Interferon Beta Modulates Endothelial Damage in Patients with Cardiac Persistence of Human Parvovirus B19 Infection

Caroline Schmidt-Lucke,[1,a] Frank Spillmann,[1,a] Thomas Bock,[3,b] Uwe Kühl,[1] Sophie Van Linthout,[1] Heinz-Peter Schultheiss,[1] and Carsten Tschöpe[1,2]

[1]Department of Cardiology and Pneumology, Charité–University Medicine Berlin, Campus Benjamin Franklin, and [2]Berlin-Brandenburg Center of Regenerative Medicine, Berlin, and [3]Department of Molecular Pathology, University Hospital of Tübingen, Tübingen, Germany

Background. In a phase 1 study, we investigated whether interferon beta reduced endothelial damage in patients with cardiac persistence of human parvovirus B19 (B19V) infection.

Methods and results. In vitro, B19V infected cultivated endothelial cells (ECs), which led to a reduction in their viability ($P = .007$). Interferon beta suppressed B19V replication by 63% ($P = .008$) in ECs and increased their viability ($P = .021$). Circulating mature apoptotic ECs (CMAECs [$CD45^-CD146^+$ cells expressing von Willebrand factor and annexin V]) and circulating progenitor cells (CPCs [$CD34^+KDR^+$ cells]) were quantified by flow cytometry in 9 symptomatic patients with cardiac B19V infection before and after 6 months of interferon beta therapy (16 MU) and were compared to levels in 9 healthy control subjects. Endothelial dysfunction was measured using flow-mediated dilatation of the forearm. Patients with B19V persistence had significantly higher ($P = .004$) levels of CMAECs than did control subjects, which normalized after treatment (mean $\pm$ standard deviation, 0.06% $\pm$ 0.08% vs 0.01% $\pm$ 0.006%; $P = .008$). Similar improvement was shown for flow-mediated dilatation ($P = .04$) in the treatment group only ($P = .017$ for the comparison with untreated patients with B19V persistence [$n = 5$]). There were significantly higher numbers of CPCs in patients with B19V persistence before therapy (mean $\pm$ standard deviation, 0.04% $\pm$ 0.05% vs 0.01% $\pm$ 0.004%; $P = .02$) than in control subjects, which normalized after treatment ($P = .03$).

Conclusion. Thus, we present (for the first time, to our knowledge) a modulation of virally induced chronic endothelial damage—specifically, EC apoptosis and endothelial regeneration.

Human parvovirus B19 (B19V), a erythrovirus and the infectious agent of fifth disease in children, is often detected in the tissues of asymptomatic individuals, and there is ongoing debate about the functional relevance of this finding [1–3]. On the other hand, B19V infection is diagnosed [4] in the majority of patients with the clinical symptoms of atypical angina [5] and impaired exercise capacity [6] who undergo endomyocardial biopsies. Furthermore, we and other have shown that this vasculotropic virus targets endothelial cells (ECs) with consequent endothelial dysfunction [7–9], which indeed suggests a pathogenetic role for B19V. Little is known about the mechanism of endothelial damage in humans with persistent B19V infection. The underlying mechanisms by which B19V might cause endothelial damage involves the cytotoxicity of nonstructural protein 1 [10], transactivation of interleukin 6 [11] and tumor necrosis factor α [12], and induction of apoptosis (which has been demonstrated in vitro) [13–15].

Endothelial vasodilator dysfunction predicts long-term disease progression in chronic heart failure [16] and atherosclerosis [17]. The close relationship between coronary artery endothelium–dependent vasomotor responses to acetylcholine and the noninvasive flow-me-

diated dilatation (FMD) in the brachial artery made FMD a useful surrogate measure [18]. Vessel wall integrity is a result of EC damage and regeneration. In vitro, EC apoptosis may be induced by proatherosclerotic agents, whereas atheroprotective substances prevent EC apoptosis [19]. EC apoptosis paralleled early endothelial vasodilator dysfunction in an animal model [20]. Furthermore, levels of shed apoptotic microparticles, which exert procoagulatory properties, are elevated in acute coronary syndromes [21, 22]. Apoptotic ECs may detach from the vessel, leading to a denudation of the vessel wall and the need for replacement with either neighboring ECs or circulating progenitor cells (CPCs) [23]. Increased EC apoptosis will ultimately lead to either an increased replication of vessel wall–derived mature ECs, with the consequence of premature aging of vessel wall ECs [24], or the consumption of a presumably finite pool of CPCs [25]. EC regeneration through CPCs plays an essential role in the maintenance of endothelial integrity [26, 27]. Ischemia-mobilized CPCs from bone marrow and other organs [28] home to sites of endothelial damage, where they incorporate into newly formed vessels and participate in reendothelialization of denuded vessels by either replacement [23] or paracrine actions [28].

We have previously shown that interferon beta was safe in patients with myocardial enteroviral or adenoviral persistence and left ventricular dysfunction and resulted in (1) elimination of viral genomes and improved left ventricular function [29] and in (2) reduced B19V load and improved clinical symptoms of B19V infection [30]. Interferon beta therapy initiated after vascular damage had occurred inhibited vascular leakage and concurrently decreased vascular permeability in ECs, showing a new mechanism for the anti-inflammatory action of interferon beta [31]. Therefore, we tested the hypothesis that a reduction in endothelial damage occurs in patients with cardiac persistence of B19V infection who have been symptomatic for at least 0.5 years despite receiving optimal symptomatic medications.

METHODS

Cell culture and infection with B19V. The immortalized human microvascular EC line HMEC-1 [32] was grown in medium 199 (Invitrogen) supplemented with 1% penicillin-streptomycin, 10 ng/mL EC growth factor (Invitrogen), 1 μg/mL hydrocortisone, and 10% fetal calf serum. HMEC-1 cells were seeded in 6-well cell culture dishes at 2.5×10^5 cells/well. Infection of HMEC-1 cells was done with prepurified B19V virions (multiplicity of infection, 100), in accordance with a method described elsewhere [13, 33]. All experiments were performed at least in triplicate.

B19V replication in mature ECs undergoing interferon beta treatment. HMEC-1 cells were infected with B19V virions as described elsewhere [33]. The cell supernatant was decanted, and medium 199 containing 100–200 U/mL interferon beta (PBL Biomedical Laboratories) was added 24 h after infection. Cells were then harvested 96 h after infection. Total RNA was isolated using the NucleoSpin RNA II kit (Macherey-Nagel), in accordance with the manufacturer's instructions. Complementary DNA of B19V RNA was obtained using Superscript II reverse transcriptase (Invitrogen) and a B19-VP2–specific primer (VP5; 3′-ATTTCTTTAGATAATCCCCTAGA-5′), in accordance with quantitative real-time TaqMan polymerase chain reaction (PCR) protocol described elsewhere [7].

Cell viability and proliferation assay. HMEC-1 cells were infected with B19V virions, and interferon beta (100–200 U/mL) was added to the cell culture medium 24 h after infection, as described above. Cell proliferation was analyzed using the ViaLight Cell Proliferation kit (Cambrex Bio Science Rockland), in accordance with the manufacturer's instructions.

Patients and control subjects. Inclusion criteria were age 18–75 years, documented presence of B19V genome and increased endothelial activation in at least 1 endomyocardial biopsy sample, onset of clinical symptoms (atypical angina, fatigue, and impaired exercise capacity), and stable clinical condition and receipt of medication for chronic heart failure for at least 6 months. Nine consecutive patients with a prolonged history of cardiac symptoms (>0.5 years) despite receiving symptomatic medications and with high numbers of B19V copies (median [range], 300 [189–896] copies/μg of DNA) in their endomyocardial biopsy samples, as measured by real-time PCR, were allocated for interferon beta therapy (Beneferon; Schering) and prospectively studied between September 2005 and November 2006 in a phase 1 study. To assess the natural course of disease with regard to endothelial dysfunction in B19V persistence, 5 consecutive patients (median [range] B19V copy number, 316 [214–360] copies/μg of DNA) were recruited between April 2006 and November 2007 after completion of the initial pilot study. In these patients, only FMD was performed.

Exclusion criteria were diabetes; acute myocarditis; other causes of left ventricular dysfunction; coronary heart disease; pregnancy; antiviral, immunmodulatory, or immunosuppressive therapy within the last 6 months; severe heart failure; clinical or biochemical evidence of the presence of concomitant chronic inflammatory disease; chronic renal insufficiency (creatinine level, ≥1.4 mmol/L); inability to understand the consent form; participation in or consent to participate in another study; or malignant disease.

Nine patients without signs or symptoms of chronic heart failure or coronary artery disease, diabetes, severely impaired left ventricular function, and no more than 1 arteriosclerotic risk factor were recruited as control subjects. Cardiovascular

risk factors were defined as follows: active smoking, hypercholesterinemia, positive family history (cardiovascular events in <55-year-old male or <65-year-old female next of kin). There were no changes in medication during the last 6 months (ie, anti-hypertensive drugs).

All study participants provided written informed consent, and the study was approved by the ethics committee of the Charité, Berlin.

Physical examination, clinical assessments (including echocardiography and electrocardiography), and laboratory controls were conducted every second month to monitor for interferon beta–associated side effects and adverse cardiac events. Assessment of clinical complaints (patient diary) and heart failure symptoms (according to the New York Heart Asssociation classification system) and the completion of a questionnaire inquiring about both interferon beta–associated adverse effects (flulike symptoms, headache, and signs of inflammation) and specific adverse cardiac symptoms (eg, angina and dyspnea) were done at every visit.

Methods for hemodynamic, histological, and immunohistological evaluations and endothelial function measurements are given in the Appendix, which appears only in the online version of the *Journal*.

Interferon beta treatment. Interferon beta therapy followed a stepped regimen to reduce the flulike side effects typical of the initial phase of interferon beta therapy. Subcutaneous administration was initiated at a dose of 4×10^6 U of interferon beta 3 times a week on alternate days and was increased to 8×10^6 U during the second week and to 16×10^6 U after the fourth week. By the end of week 24, interferon beta therapy was discontinued. Follow-up visits after completion of treatment were prespecified to occur after 7 months (± 30 days; visit 1) and after 12 months (± 15 days; visit 2).

Isolation of mononuclear cells and flow cytometry. Mononuclear cells (MNCs) were isolated from peripheral blood by means of a ficoll gradient (Histopaque 1077; Sigma). Circulating mature apoptotic ECs (CMAECs) were analyzed by 4-channel flow cytometry performed in accordance with the methods of Mancuso et al [34] and Monstiroli et al [35] (Figure 1). After exclusion of debris and platelets, only CD45$^-$ cells (CD45–peridinin chlorophyll protein [PerCP]; Becton Dickinson) were further analyzed by flow cytometry via double staining with antibodies against the EC-specific epitopes CD146 (fluorescein isothiocyanate [FITC] conjugated; Chemicon) and against von Willebrand factor (vWF) (goat; Calbiochem), followed by a phycoerythrin (PE)–conjugated secondary antibody. These CD45$^-$CD146$^+$vWF$^+$ cells were then assessed for apoptosis with primary annexin V–allophycocyanin (APC) conjugates (BD Pharmingen) [36]. As an individual negative control for the CD146$^+$vWF$^+$ double-positive cells, a gate was set on lymphocytes. Annexin binding was controlled by incubation

with streptavidin-coupled APC. At least 100,000 events were counted. Measurements were done in duplicate.

CPCs were detected by flow cytometry, as described elsewhere [26]. In brief, MNCs were double stained with PE-conjugated monoclonal antibodies against human KDR (R&D Systems) and against PerCP-conjugated CD34 (Becton Dickinson) to depict CPCs, in accordance with the methods of Rafii [37]. Additionally, the more immature CD133$^+$KDR$^+$ CPCs were stained with PE-conjugated anti–human KDR (R&D Systems) and APC-conjugated anti–human CD133 (Becton Dickinson). Isotype-identical antibodies served as controls (immunoglobulin G [IgG] 2a–FITC and IgG1-PE; Becton Dickinson). Each analysis included 200,000 events.

Cytokines. Levels of cytokines (stromal-derived factor 1 [SDF-1] and vascular endothelial growth factor [VEGF]) were measured using commercially available enzyme-linked immunosorbent assay kits (R&D Systems), in accordance with the manufacturer's instructions.

Statistical analysis. Continuous variables were tested for normal distribution by the Kolmogorov-Smirnov test. Comparisons between the 2 groups were analyzed by the *t* test (2-sided) for normally distributed variables. Nonnormally distributed continuous variables (FMD, CMAECs, and CD34$^+$KDR$^+$ CPCs) were compared by the Mann-Whitney *U* test. Comparison of categorical variables was generated by the Pearson χ^2 test. Statistical significance was assumed if a null hypothesis could be rejected at $P \leq .05$. All statistical analysis was performed with SPSS software (version 14; SPSS).

RESULTS

Infection of cultured ECs. As shown in Figure 2*A*, there was a statistically significant increase in replication intermediates of B19V in HMEC-1 cells that was significantly ($P = .008$) suppressed by interferon beta. B19V infection resulted in significantly reduced cell viability and proliferation ($P = .007$) of HMEC-1 cells. However, after treatment with interferon beta the viability of infected HMEC-1 cells was comparable to that of uninfected cells, as shown in Figure 2*B*.

Patient characteristics. The baseline characteristics of the 23 subjects are summarized in Table 1. The 14 patients and 9 control subjects were matched for age and sex. As expected, patients with B19V persistence had a higher incidence of angina ($n = 7$ for B19V with treatment; $n = 1$ for B19V without treatment), arrhythmias ($n = 4$ for B19V with treatment; $n = 1$ for B19V without treatment), and fatigue ($n = 7$ for B19V with treatment; $n = 3$ for B19V without treatment). The control subjects either were healthy individuals recruited from the outpatient clinic after exclusion of signs or symptoms of cardiac disease ($n = 6$) or had intermittent atrio-ventricular block 2 ($n = 1$) or intermittent atrial fibrillation ($n = 2$; last episode, ≥ 1 month ago; duration of episode, <4 h). Control subjects
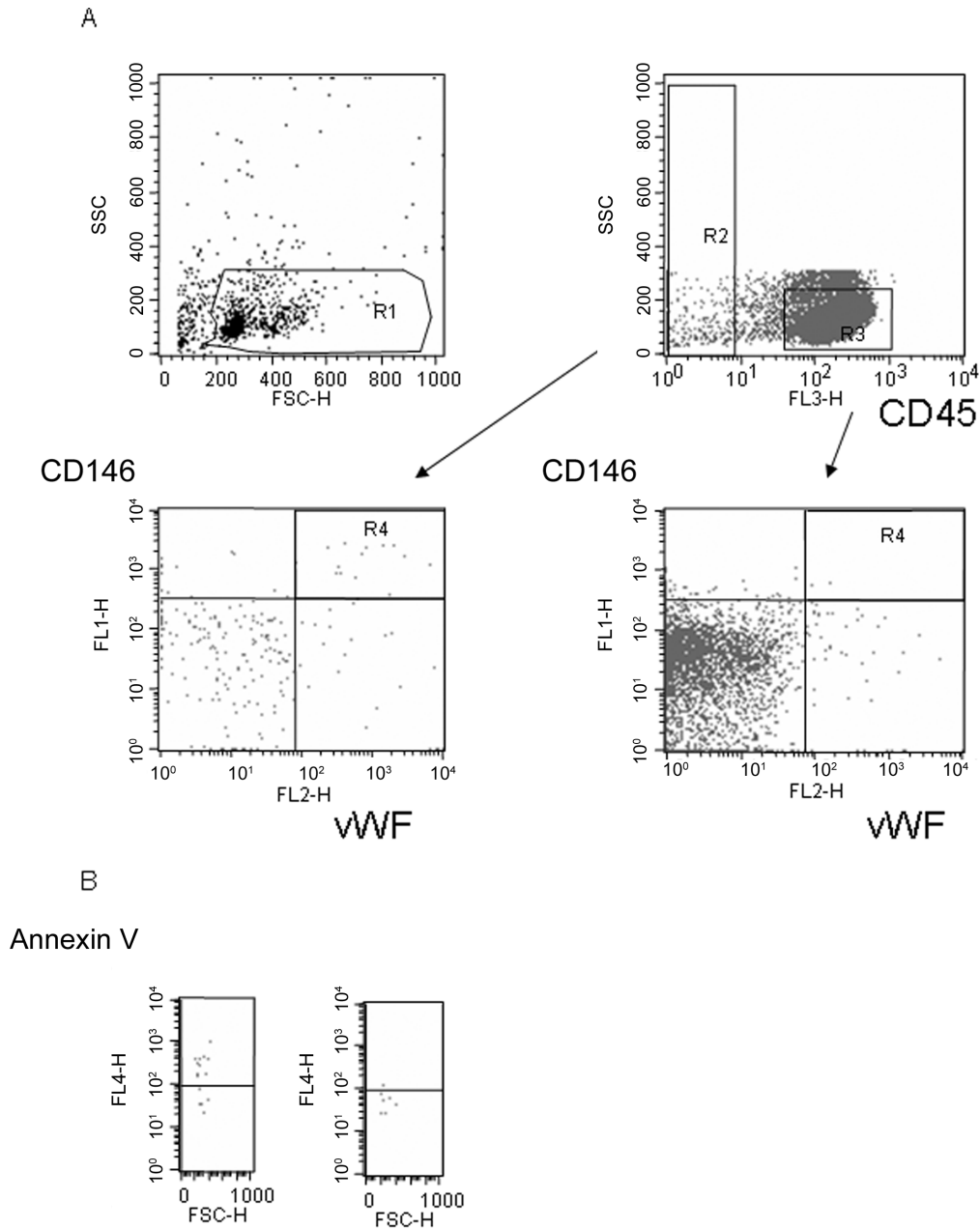
Figure 1. Flow cytometry measurement of circulating endothelial cells (ECs). Circulating cells from peripheral blood were analyzed after density gradient separation. *A*, Identification of circulating mature ECs. Shown are exclusion of platelets and debris from further analysis by means of the forward scatter (FSC) and sidescatter (SSC) view (region R1; *upper left*); anti–CD45–peridinin chlorophyll protein staining to identify circulating cells other than white blood cells for further analysis (region R2; *upper right*); gating of CD45⁻ cells for quantification of cells positive for the endothelial-specific surface markers CD146–fluorescein isothiocyanate and von Willebrand factor (vWF)/goat anti-mouse–phycoerythrin (region R4; *lower left*); and the negative control for setting the gate for CD146⁺vWF⁺ double-positive cells, as acquired from lymphocytes (region R3; *lower right*). The resulting EC number was normalized for peripheral mononuclear cells. *B*, Measurement of EC apoptosis. Shown are annexin V–allophycocyanin (APC) fluorescence of selectively gated mature ECs (*left*) and APC fluorescence of these cells after incubation with APC-labeled probes of the synthetic ligand streptavidin as a negative control (*right*). Cells positive for annexin V were normalized for false-positive streptavidin signals.

had a mean ± standard deviation (SD) number of cardiovascular risk factors of 0.7 ± 1.1, patients with B19V persistence who received treatment had 1.8 ± 1.1, and patients with B19V persistence who did not received treatment had 1.8 ± 1.3 (Table

1). There were no differences with respect to concomitant medication. In endomyocardial biopsy samples, 70% of patients had elevated levels of the endothelial activation marker intercellular adhesion molecule 1 (ICAM-1) and HLA. Interferon
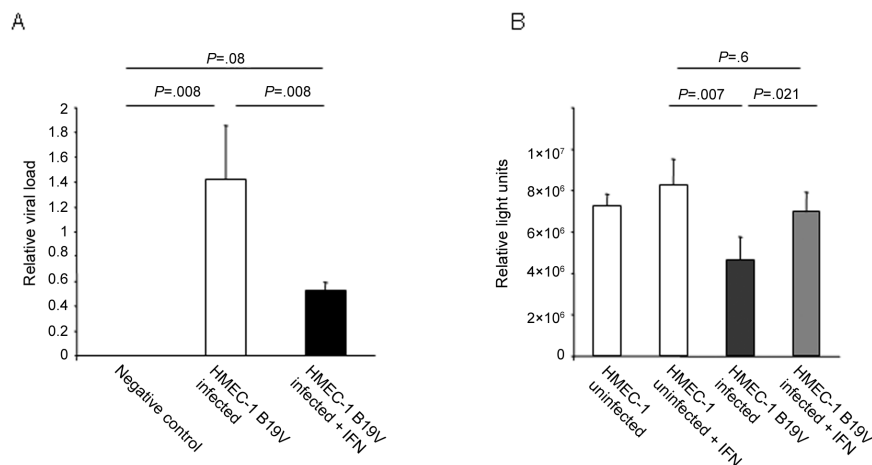
Figure 2. *A*, Suppression of human parvovirus B19 (B19V) replication by interferon beta in human endothelial cells (ECs). HMEC-1 cells were infected with serum-derived B19V virions (multiplicity of infection, 100), and interferon beta (100 U/mL) was added 24 h after infection. Cells were harvested 96 h after infection, and B19V RNA was isolated to demonstrate viral replication intermediates. *B*, Cell viability and proliferation of B19V-infected human ECs undergoing interferon beta treatment. HMEC-1 cells were infected with serum-derived B19V virions (multiplicity of infection, 100). Interferon beta (100 U/mL) was added 48 h after infection, and cells were harvested 96 h after infection. Cell proliferation was measured using the ViaLight assay. Error bars represent standard deviations. IFN, interferon beta.

beta therapy was mostly well tolerated by all patients. All of the patients experienced at least 1 symptom classified as moderate associated with interferon; in 2 patients, symptoms were classified as severe (leukopenia and thrombocytopenia). Interferon therapy had to be interrupted in 4 patients for between 5 days and 2 weeks because of adverse events—namely, leukopenia ($n = 2$), anemia ($n = 1$), and local skin reaction ($n = 1$). Adverse effects in the 9 patients were flulike symptoms ($n = 8$), leukopenia ($n = 4$), local skin reaction ($n = 6$), headache ($n = 3$), fever ($n = 2$), dyspnea ($n = 2$), palpitations ($n = 2$), fatigue ($n = 2$), and intermittent increase in atypical angina symptoms ($n = 2$). Athralgia, nausea, diarrhea, conjuctivitis, increase in liver enzyme levels, and sore throat were present in 1 patient each. All of the patients completed the full course of interferon. Anti–heart failure treatment was kept constant throughout the follow-up period.

The mean $\pm$ SD duration of follow-up in patients with B19V persistence who did not receive treatment was 8 ± 3 months.

Clinical end points and echocardiographic measurements. There was a significant reduction in the number of patients with B19V persistence reporting angina pectoris after 6 months of therapy (from 7 to 2; $P = .046$), which persisted over the actual treatment period, whereas the frequency of the symptoms of fatigue, dyspnea, and arrhythmias remained unchanged. In patients with B19V persistence who were not treated, symptoms remained unchanged. There were 3 patients with B19V persistence in the therapy arm and 2 patients with B19V persistence in the no-therapy arm who experienced suppressed (<45%) left ventricular ejection fraction (LVEF), whereas none of the control subjects had a LVEF of <45% (Table 1).

Cardiac measures were similar between groups (mean $\pm$ SD angiographic left ventricular end diastolic pressure, not available for control subjects, 7.25 ± 3.2 mm Hg for B19V with treatment, and 17 ± 9 mm Hg for B19V without treatment; mean $\pm$ SD left ventricular end diastolic diameter, 48% $\pm$ 2% for control subjects, 55% $\pm$ 9% for B19V with treatment, and 51% $\pm$ 13% for B19V without treatment; and mean $\pm$ SD intraventricular septal thickness, 10 ± 1 mm for control subjects, 10 ± 1 mm for B19V with treatment, and 11 ± 2 mm for B19V without treatment). There was a strong trend between the 2 subgroups of patients with B19V persistence in E/A value (mean [range], 1.1 [1.1–2.2] for control subjects, 1.33 [0.69–1.4] for B19V with treatment, and 1.7 [1.4–1.9] for B19V without treatment; $P = .05$). After interferon beta therapy, there was no significant improvement in echocardiographic left ventricular systolic or diastolic function.

Endothelial dysfunction. As illustrated in Figure 3*A*, highly impaired FMD in patients with B19V persistence compared with that in control subjects ($P = .002$ for B19V with treatment and $P = .001$ for B19V without treatment; data not shown) was observed in this study. Flow-mediated dilatation normalized after 6 months of interferon beta therapy (mean [range], 4.7% [2.3%–5.8%] vs 12.3% [11.3%–14.6%]; $P = .04$). In contrast, endothelium-independent dilatation was similar in control subjects and both patient groups, as depicted in Figure 3*B*, and remained unchanged throughout the course of treatment. In contrast to patients with B19V persistence who received interferon beta therapy ($P = .004$), patients with B19V persistence who did not receive therapy showed persistently impaired

Table 1. Baseline Characteristics

Characteristic	Control subjects (n = 9)	Patients with B19V persistence	
		Before therapy (n = 9)	No therapy (n = 5)
Age, mean ± SD, years	48 ± 15	50 ± 14	48 ± 9
Male sex	1 (11)	3 (33)	4 (80)
Clinical characteristics			
NYHA functional class			
I	0 (0)	4 (44)	3 (60)
II	0 (0)	3 (33)	1 (20)
III	0 (0)	2 (22)	1 (20)
IV	0 (0)	0 (0)	0 (0)
Atypical angina	0 (0)	7 (78)	1 (20)
Arrhythmias	3 (33)	4 (44)	1 (20)
Fatigue	0 (0)	7 (78)	3 (60)
Cardiovascular risk factors			
Smoking	1 (11)	3 (33)	1 (20)
Diabetes mellitus	0 (0)	0 (0)	1 (20)
Hypercholesterinemia	3 (33)	5 (55)	2 (40)
Family history	1 (11)	4 (44)	0 (0)
Arterial hypertension	3 (33)	4 (44)	3 (60)
Medication			
ACE inhibitors/AT1 blockers	2 (22)	8 (89)	5 (100)
β-Blockers	3 (33)	5 (56)	5 (100)
Statins	0 (0)	3 (33)	3 (60)
Hemodynamic and echocardiographic measurements, mean ± SD			
LVEF echocardiography, %	60 ± 7	54 ± 14	46 ± 25
Laboratory parameters, mean ± SD			
VEGF level, pg/mL	81 ± 51	292 ± 644	…
SDF-1 level, pg/mL	105 ± 35	125 ± 75	…

NOTE. Data are no. (%) of participants, unless otherwise indicated. There were no significant differences between the 3 groups. ACE, angiotensin-converting enzyme; AT1, angiotensin II receptor 1; LVEF, left ventricular ejection fraction; NYHA, New York Heart Asssociation; SD, standard deviation; SDF-1, stromal cell derived factor 1; VEGF, vascular endothelial growth factor.

FMD. There was no correlation between systolic or diastolic dysfunction and FMD.

CMAECs and CPCs. Figure 4A illustrates that, at baseline, patients with B19V persistence before therapy had significantly higher levels of CMAECs than did control subjects ($P = .004$). EC apoptosis was significantly reduced after 6 months of interferon beta therapy in patients with B19V persistence ($P = .008$) and reached values comparable to those in control subjects. As shown in Figure 4B, there were significantly higher numbers of CPCs in patients with B19V persistence before therapy ($P = .02$), compared with those in control subjects. Similarly, the more premature subset of CPCs, characterized by the coexpression of CD133 and KDR, was significantly increased in patients with B19V persistence (mean ± SD, 0.0049% ± 0.003% vs 0.0013% ± 0.0009% of peripheral MNCs; $P = .007$). Further analysis showed that only patients with a history of angina ($n = 7$) showed a strong trend toward elevated levels of CD34+KDR+ CPCs (mean ± SD, 0.52% ± 0.48% vs 0.01% ± 0.009% of peripheral MNCs; $P = .059$). Indeed, after 6 months of treatment with interferon beta the number of CD34+KDR+ CPCs returned to normal ($P = .03$), which persisted over the actual treatment period. None of the cardiac medications at baseline were seen to have any effect on EC apoptosis.

Levels of growth factors. To assess potential mobilization of CPCs, VEGF and SDF-1 were analyzed. Plasma VEGF levels were nonsignificantly higher in patients with B19V persistence (mean ± SD, 292 ± 644 vs 81 ± 51 pg/mL; $P = .2$) and were lowered by interferon beta therapy (mean ± SD, 292 ± 644 vs 30 ± 21 pg/mL; $P = .038$). SDF-1 levels were similar in both groups and were not altered by treatment.

Correlations among cytokines, circulating ECs, and endothe-
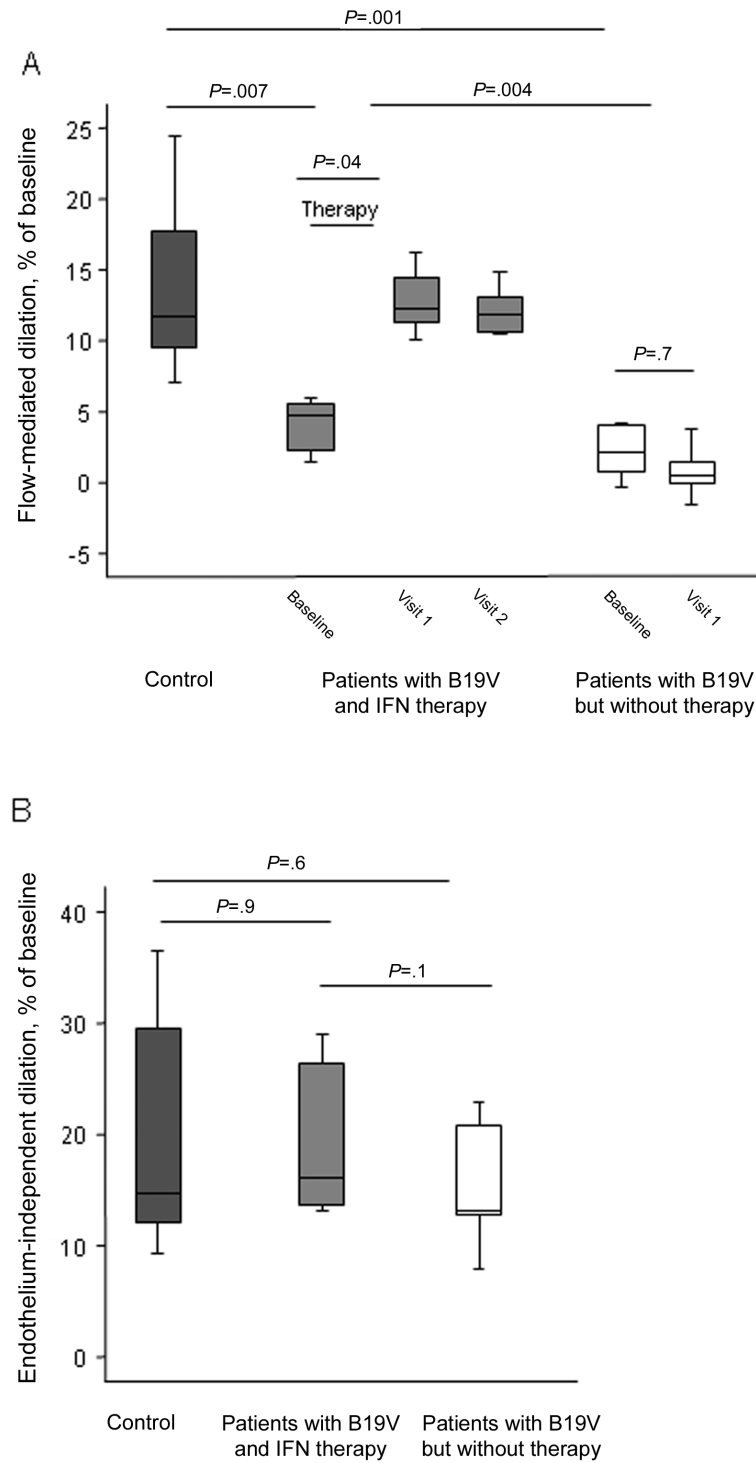
Figure 3. *A*, Flow-mediated dilatation in 9 control subjects, in 9 patients with human parvovirus B19 (B19V) persistence before and after a 6-month course of interferon beta therapy, and in 5 patients with B19V persistence who did not receive interferon beta therapy. Significance levels are given for group comparisons and for changes in patients during interferon beta therapy. *B*, Endothelial-independent dilatation in control subjects and in patients with B19V persistence at baseline. The horizontal line in the middle of each box indicates the median; the top and bottom borders of the box mark the 75th and 25th percentiles, respectively; and the whiskers above and below the box mark the range. IFN, interferon beta.
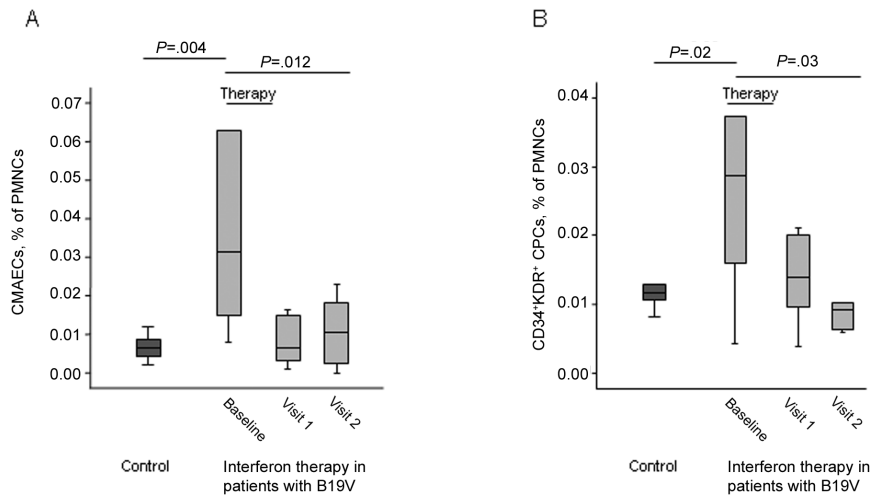
Figure 4. Percentage of circulating mature apoptotic endothelial cells (CMAECs) (*A*) and of circulating CD34⁺KDR⁺ progenitor cells (CPCs) (*B*) among peripheral mononuclear cells (PMNCs) in 9 control subjects and in 9 patients with human parvovirus B19 (B19V) persistence before and after a 6-month course of interferon beta therapy. Significance levels are given for group comparisons and for changes in patients during interferon beta therapy. The horizontal line in the middle of each box indicates the median; the top and bottom borders of the box mark the 75th and 25th percentiles, respectively; and the whiskers above and below the box mark the range. IFN, interferon beta.

lial vasodilator dysfunction. There was an inverse correlation between circulating mature ECs and FMD ($r = -0.53$; $P = .02$). The CPC-mobilizing factors SDF-1 and VEGF ($r = .55$; $P = .02$) were correlated with each other, but no correlation was seen between the 2 cytokines and the 2 subsets of CPCs or FMD.

DISCUSSION

The results of the present phase 1 study confirm and extend previous findings of B19V-associated vascular damage. B19V persistence is associated with endothelial dysfunction [7], atypical angina pectoris [5], and impaired exercise capacity. However, the underlying complex pathogenetic mechanisms of B19V-induced endothelial dysfunction are unclear. To evaluate whether immunomodulation could improve HMEC-1 cell fate in B19V persistence, the effect of interferon beta was assessed. The viability of HMEC-1 cells was markedly reduced after infection with B19V. In vitro, interferon beta significantly reduced viral replication in HMEC-1 cells and significantly increased the viability of ECs, providing strong evidence that B19V-induced damage of HMEC-1 cells is at least partially mediated by direct virus-cell interaction. Clearly, the results of this study do not provide detailed insight into the pathogenetic mechanisms, which will be the aim of future studies.

To assess whether these preliminary in vitro data translate into better endothelial function in patients with B19V infection after interferon beta therapy, we designed a human phase 1 study to analyze different component of endothelial damage in vivo. The inverse correlation between a significantly increased level of circulating ECs and impaired FMD suggest that similar

pathophysiological mechanisms led to EC apoptosis and endothelial dysfunction in this (albeit small) group of patients. Given that previous studies have shown that a procoagulant state is associated with the circulation of apoptotic microparticles [21, 22], we assume that, apart from vessel wall erosion, circulation of increased numbers of apoptotic mature ECs might be another component in the multifactorial pathogenesis of endothelial dysfunction and activation. Simultaneous measurements of different stages of maturation of CPCs and mature ECs allowed us to differentiate between vessel wall damage and regeneration capacity. Increased levels of CD34⁺KDR⁺ CPCs—as well as the more immature subset (CD133⁺KDR⁺)—in patients with B19V infection and angina denote a potentially ischemia-triggered mobilization of these cells. Notably, patients with virally induced endothelial damage and angina mobilize CPCs, in contrast to the vascular damage evoked by classical risk factors [38] or typical coronary heart disease [26]. Future studies will be needed to address the functional capacity of CPCs in B19V infection. There may be secondary mechanisms responsible for myocardial damage in these patients.

Indeed, the results of our pilot study demonstrated that a 6-month course of immunomodulation significantly reduced the numbers of CMAECs and led to normalization of endothelial dysfunction in patients with B19V infection over the actual treatment period, pointing to a disease-modifying property of interferon beta therapy. In contrast, we documented persistent endothelial dysfunction in patients with B19V persistence who did not receive treatment, hence excluding the possibility of spontaneous improvement of endothelial damage. The underlying protective mechanisms of interferon beta remain to be

further elucidated. Simultaneously, after interferon beta therapy the clinical symptom of angina subsided in the majority of patients with B19V persistence, and the increased numbers of CPCs normalized. Further supporting that mobilization indeed influenced the increased numbers of CPCs in patients with B19V persistence was the reduction in the elevated levels of the ischemia-induced mobilizing growth factor VEGF [28] after interferon beta therapy. Clearly, the nature of our clinical study does not permit us to dissect the individual components leading to the presumably increased mobilization of CPCs in patients with angina associated with B19V persistence.

Thus, our data provide clinical evidence for the hypothesis that B19V contributes to ongoing vascular injury and that chronic endothelial damage evoked by an extrinsic viral stimulus can be improved by immunomodulation. However, the present study is a phase 1 study, and even if it is unlikely that the natural process of healing accounts for our findings after the long period of symptoms in these patients, a randomized, placebo-controlled phase 2 trial is warranted to confirm our results. For ethical reasons a second biopsy was not performed after the treatment course, so no information on the clearance of B19V or a reduction in viral load in endomyocardial biopsy samples after treatment can be provided. Given than the majority of our patients had a normal or only slightly reduced ejection fraction and that there was a low incidence of concomitant cardiovascular risk factors as inducers of endothelial dysfunction, we presume, but cannot prove, that B19V was the cause of endothelial dysfunction.

Acknowledgments

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