing the highest concentrations of B19V DNA in CD34+/KDR+ cells are in line with findings from other studies, which reported that CACs from blood specimens may serve as viral carriers mediating hepatitis B virus infection of the endothelium in experimental models [41]. Significantly extending the knowledge of viral infection and transport resulting in dysfunction of CACs, we demonstrated B19V gene expression in angiogenic cells from bone marrow and peripheral circulation specimens. Constant spreading of B19V with CAC is associated with cardiomyopathy. This was not shown in patients after a previously unnoticed B19V infection.

Highly reduced numbers of eo-EPCs in patients with B19V-associated cardiomyopathy indicate a functional impairment of endogenous cellular vascular regeneration. Very small numbers of circulating ECFCs in peripheral blood specimens from healthy adults [25] prevented further functional and quantitative analysis. Although B19V-associated cardiomyopathy is linked to endothelial dysfunction and associated with higher numbers of CD34+/KDR+ cells [28], our data provide evidence for considerably reduced functional endothelial regeneration. Specifically, the presence of B19V but not reduced left ventricular function was associated with impaired regenerative potential in our patients.

Showing high in vitro susceptibility to infection, cells involved in endothelial regeneration, rather than endothelial cells in the vessel wall, are the focus of B19V infection. Initially, both eo-EPCs and ECFCs showed similar and greater B19V infectivity, compared with UT7/Epo-S1 cells. When detecting single B19V DNA and RNA transcripts with the in situ hybridization probe, there was a time-dependent increase of B19V in eo-EPCs. Induction of apoptosis in ECFCs after B19V infection and proliferation of uninfected viable ECFCs may contribute to the time-dependent reduction of infected ECFCs. However, findings from subsequent studies that quantified total B19V DNA copy numbers precluded viral replication in eo-EPCs or ECFCs in vitro.

A strong induction of apoptosis was demonstrated not only after direct infection of eo-EPCs and ECFCs, but also after single expression of NS1 and 11-kDa protein in ECFCs. As described for CD36+ ErPCs [42], our data demonstrate death receptor signaling with activation of initiator caspase-8 and caspase-10 for apoptosis induction. Much to our surprise, VP1 alone led to the same extent of apoptosis, implying a novel role for this protein in apoptosis induction. Higher numbers of apoptotic cells than transfected cells indicate that indirect mechanisms also lead to apoptosis of CACs. It is tempting to speculate that induction of apoptosis through B19V leads to dysfunction and depletion of a probably finite pool of CACs [31].

Finally, proof of a causal relation between B19V infection and dysfunctional endothelial regenerative capacity in vivo was revealed by a significant reduction in experimental reendothelialization among B19V-infected eo-EPCs. This occurred despite proliferation and mobilization of endogenous c-kit+ilk-1+ progenitor cells as a compensatory effect, resembling the analogous finding of increased numbers of CACs in patients with chronic B19V infection [28]. The presence of human B19V-infected eo-EPCs attached to damaged murine vessels indicates a possible mechanism of viral dissemination through cellular transport. Whether these findings translate into development of cardiomyopathy will have to be confirmed in future experiments.

Our studies merit some critical considerations. Because of limited quantities of specimens available in the clinical study, low B19V DNA copy numbers, or infection of a rather small number of eo-EPCs, we might have missed B19V DNA in patients with B19V-associated cardiomyopathy. Another explanation for missing B19V DNA in eo-EPC could be the induction of apoptosis in B19V-infected cells or the failure. Furthermore, patients with chronic B19V infection have substantially fewer viral particles available for infection of CACs, compared with the B19V genome equivalents used in our in vitro experiments.

We postulate that primary infection of bone marrow–derived angiogenic cells causes a secondary chronic vascular disorder, with spreading of B19V-infected angiogenic cells into the
circulation and aggregation to sites of endothelial damage. This mechanism may explain why B19V appears to persist in the heart, and it suggests that the bone marrow is the proper therapeutic target in patients with this seemingly cardiac disorder.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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