Endothelium-Dependent Flow-Mediated Vasodilation of Systemic Arteries Is Impaired in Patients With Myocardial Virus Persistence

Katja B. Vallbracht, MD; Peter L. Schwimmbeck, MD; Uwe Kühl, MD, PhD; Bettina Seeberg, MD; Heinz-Peter Schultheiss, MD

Background—Myocardial virus persistence is frequently observed in patients with cardiomyopathy. Endothelial dysfunction in patients with cardiomyopathy is associated with inflammatory immunoresponses in myocardial biopsies. The aim of this study was to investigate the impact of myocardial virus persistence on endothelial function.

Methods and Results—In 124 patients with suspected cardiomyopathy, myocardial biopsies were examined for virus persistence (by polymerase chain reaction) and inflammation (by immunohistology). Endothelial function of the radial artery was examined by high-resolution ultrasound. Diameter changes in response to reactive hyperemia (flow-mediated dilation [FMD]) compared with glycerol trinitrate (GTN-MD) were measured. Mean age of the patients (55 men, 69 women) was 45±13 years; ejection fraction was 57±17%. In 73 patients, adenovirus, enterovirus, parvovirus, or HHV6 virus (V) was detected; in 51, no virus was detected. FMD was significantly impaired in patients with myocardial virus persistence compared with control subjects (Co): FMD-V, 3.38±2.67%; FMD-Co, 7.34±3.44 (P<0.001). In 86 patients, myocardial inflammation was confirmed (Inf). Of those, 57 had virus, and 29 did not. FMD was significantly impaired in patients with virus compared with controls: FMD-Inf-V, 3.24±2.66%; FMD-Inf-Co, 6.07±3.00 (P<0.001). In 38 patients, immunohistology of the myocardial biopsies was normal (Co); of those, 16 had virus, and 22 did not. FMD was impaired in patients with virus compared with control subjects: FMD-Co-V, 3.38±2.72%; FMD-Co, 9.00±3.32% (P<0.001). Endothelium-independent vasodilation (GTN-MD) was not significantly affected.

Conclusions—Myocardial virus persistence is associated with endothelial dysfunction. Endothelial dysfunction in patients with myocardial virus persistence can occur independently of endothelial activation or myocardial inflammation but is more pronounced in patients with concurrent inflammation. (Circulation. 2004;110:2938-2945.)

Key Words: cardiomyopathy • endothelium • inflammation • viruses • vasodilation

Clinical symptoms of patients with nonischemic heart disease such as dilated or inflammatory cardiomyopathy are often poorly understood. Chest pain that may, with corresponding ECG changes, even mimic myocardial infarction could be explained by endothelial dysfunction. Exercise intolerance, which may occur even in patients with only mildly impaired left ventricular function, could be attributed to endothelial dysfunction. In the absence of ischemic heart disease or risk factors for atherosclerosis, the causative agent for endothelial dysfunction may be myocardial virus persistence or myocardial inflammation.

Myocardial inflammation is associated with endothelial dysfunction of systemic arteries.1 A correlation between endothelial dysfunction and endothelial expression of HLA and adhesion molecules in myocardial biopsies has been described.1 These findings are in line with data from other groups that have demonstrated endothelial dysfunction in systemic infections such as after typhoid vaccination,2 after Kawasaki disease,3 in systemic vasculitis,4 and in association with elevated C-reactive protein levels.5

Myocardial virus persistence is frequently observed in patients with nonischemic heart disease. In patients with clinically suspected myocarditis, myocardial enterovirus persistence has been demonstrated in 40%, with 56% of those actively replicating.6 In patients with so-called dilated cardiomyopathy, adenovirus (AdV) could be demonstrated in myocardial biopsies in 13% and enterovirus (EnV) in another 13% of patients.6 In acute myocarditis, parvovirus (PVB19) has been demonstrated in myocardial biopsies in 71% of patients.7

Inflammatory parameters can be associated with an increased risk of cardiovascular events8,9 or the progression of heart failure.10 Endothelial function, which is impaired in inflammatory processes, is a relevant marker of prognosis, as has been demonstrated for patients with atherosclerosis11–13 and after transplantation.14 Therefore, it is important to know whether myocardial virus persistence is associated with endothelial dysfunction.

The aim of this study was to investigate the impact of myocardial virus persistence on endothelial function in pa-
tients with nonischemic cardiomyopathy. We focused on the question of whether myocardial virus persistence, as demonstrated by detection of the virus genome in myocardial biopsies, was associated with endothelial dysfunction and whether this endothelial dysfunction was due to inflammatory processes or was caused by direct damage by the virus.

Methods

We included 124 consecutive patients with nonischemic cardiomyopathy suspected from history, physical examination, and noninvasive tests. Mandatory criteria for inclusion in the study were (1) clinically suspected cardiomyopathy indicated by history and symptoms (chest pain [angina], dyspnea, palpitations, or exercise intolerance) or by history and ECG changes (ST-segment or T-wave deviations or rhythm disturbances) and (2) echocardiographic findings of left ventricular dysfunction (regional wall motion abnormalities or global left ventricular dysfunction). The time period between symptom onset and inclusion in our study varied between 3 and 12 months. Because our intention was to examine patients with chronic cardiomyopathy and not acute myocarditis, patients with earlier onset of symptoms (<3 months) were excluded. Through left ventricular catheterization and angiography, coronary artery disease was excluded and left ventricular ejection fraction and pressures were measured. Right ventricular catheterization was performed to obtain endomyocardial biopsies and hemodynamic measurements. To minimize other confounding factors on endothelial dysfunction, patients with coronary artery disease, diabetes, obesity or other risk factor for arteriosclerosis, overt arteriosclerosis, or other severe disease were excluded. Because heart failure is known to affect endothelial function, we excluded patients with severely impaired left ventricular contractility (ejection fraction <35%). At the time of the study, most patients were already on cardiovascular medication known to influence endothelial function. To exclude acute effects, all cardiovascular medication was ceased according to half-life before the study, although this may not be necessary. Patients were required to have sinus rhythm, and patients did not receive any immunomodulatory therapies.

Informed consent was obtained from all patients. The local ethics committee of the Free University of Berlin approved the study protocol.

Myocardial Biopsies

Endomyocardial biopsies from the right ventricular septum were obtained by standard percutaneous transvenous femoral approach with a standard bioptome.

Immunohistology

For immunohistologic evaluation, samples were prepared and evaluated as published previously. Immunohistologically stained leukocytes (CD2+, CD3+, CD4+, CD8+, activated CD45RO+ lymphocytes, macrophages) were counted per high-power field (400-fold magnification, equivalent to 0.28 mm²). Endothelial expression of HLA-1, HLA-DR, and ICAM-1 was semiquantitatively scaled (1 = normal, 2 = intense, 3 = abundant) according to intensity of immunoperoxidase staining. Endothelial activation was graded according to the sum of endothelial expression of HLA-1, HLA-DR, and ICAM-1: 3 = mild to normal, 5 = mild to moderate, 8 = moderate to abundant. Myocardial inflammation was confirmed in myocardial biopsies if > 7 CD3+ lymphocytes per 1 mm² tissue were identified and/or if endothelial expression of cell adhesion molecules was enhanced. Myocardial biopsies were examined and analyzed by 2 independent blinded observers.

Viral Genome Evaluation

For viral genome evaluation of AdV, EnV (including coxsackievirus and echoviruses), PVB19, Epstein-Barr virus (EBV), and human herpes virus (HHV-6), the samples were examined as published previously. DNA and RNA were extracted simultaneously from frozen myocardial tissue probes. Polymerase chain reaction (PCR) or reverse transcriptase–PCR was performed for the detection of viruses. As a control for successful extraction of nucleic acids, primer sequences were chosen from the sequence of the glyceraldehyde-3-phosphate dehydrogenase genes. Sequence analysis of PVB19 PCR fragments was performed by an automatic ABI Prism 310 Genetic Analyzer and BigDye Cycle Sequencing Kit according to the manufacturer instructions (Applied Biosystems).

Endothelial Function

Endothelial function of the radial artery was assessed as published previously. By means of high-resolution ultrasound, diameter changes in response to reactive hyperemia (flow-mediated dilation [FMD]) compared with glycerol trinitrate–mediated dilation (GTN-MD) were detected using standard protocols. FMD represents endothelium-dependent vasoreactivity, whereas GTN-MD indicates smooth muscle cell function.

Calculations

FMD represents the percentage of diameter increase caused by shear stress compared with baseline. GTN-MD represents the percentage of diameter increase induced by GTN compared with baseline.

Statistical Analysis

Statistical analysis was performed with SPSS Inc software, version 11.0 for IBM-PC. Descriptive data are expressed as mean±SD. Quantitative data were compared with qualitative data by use of the Kruskal-Wallis test on rank sums for independent samples, followed by a post hoc multiple comparison procedure if appropriate. Multivariate analysis was accomplished by linear regression ANOVA. To compare quantitative data of 2 groups, the Mann-Whitney U test was applied. Quantitative data were correlated by use of the Spearman p rank-order analysis, calculating the coefficient of correlation, r. Statistical significance was inferred at values of P<0.01.

Results

Patients Characteristics

General Characteristics

The mean age of the 124 patients was 43±13 years; 55 were male, and 69 were female. At the time of investigation, all patients were normotensive and had normal lipid levels; 22 were treated for hypertension; 26 were treated for hypercholesterinemia (with statins); and 15 were smokers. The patients were on standard cardiovascular medication (ACE inhibitors, AT1 antagonists, β-blockers, calcium antagonists, digitalis glucosides, 33). There were no significant differences in these parameters between patient groups.

Clinical Presentation and History

Sixty-four patients presented with chest pain (angina), 40 with palpitations, and 93 with fatigue or exercise intolerance. Previously, 32 patients had been treated for heart failure symptoms, 67 had experienced exertional dyspnea, and 59 reported an antecedent viral illness. At the time of inclusion, all patients were in NYHA stage II or III (no significant differences).

Noninvasive Examinations

ST-segment changes were documented in the ECGs of 26 patients; arrhythmias were found in 10 patients. Echocardiography revealed regional wall motion abnormalities or an impaired global systolic left ventricular function (Table 1). Pericardial effusions were not observed. Hemodynamic measurements are given in Table 1.
C-reactive protein levels were <6 mg/L and white cell counts were normal in the study population. Most patients had low IgG titers for various virus species (EnV, AdV, CMV, EBV, PVB19); however, none of the patients had signs of any acute virus infection determined by IgM titers (no significant differences).

### TABLE 1. Patient Characteristics and Endothelial Function

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total Population (n = 124)</th>
<th>No Virus (n = 51)</th>
<th>Virus (n = 73)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation, n</td>
<td>86</td>
<td>29</td>
<td>57</td>
<td>...</td>
</tr>
<tr>
<td>No inflammation, n</td>
<td>38</td>
<td>22</td>
<td>16</td>
<td>...</td>
</tr>
<tr>
<td>Age, y</td>
<td>44.99 ± 13.50</td>
<td>47.01 ± 14.68</td>
<td>43.57 ± 12.70</td>
<td>0.140</td>
</tr>
<tr>
<td>LVED, mm</td>
<td>55.44 ± 9.8</td>
<td>57.20 ± 10.47</td>
<td>54.32 ± 9.24</td>
<td>0.140</td>
</tr>
<tr>
<td>FMD, %</td>
<td>5.01 ± 3.58</td>
<td>7.34 ± 3.44</td>
<td>3.38 ± 2.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GTN-MD, %</td>
<td>25.93 ± 9.02</td>
<td>27.18 ± 7.48</td>
<td>25.06 ± 9.91</td>
<td>0.061</td>
</tr>
<tr>
<td>RelFMD, %</td>
<td>19.93 ± 13.48</td>
<td>25.85 ± 15.52</td>
<td>17.31 ± 11.64</td>
<td>0.005</td>
</tr>
<tr>
<td>D-base, mm</td>
<td>2.47 ± 0.47</td>
<td>2.43 ± 0.47</td>
<td>2.49 ± 0.48</td>
<td>0.597</td>
</tr>
<tr>
<td>D-FMD, mm</td>
<td>2.59 ± 0.49</td>
<td>2.60 ± 0.48</td>
<td>2.57 ± 0.49</td>
<td>0.659</td>
</tr>
<tr>
<td>D-GTN, mm</td>
<td>3.09 ± 0.49</td>
<td>3.09 ± 0.53</td>
<td>3.09 ± 0.46</td>
<td>0.758</td>
</tr>
<tr>
<td>EF, %</td>
<td>57.08 ± 17.56</td>
<td>58.22 ± 19.44</td>
<td>56.30 ± 16.23</td>
<td>0.141</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>8.61 ± 4.99</td>
<td>7.97 ± 4.53</td>
<td>8.97 ± 5.24</td>
<td>0.204</td>
</tr>
<tr>
<td>RAP, mm Hg</td>
<td>3.94 ± 2.83</td>
<td>3.53 ± 1.95</td>
<td>4.17 ± 3.22</td>
<td>0.390</td>
</tr>
<tr>
<td>RVP sys, mm Hg</td>
<td>27.14 ± 6.53</td>
<td>28.5 ± 6.65</td>
<td>26.35 ± 6.38</td>
<td>0.231</td>
</tr>
<tr>
<td>RVP diast, mm Hg</td>
<td>4.98 ± 2.54</td>
<td>4.25 ± 2.10</td>
<td>5.40 ± 2.68</td>
<td>0.019</td>
</tr>
<tr>
<td>PAP mean, mm Hg</td>
<td>14.31 ± 5.88</td>
<td>15.70 ± 6.61</td>
<td>13.61 ± 5.40</td>
<td>0.130</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>7.59 ± 4.69</td>
<td>8.26 ± 5.38</td>
<td>7.20 ± 4.23</td>
<td>0.632</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>6.2 ± 1.9</td>
<td>5.75 ± 1.51</td>
<td>6.47 ± 2.03</td>
<td>0.088</td>
</tr>
<tr>
<td>SVI, mL/m²</td>
<td>44.68 ± 15.69</td>
<td>40.63 ± 13.85</td>
<td>47.53 ± 16.20</td>
<td>0.068</td>
</tr>
<tr>
<td>CI, L/min⁻¹·m⁻²</td>
<td>3.31 ± 1.01</td>
<td>3.01 ± 0.79</td>
<td>3.48 ± 1.08</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Inflammation indicates myocardial inflammation/endothelial activation (immunohistology); LVED, left ventricular end-diastolic diameter (echocardiography); D, diameter; base, baseline; EF, ejection fraction (angiography); LVEDP, left ventricular end-diastolic pressure; RAP, right atrial pressure; RVP, right ventricular pressure (systolic/diastolic); PAP, pulmonary artery pressure (mean); PCWP, pulmonary capillary wedge pressure; CO, cardiac output; SVI, stroke volume index; and CI, cardiac index. Data are expressed as mean ± SD or absolute numbers. Probability value describes statistical differences between patients with myocardial virus persistence and control subjects.

*Statistically significant difference (P<0.001).

### Markers of Systemic Inflammation

C-reactive protein levels were <6 mg/L and white cell counts were normal in the study population. Most patients had low IgG titers for various virus species (EnV, AdV, CMV, EBV, PVB19); however, none of the patients had signs of any acute virus infection determined by IgM titers (no significant differences).

### Myocardial Biopsies

We included 124 patients with suspected inflammatory cardiomyopathy. Myocardial inflammation or endothelial activation was confirmed by immunohistology in myocardial biopsies in 86 patients according to the criteria described above (Table 2). In 38 patients, no inflammatory immune response was detected.

### TABLE 2. Immunohistology of Myocardial Biopsies

<table>
<thead>
<tr>
<th></th>
<th>Total Population (n = 124)</th>
<th>No Virus (n = 51)</th>
<th>Virus (n = 73)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENA</td>
<td>5.41 ± 2.21</td>
<td>4.92 ± 2.20</td>
<td>5.74 ± 2.16</td>
<td>0.029</td>
</tr>
<tr>
<td>HLA-1</td>
<td>2.21 ± 0.83</td>
<td>2.07 ± 0.82</td>
<td>2.29 ± 0.69</td>
<td>0.153</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>1.70 ± 0.81</td>
<td>1.68 ± 0.79</td>
<td>1.71 ± 0.83</td>
<td>0.943</td>
</tr>
<tr>
<td>ICAM</td>
<td>1.71 ± 0.85</td>
<td>1.63 ± 0.81</td>
<td>1.76 ± 0.87</td>
<td>0.453</td>
</tr>
<tr>
<td>CD-2</td>
<td>1.41 ± 2.69</td>
<td>0.88 ± 0.68</td>
<td>1.62 ± 3.06</td>
<td>0.506</td>
</tr>
<tr>
<td>CD-3</td>
<td>1.52 ± 2.90</td>
<td>0.84 ± 0.74</td>
<td>1.85 ± 3.48</td>
<td>0.257</td>
</tr>
<tr>
<td>CD-4</td>
<td>0.68 ± 1.26</td>
<td>0.48 ± 0.64</td>
<td>0.78 ± 1.47</td>
<td>0.211</td>
</tr>
<tr>
<td>CD-8</td>
<td>0.56 ± 1.06</td>
<td>0.39 ± 0.52</td>
<td>0.65 ± 1.25</td>
<td>0.202</td>
</tr>
<tr>
<td>CD-4S-R0</td>
<td>1.39 ± 2.58</td>
<td>0.92 ± 1.02</td>
<td>1.62 ± 3.06</td>
<td>0.383</td>
</tr>
<tr>
<td>Macrophages</td>
<td>1.61 ± 2.95</td>
<td>1.22 ± 1.00</td>
<td>1.81 ± 3.57</td>
<td>0.600</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD or absolute numbers (n). Probability value describes statistical differences between patients with myocardial virus persistence and control subjects.
(Table 2). In 73 of the 124 patients, myocardial virus persistence was demonstrated in myocardial biopsies (Table 2), and 51 had no myocardial virus persistence (Table 2). AdV was detected in 8 patients, EnV in 17, PVB19 in 46, EBV in 3, and HHV-6 in 17 (coinfections in Figure 3). Of the 86 patients with myocardial inflammation or endothelial activation, 57 had myocardial virus persistence, and 29 did not. Of the 38 patients without inflammatory immune response detectable in myocardial biopsies, 16 had myocardial virus persistence, and 22 did not.

Leukocyte counts tended to be higher in patients with myocardial virus persistence, but the differences did not reach statistical significance (Table 2). Myocyte necrosis was not observed in this study population.

Endothelial activation was normal in 54 patients, moderate in 41 patients, and abundant in 29 patients; it was enhanced in patients with myocardial virus persistence compared with control subjects ($P=0.029$). However, endothelial expression of HLA-1, HLA-DR, or ICAM-1 alone was not significantly increased in the patients with myocardial virus persistence compared with patients without virus (Table 2). Subgroup analysis revealed that for the subgroup of patients with myocardial inflammation ($P=0.603$) and the subgroup without myocardial inflammation ($P=0.356$), endothelial activation was not significantly enhanced in patients with virus persistence compared with patients without virus.

### Endothelial Function

#### General Characteristics
Heart rate and blood pressure (systolic and diastolic) did not change significantly during measurements with reactive hyperemia and after application of GTN. Adequate reactive hyperemia was achieved in all subjects. Diameter changes are given in Table 1.

#### Flow-Mediated Vasodilation
Endothelial function, as determined by FMD of the radial artery, was significantly impaired in patients with myocardial virus persistence (FMD-V, 3.38±2.67%) compared with patients without myocardial virus detection (FMD-Co, 7.34±3.44%; Table 1 and Figure 1; $P<0.001$). For the inflammatory patient subgroup (n=86), FMD was significantly impaired in patients with myocardial virus persistence (GTN-MD-V, 2.72%; $P=0.005$) and not significantly impaired in patients without virus (GTN-MD-Co, 2.78%; $P=0.806$). The different patterns of myocardial virus infection are described above. It is evident that the number of patients for each type of infection is too small to make a definite statement on the effect of a special virus infection on endothelial function. However, statistical overall analysis for different virus types (including patients without virus) revealed a significant-difference testing for endothelial function (FMD) ($P<0.001$) (Figure 3). For endothelium independent vasodilation (GTN-MD) ($P=0.065$) and for endothelial activation ($P=0.075$), statistical overall analysis revealed only a tendency toward a difference. No relation was observed for different virus types and left ventricular contractility (ejection fraction) ($P=0.806$).

When only patients with myocardial virus persistence (n=73) were considered, FMD was not significantly influ-
enced by virus type ($P=0.416$) (Figure 3). Of those, virus type had slightly more impact on FMD in patients without myocardial inflammation/endothelial activation ($n=16$) ($P=0.173$) than in patients with myocardial inflammation or endothelial activation ($n=57$) ($P=0.238$). In patients without myocardial inflammation or endothelial activation, only AdV, PVB19, or HHV-6 was observed to persist in the myocardium. In patients with myocardial inflammation/endothelial activation, all virus types and combined virus infections were observed to persist in the myocardium.

**Impact of Other Factors**

All subjects in our study population were middle-aged, with only small variations. Ejection fraction and other hemodynamic measurements did not vary extensively among the study population because patients with severely impaired left ventricular function were excluded. Therefore, in this study, age, left ventricular ejection fraction, end-diastolic diameter, end-diastolic pressure, pulmonary capillary wedge pressure, cardiac output, cardiac index, and stroke volume index had no impact on endothelial function, endothelial activation, or myocardial inflammation.

We performed a multivariate analysis (linear regression ANOVA) with age, ejection fraction, GTN-MD, endothelial activation, and virus considered potential candidates to influence endothelial function (FMD) ($r=0.650$, $r^2=0.423$, $P<0.001$). FMD was found to be significantly influenced by myocardial virus persistence (coefficient $\beta=-0.473$, $P<0.001$) and endothelial activation ($\beta=-0.261$, $P=0.001$) and to a lesser extent by GTN-MD ($\beta=0.187$, $P=0.015$) but not by ejection fraction ($\beta=0.039$, $P=0.601$) or age ($\beta=0.081$, $P=0.817$).

**Discussion**

In this study, we have demonstrated for the first time a relationship between viral myocardial disease and endothelial...
dysfunction. Acute, generalized inflammatory immune responses such as that occurring after typhoid vaccination are known to be associated with endothelial dysfunction. In this study, we focused on myocardial virus persistence in which serological signs of acute virus infections are lacking. According to our observations, discussed in detail below, myocardial virus persistence influences endothelial function. We demonstrated that endothelial function is impaired in patients with compared with patients without myocardial virus persistence, even in the absence of other discernible inflammatory immune responses.

The terms used to describe nonischemic heart disease remain controversial. “Cardiomyopathy” is usually applied if left ventricular systolic function is impaired, but what about the patients with regional wall motion disturbances that do not lead to an impaired ejection fraction? These patients cannot be considered healthy; rather, they may represent a group with a diagnosis made early in the course of the disease, and progression may be imminent. In this study, to facilitate the description, we also apply “cardiomyopathy” to patients with only mildly or regionally impaired left ventricular function. Regardless of definitions and terms, the main point of this study is that we consider myocardial virus persistence to influence endothelial function and that this may be, but is not necessarily, associated with inflammatory processes in the myocardium or endothelium. With myocardial biopsies, the underlying disease can be identified. Especially in patients with symptoms but only mild or regional left ventricular dysfunction, endothelial function may be an important predictor of prognosis.

To examine the extent to which myocardial virus persistence itself affects endothelial function (FMD), we focused on the subgroup of patients with myocardial inflammation. In this subgroup in which differences in myocardial inflammation and endothelial activation were excluded, endothelial function (FMD) was significantly impaired in patients with
myocardial virus persistence compared with control subjects. This may indicate that myocardial virus persistence, independently of inflammatory responses, may be associated with endothelial dysfunction. This is further supported by the findings that, in patients with myocardial virus persistence, endothelial function is severely impaired where the additional presence of myocardial inflammation or endothelial activation can obviously further impair endothelial function, even though these differences (between severely and very severely impaired endothelial function in the presence of myocardial virus persistence) do not reach statistical significance.

To further support our hypothesis, we focused on the subgroup of patients without myocardial inflammation and endothelial activation. Per definition, in this subgroup, there was no difference in myocardial inflammation and endothelial activation between patients with and without myocardial virus persistence. Even after elimination of these confounding factors of endothelial dysfunction, endothelial function (FMD) was significantly impaired in patients with myocardial virus persistence compared with control subjects. This, as well as the multivariate analysis considering various factors with potential impact on endothelial function, strengthens our hypothesis that myocardial virus persistence itself is associated with endothelial dysfunction.

In patients without myocardial inflammation, GTN-MD was equal in patients with and without myocardial virus persistence. In patients with myocardial inflammation or endothelial activation, endothelium-independent vasodilation was slightly impaired in patients with concurrent myocardial virus persistence. This may reflect structural changes of the vessel wall and endothelial cell damage that may occur with virus infection and inflammatory immune response but not without immune response.

Myocardial virus persistence without detectable inflammatory immune response is not an uncommon finding. It may partly be explained by the fact that different viruses induce different immunologic pathomechanisms. The inflammatory infiltrate is often less in patients with myocardial AdV persistence compared with EnV. AdV proteins can, for example, protect cells from tumor necrosis factor–mediated lysis and can downregulate MHC class I antigen expression. In myocardial PVB19 persistence, myocardial inflammation, considering lymphocyte infiltrates, is frequently only low grade, whereas macrophages are increased. Endothelial cells, in addition to proliferating erythroid progenitor cells, have been recognized as targets for PVB19 infection, with blood group p antigen serving as a cellular receptor for the virus. Therefore, PVB19 infection is likely to be associated with endothelial dysfunction even in the absence of a lymphocyte infiltrate in the myocardium. To differentiate endothelial function for the various viruses, the number of patients in the present study was too small, and coinfection with different viruses was too common. Thus, a definite answer as to which virus displays which effect on endothelial function is not possible but warrants further exploration.

In the present study, the patients did not have any signs of acute virus infection; however, low-grade IgG titers of various viruses, induced by antecedent virus infections, were detectable. Comparable to the findings in other study populations, no conclusions can be drawn from virus serology for viral heart disease because virus serology does not correlate with myocardial virus persistence. To what extent other serological markers, potentially induced by myocardial virus persistence, may affect endothelial function in this context remains speculative. Our recent research is focused on further elucidating these questions.

With the present study, we cannot explain which mechanisms lead to peripheral endothelial dysfunction in patients with myocardial virus persistence. On the one hand, even localized myocardial virus persistence may induce circulating cytokines, which lead to endothelial dysfunction, also in the peripheral circulation. On the other hand, we currently cannot exclude that virus persistence is not localized to the heart but also is present in the endothelium of the peripheral vasculature, thereby causing endothelial dysfunction. Finally, we cannot rule out an unknown systemic or endothelial pathology that may cause both virus persistence and endothelial dysfunction. Our current research is focused on the identification of circulating cytokines associated with endothelial dysfunction in nonischemic cardiomyopathy.

We conclude that myocardial virus persistence is associated with endothelial dysfunction. This finding is clinically important because endothelial dysfunction represents a marker of prognostic relevance and may influence therapeutic decisions. Endothelial dysfunction may partly explain the symptoms of patients with myocardial virus persistence or inflammatory cardiomyopathy.

Conclusions

Endothelial function is impaired in patients with myocardial virus persistence. Because we aimed to exclude other factors with an impact on endothelial function, we consider myocardial
vanilla virus persistence, myocardial inflammation, and endothelial activation to directly influence endothelial function. Endothelial function and endothelial activation are related but can be observed independently from each other. Myocardial virus persistence itself can lead to endothelial dysfunction. Our findings are clinically important because endothelial function represents a marker of prognostic relevance.

References

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