Differential Aspects of Endothelial Function of the Coronary Microcirculation Considering Myocardial Virus Persistence, Endothelial Activation, and Myocardial Leukocyte Infiltrates

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

Background—Viral cardiomyopathy resulting from myocardial virus persistence can be associated with inflammatory immune responses that involve the myocardium and coronary blood vessels. The aim of this study was to investigate the differential impact of myocardial virus persistence and inflammation on endothelial function of the coronary microcirculation.

Methods and Results—In 71 patients with nonischemic cardiomyopathy, myocardial biopsies were examined for virus persistence (by polymerase chain reaction) and inflammation (by immunohistology). Endothelial function of the coronary microcirculation was examined during heart catheterization by measuring diameter (by quantitative coronary angiography) and velocity changes (by intracoronary Doppler) of the left anterior descending artery in response to acetylcholine. Coronary blood flow (CBF) was calculated. Endothelium-independent vasoactivity to adenosine was assessed. Mean age of the patients (37 men, 34 women) was 43 ± 13 years; mean ejection fraction was 64 ± 11%. In 43 patients, adenovirus, enterovirus, parvovirus, or HHV-6 was detected; 28 had no virus. Endothelial function of the coronary microcirculation was significantly impaired in patients with myocardial virus persistence (V) compared with patients without virus (Co) (ΔCBF-V, 22 ± 86%; ΔCBF-Co, 110 ± 113%; P = 0.001), which was confirmed in 51 patients with myocardial inflammation (MC) (32 with virus, 19 with no virus) (ΔCBF-MC-V, 12 ± 89%; ΔCBF-MC-Co, 81 ± 109%; P = 0.034) and in 20 patients with normal immunohistology of the myocardial biopsies (Co) (11 with virus, 9 with no virus) (ΔCBF-Co-V, 51 ± 72%; ΔCBF-Co-Co, 175 ± 97%; P = 0.006). Endothelial function of the coronary microcirculation was also significantly impaired in patients with myocardial inflammation/endothelial activation compared with patients without inflammatory immune response. Endothelium-independent vasodilation was not influenced significantly.

Conclusions—Myocardial virus persistence and myocardial inflammation/endothelial activation are associated with endothelial dysfunction of the coronary microcirculation. Endothelial dysfunction in patients with myocardial virus persistence can occur independently of myocardial inflammation/endothelial activation but is more pronounced in patients with concurrent inflammation. (Circulation. 2005;111:1784-1791.)

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

Clinical symptoms of patients with nonischemic heart disease like dilated or inflammatory cardiomyopathy are often poorly understood. Chest pain that may, with corresponding ECG changes, mimic myocardial infarction could be explained by endothelial dysfunction. Exercise intolerance that 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. A correlation has been described between endothelial dysfunction and endothelial expression of HLA and adhesion molecules in myocardial biopsies. These findings are in line with data from other groups that have demonstrated endothelial dysfunction in systemic inflammatory states.

Myocardial virus persistence is frequently observed in patients with nonischemic heart disease. In patients with so-called dilated cardiomyopathy, adenovirus (AdV) could be demonstrated in myocardial biopsies of 13% and enterovirus (EnV) in another 13% of patients. Parvovirus has been demonstrated in myocardial biopsies in 71% of patients with acute myocarditis.

Different viruses induce different immunological pathomechanisms; not all myocardial virus infections are associated with myocardial inflammatory infiltrates or endothelial

Circulation is available at http://www.circulationaha.org DOI: 10.1161/01.CIR.0000160863.30496.9B
The inflammatory infiltrate is often less in patients with myocardial AdV persistence compared with those with EnV. In parvovirus persistence, macrophages are increased, whereas lymphocyte infiltrates are sparse.9 Endothelial cells are the targets for PVB19 infection, with blood group p antigen serving as a cellular receptor for the virus.10 Therefore, PVB19 infection is likely to be associated with endothelial dysfunction, even in the absence of a lymphocyte infiltrate in the myocardium. We have demonstrated endothelial dysfunction of systemic arteries in patients with myocardial virus persistence.11

Inflammatory parameters can be associated with an increased risk of cardiovascular events12 or the progression of heart failure.13 Endothelial function, which is impaired in inflammatory processes, is a relevant marker of prognosis, as has been demonstrated for patients with atherosclerosis14–16 and in those after transplantation.17,18 Therefore, it is important to know whether myocardial virus persistence, either in association with myocardial inflammation or by direct effects on the endothelium, is associated with endothelial dysfunction.

The coronary microcirculation appears to be most prone to inflammatory and viral changes in the myocardium. In this vascular region most adjacent to the specific findings in myocardial biopsies, we intend to elucidate differential effects of inflammatory parameters and virus infections on endothelial dysfunction.

The aim of this study was to investigate the impact of myocardial virus persistence, endothelial activation, and myocardial leukocyte infiltrates on endothelial function of the coronary microcirculation in patients with nonischemic cardiomyopathy.

Methods

We included 71 consecutive patients with suspected nonischemic cardiomyopathy, considering history, physical examination, and noninvasive tests. Patients were included in the study if they had both clinically suspected cardiomyopathy from history and symptoms (chest pain [angina], dyspnea, palpitations, or exercise intolerance) or from history and ECG changes (ST-segment or T-wave deviations or rhythm disturbances) and echocardiographic findings of left ventricular dysfunction (regional wall motion abnormalities ≥3 segments) or global left ventricular dysfunction (ejection fraction <55%, Simpson). The time period between onset of symptoms and inclusion in our study was 3 to 12 months; patients with earlier onset of symptoms (<3 months) and acute myocarditis were excluded. Through left ventricular catheterization and angiography, coronary artery disease was excluded, endothelial function was measured, and left ventricular ejection fraction and pressures were documented. Right ventricular catheterization was performed to obtain endomyocardial biopsies and to carry out hemodynamic measurements. To minimize other confounding factors on endothelial dysfunction, patients with coronary artery disease,14–16 diabetes,19 >1 other risk factor for arteriosclerosis,19–21 overt arteriosclerosis, severely impaired left ventricular contractility (ejection fraction <35%),22,23 or other severe disease were excluded from this study. Of the 87 patients screened, 16 were excluded for the reasons listed above. At the time of the study, most patients were already on cardiovascular medication known to influence endothelial function.24 All cardiovascular medication was ceased according to half-life before the study, although this may not be required.24 At the time of the study, the 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 bioprobe.

Immunohistology

For immunohistological evaluation, the samples were prepared and evaluated as published previously.1,25–26 Immunohistologically stained leukocytes (CD2+, CD3+, CD8+, and activated CD45RO+ lymphocytes and macrophages) were counted by high-power field (×400 magnification, equivalent to 0.28 mm²) by use of a Leica MDRD microscope in all available fields (>10 fields per antibody). Endothelial expression of HLA-1, HLA-DR, and ICAM-1 was semiquantitatively scaled as 1 (normal), 2 (intense), or 3 (abundant), according to intensity of immunoperoxidase staining of endothelial cells. Endothelial activation was graded according to the sum of endothelial expression of HLA-1, HLA-DR, and ICAM-1: 3 to 4 = normal, 5 to 7 = moderate, and 8 to 9 = 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), parvoviruses (PVB19), Ebstein Barr virus (EBV), and human herpes virus (HHV-6), samples were examined as published previously.8,27–31 DNA and RNA were extracted simultaneously from frozen myocardial tissue probes. Polymerase chain reaction (PCR) or reverse-transcriptase PCR was performed to detect the 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 with the automatic ABI Prism 310 Genetic Analyzer and BigDye Cycle Sequencing Kit according to the manufacturer’s instructions (Applied Biosystems).29

Endothelial Function

Endothelial function of the coronary microcirculation was assessed. Through coronary angiography and intracoronary Doppler, diameter and flow velocity changes of the left anterior descending coronary artery (LAD) in response to acetylcholine compared with adenosine were detected, following standard protocols.20,32,34,35

Diagnostic left-heart catheterization and coronary angiography were performed by a standard percutaneous femoral approach. After completion of the diagnostic catheterization, an additional 5000 U heparin and 500 mg acetylsalicylate was given intravenously, and an 8F guiding catheter was inserted into the left main coronary artery (LAD). A Doppler-tipped guidewire (FloWire; floppy, 0.014 in, 15 MHz; pulse repetition frequency, 15 to 120 kHz; Cordis) was introduced into the mid segment of the LAD and carefully positioned to obtain a stable flow velocity signal. Via a monorail system, an infusion catheter (multifunctional probing catheter, 0.018+0.014 in; Boston Scientific) was inserted into the mid segment of the LAD just proximal to the tip of the Doppler wire for local application of acetylsalicylate and adenosine.

After stable baseline conditions were obtained, increasing dosages of acetylcholine (0.036, 0.36, and 3.6 µg/mL) were infused at a rate of 2 mL/min via the infusion catheter to the mid segment of the LAD, lasting for 3 minutes for each concentration. Acetylsalicylate causes vasodilation and an increase in velocity when endothelial function is normal but vasocostriction (or inadequate vasodilation) and an inadequate increase in velocity when endothelial function is impaired. Five minutes after the end of the acetylsalicylate infusion, adenosine (0.12 mg/mL) was infused at a rate of 2 mL/min for 3 minutes via the infusion catheter to the mid segment of the LAD to
assess maximal endothelium-independent dilator capacity of the microcirculation, indicated by maximal velocity and blood flow increase.

Throughout the study, phasic and mean intracoronary blood flow velocity (via the FloWire), heart rate, and aortic pressure (via guiding catheter) were continuously measured. Serial hand injections of nonionic contrast medium were performed at baseline and at the end of the acetylcholine and adenosine infusions. Diameter changes of the LAD were measured by quantitative coronary analysis (Philips Inturis CardioView, QCA V3.3, Pie Medical Imaging). Coronary angiography was performed with a simultaneous biplane, multidirectional, and isocentric x-ray system, and videodigitized end-diastolic frames were analyzed. Velocity changes were measured by intracoronary Doppler (FloMap, Endosonics/Volcano). Diameter and flow changes in response to infusion of vasoactive medication were expressed as percent change from baseline.

### Calculations

Coronary blood flow (CBF) was calculated, considering average peak velocity (measured by intracoronary Doppler) and luminal area calculated from epicardial vessel diameter (measured by quantitative coronary analysis): CBF=(diameter/2)^2 × π × velocity/2.

Changes in CBF (ΔCBF) in response to acetylcholine represent endothelium-dependent vasoreactivity of the coronary microcirculation; changes in CBF in response to adenosine represent endothelium-independent vasodilation of the coronary microcirculation: ΔCBF (%)=(CBF max−CBF base)/CBF base.

### Statistical Analysis

Statistical analysis was performed with SPSS Inc software, version 11.0 for IBM PCs. Descriptive data are expressed as mean±SD. After testing for homogeneity of variances, a t test was applied to compare the quantitative data of the 2 groups. Quantitative data were correlated by Pearson’s analysis, calculating the coefficient of correlation (r). Multivariate analysis was accomplished by linear regression ANOVA after testing for homogeneity of variances. Statistical significance was inferred at P<0.05.

### Results

#### Patient Characteristics

#### General Characteristics

The mean age of the 71 patients was 43±13 years; 37 were male, and 34 were female. At the time of investigation, all patients were normotensive and had normal lipid levels, 15 were treated for hypertension, 13 were treated for hypercholesterolemia (with statins), and 8 were smokers. The patients were on standard cardiovascular medication (54 on ACE inhibitors, 4 on angiotensin type 1 antagonists, 44 on β-blockers, 10 on calcium antagonists, and 15 on digitalis glucosides). There were no significant differences in these parameters between patient groups.

#### Clinical Presentation and History

Forty-seven patients presented with chest pain (angina), 25 with palpitations, and 59 with fatigue or exercise intolerance. Previously, 20 patients had been treated for heart failure symptoms, 34 had experienced exertional dyspnea, and 36 reported an antecedent viral illness. At the time of inclusion in the study, all patients were in NYHA stage II or III (no significant differences).

#### Noninvasive Examinations

ST-segment changes were documented in the ECGs of 14 patients; arrhythmias changes were noted in 10 patients. In all patients, regional wall motion abnormalities or an impaired global systolic left ventricular function was demonstrated by echocardiography (Table 1). Pericardial effusions were not observed.

#### Hemodynamic Measurements

Table 1 gives left ventricular end-diastolic diameter (by echocardiography), ejection fraction (by angiography), left ventricular diastolic pressure, right atrial pressure, right ventricular pressure (systolic/diastolic), pulmonary artery pressure (systolic/diastolic/mean), pulmonary capillary wedge pressure, cardiac output, stroke volume index, and cardiac index. Hemodynamic parameters were not significantly different between patient groups.

#### Markers of Systemic Inflammation

C-reactive protein levels were <6 mg/L and white cell counts were normal in the study sample. 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

### Table 1. Patient Characteristics and Endothelial Function

<table>
<thead>
<tr>
<th></th>
<th>Total Population (n=71)</th>
<th>No Virus (n=28)</th>
<th>Virus (n=43)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation, n</td>
<td>51</td>
<td>19</td>
<td>32</td>
<td>...</td>
</tr>
<tr>
<td>No inflammation, n</td>
<td>20</td>
<td>9</td>
<td>11</td>
<td>...</td>
</tr>
<tr>
<td>Age, y</td>
<td>43.06 ± 12.99</td>
<td>41.30 ± 13.57</td>
<td>43.82 ± 12.70</td>
<td>0.75</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>53.90 ± 8.58</td>
<td>54.04 ± 8.74</td>
<td>53.79 ± 8.58</td>
<td>0.67</td>
</tr>
<tr>
<td>ΔCBF-ACh, %</td>
<td>57.15 ± 105.96</td>
<td>110.81 ± 112.88</td>
<td>22.21 ± 85.74</td>
<td>0.002</td>
</tr>
<tr>
<td>ΔCBF-Ad, %</td>
<td>324.95 ± 169.28</td>
<td>345.88 ± 176.41</td>
<td>311.32 ± 165.11</td>
<td>0.22</td>
</tr>
<tr>
<td>D baseline, mm</td>
<td>2.29 ± 0.54</td>
<td>2.38 ± 0.58</td>
<td>2.23 ± 0.52</td>
<td>0.31</td>
</tr>
<tr>
<td>D ACh, mm*</td>
<td>1.98 ± 0.64</td>
<td>2.32 ± 0.80</td>
<td>1.76 ± 0.78</td>
<td>0.026</td>
</tr>
<tr>
<td>D Ad, mm</td>
<td>2.65 ± 0.59</td>
<td>2.77 ± 0.73</td>
<td>2.58 ± 0.47</td>
<td>0.30</td>
</tr>
<tr>
<td>V baseline, mm</td>
<td>28.69 ± 10.46</td>
<td>26.96 ± 11.74</td>
<td>29.81 ± 9.51</td>
<td>0.30</td>
</tr>
<tr>
<td>V ACh, mm</td>
<td>48.74 ± 21.56</td>
<td>50.38 ± 22.57</td>
<td>47.67 ± 21.11</td>
<td>0.75</td>
</tr>
<tr>
<td>V Ad, mm</td>
<td>80.54 ± 25.08</td>
<td>77.86 ± 20.36</td>
<td>82.28 ± 27.82</td>
<td>0.22</td>
</tr>
<tr>
<td>EF, %</td>
<td>64.18 ± 10.89</td>
<td>67.64 ± 7.56</td>
<td>61.93 ± 12.15</td>
<td>0.12</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>8.18 ± 3.43</td>
<td>6.83 ± 2.66</td>
<td>9.08 ± 3.62</td>
<td>0.11</td>
</tr>
<tr>
<td>RAP, mm Hg</td>
<td>3.95 ± 3.12</td>
<td>3.33 ± 1.90</td>
<td>4.33 ± 3.05</td>
<td>0.30</td>
</tr>
<tr>
<td>RVP systolic, mm Hg</td>
<td>26.38 ± 5.51</td>
<td>26.21 ± 5.58</td>
<td>26.49 ± 5.53</td>
<td>0.69</td>
</tr>
<tr>
<td>RVP diastolic, mm Hg</td>
<td>4.84 ± 2.31</td>
<td>4.13 ± 1.96</td>
<td>5.30 ± 2.43</td>
<td>0.07</td>
</tr>
<tr>
<td>PAP mean, mm Hg</td>
<td>13.51 ± 3.64</td>
<td>13.57 ± 4.53</td>
<td>13.47 ± 3.10</td>
<td>0.73</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>7.02 ± 3.01</td>
<td>6.92 ± 3.25</td>
<td>7.08 ± 2.88</td>
<td>0.84</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>6.30 ± 2.06</td>
<td>6.54 ± 2.33</td>
<td>6.14 ± 1.87</td>
<td>0.59</td>
</tr>
<tr>
<td>SVI, mL/m²</td>
<td>45.48 ± 14.75</td>
<td>44.49 ± 14.77</td>
<td>46.13 ± 14.91</td>
<td>0.45</td>
</tr>
<tr>
<td>CI, L⋅min⁻¹⋅m⁻²</td>
<td>3.33 ± 1.03</td>
<td>3.07 ± 1.08</td>
<td>3.30 ± 1.02</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Inflammation indicates myocardial leukocyte infiltrates or endothelial activation (by immunohistology); LVEDD, left ventricular end-diastolic diameter (by echocardiography); ACh, acetylcholine (endothelium-dependent vasoreactivity); Ad, adenosine (endothelium-independent vasoreactivity); D, diameter; V, velocity; EF, ejection fraction (by 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 when appropriate. Probability values describe statistical differences between patients with myocardial virus persistence and control subjects.

*Statistically significant difference between patients with myocardial virus persistence and control subjects.
Endothelial Dysfunction in Myocardial Virus Persistence

Vallbracht et al

1787

TABLE 2. Immunohistology of Myocardial Biopsies

<table>
<thead>
<tr>
<th></th>
<th>Total Population (n = 71)</th>
<th>No Virus (n = 51)</th>
<th>Virus (n = 20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENA</td>
<td>5.63 ± 2.22</td>
<td>5.32 ± 2.21</td>
<td>5.83 ± 2.22</td>
<td>0.36</td>
</tr>
<tr>
<td>HLA-1</td>
<td>2.17 ± 0.86</td>
<td>1.93 ± 0.81</td>
<td>2.34 ± 0.87</td>
<td>0.10</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>1.78 ± 0.85</td>
<td>1.79 ± 0.83</td>
<td>1.77 ± 0.87</td>
<td>0.90</td>
</tr>
<tr>
<td>ICAM</td>
<td>1.69 ± 0.81</td>
<td>1.59 ± 0.75</td>
<td>1.74 ± 0.85</td>
<td>0.51</td>
</tr>
<tr>
<td>CD-2</td>
<td>1.55 ± 2.90</td>
<td>0.79 ± 0.65</td>
<td>2.05 ± 3.63</td>
<td>0.040</td>
</tr>
<tr>
<td>CD-3</td>
<td>1.63 ± 3.09</td>
<td>0.81 ± 0.69</td>
<td>2.18 ± 3.87</td>
<td>0.035</td>
</tr>
<tr>
<td>CD-4</td>
<td>0.62 ± 1.25</td>
<td>0.44 ± 0.58</td>
<td>0.74 ± 1.54</td>
<td>0.33</td>
</tr>
<tr>
<td>CD-8</td>
<td>0.57 ± 1.11</td>
<td>0.32 ± 0.49</td>
<td>0.74 ± 1.35</td>
<td>0.08</td>
</tr>
<tr>
<td>CD-45-R0</td>
<td>1.40 ± 2.68</td>
<td>0.83 ± 0.96</td>
<td>1.77 ± 3.33</td>
<td>0.22</td>
</tr>
<tr>
<td>Macrophages</td>
<td>1.65 ± 3.10</td>
<td>1.22 ± 0.97</td>
<td>1.94 ± 3.92</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD when appropriate. Probability values describe statistical differences between patients with myocardial virus persistence and control subjects.

Endothelial Function

Myocardial Biopsies

We included 71 patients with nonischemic cardiomyopathy. Myocardial inflammation was confirmed by immunohistology in myocardial biopsies in 51 patients, according to the criteria given above. No inflammatory immune response was detected in 20 patients (Table 2). In 43 of the 71 patients, myocardial virus persistence was demonstrated in myocardial biopsies, and 28 had no myocardial virus persistence (Table 2). AdV was detected in 3 patients, EnV in 3, CMV in 2, PVB19 in 33, EBV in 3, and HHV-6 in 12. Coinfections were observed in 11 patients. Of the 51 patients with myocardial inflammation, 32 had myocardial virus persistence, and 19 did not. Of the 20 patients without inflammatory immune response detectable in myocardial biopsies, 11 had myocardial virus persistence, whereas 9 did not. The prevalence of virus was similar in patients with and without inflammation.

Leukocyte counts (CD2+; CD3+, CD4+, CD8+, and CD45Ro lymphocytes and macrophages) per high-power field (0.28 mm²) were higher in patients with myocardial virus persistence; however, the differences were significant only for CD2+ and CD3+ lymphocytes (Table 2). Myocyte necrosis was not observed in this study sample.

Endothelial activation, according to the sum score of HLA-1, HLA-DR, and ICAM expression, was not significantly enhanced in patients with myocardial virus persistence compared with control subjects (P = 0.37) (Table 2).

Impact of Inflammation

The severity of endothelial dysfunction of the coronary microcirculation, measured as ΔCBF-ACh, correlates significantly with the intensity of lymphocyte infiltrates, considering CD2+ (r = -0.34, P = 0.005), CD3+ (r = -0.34, P = 0.004), CD4+ (r = -0.27, P = 0.025), CD8+ (r = -0.31, P = 0.011), and virus infection determined by IgM titers (no significant differences).

Endothelial cell injury in myocardial virus persistence compared with patients without myocardial virus detection (Table 1 and Figure 1) (P = 0.002). For the noninflammatory patient subgroup (n = 20) (ΔCBF-ACh-Co, 51.47 ± 71.93; ΔCBF-ACh-Co-Co, 174.50 ± 96.74; P = 0.004) in which, according to the definition, there was no endothelial activation and no myocardial leukocyte infiltrates, ΔCBF-ACh was significantly impaired in patients with myocardial virus compared with patients without myocardial virus detection (Figure 1). For the inflammatory patient subgroup (n = 51) (ΔCBF-ACh-Inf-V, 12.15 ± 88.79; ΔCBF-ACh-Inf-Co, 80.65 ± 109.39; P = 0.034), ΔCBF-ACh was also significantly impaired in patients with myocardial virus persistence (Figure 1).

Endothelial function of the coronary microcirculation (ΔCBF-ACh) is significantly impaired in patients with myocardial inflammation compared with patients without inflammatory immune response (ΔCBF-ACh-Inf, 37.67 ± 101.56; ΔCBF-ACh-Co, 106.83 ± 102.99; P = 0.012) in the total study sample (Figure 1). Subgroup analysis showed that this difference is significant only in the patient subgroup without myocardial virus persistence (n = 28; P = 0.026), not in the patient subgroup with myocardial virus persistence (n = 43; P = 0.23; Figure 1).

Endothelium-Independent Vasodilation

Endothelium-independent vasodilation in response to adenosine (ΔCBF-Ad) was not significantly impaired in patients with myocardial virus persistence compared with patients without myocardial virus detection in the total sample (P = 0.44) (Table 1) and in the inflammatory patient subgroup (ΔCBF-Ad-Inf-V, 313.19 ± 149.23; ΔCBF-Ad-Inf-Co, 327.32 ± 186.61; P = 0.77) and noninflammatory patient subgroup (ΔCBF-Ad-Co-V, 305.91 ± 213.13; ΔCBF-Ad-Co-Co, 385.06 ± 155.36; P = 0.37; Figure 2).

Endothelium-independent vasoreactivity (ΔCBF-Ad) also was not significantly different in patients with myocardial inflammation compared with patients without inflammatory immune response in the total sample (P = 0.61; Table 1) and in the patient subgroups with myocardial virus persistence (P = 0.80) and without myocardial virus persistence (P = 0.32; Figure 2).

There is a significant correlation between endothelium-dependent vasodilation of the coronary microcirculation (ΔCBF-ACh) and endothelium-independent vasodilation of the coronary microcirculation (ΔCBF-Ad) (r = 0.44, P < 0.001).

Impact of Inflammation

The severity of endothelial dysfunction of the coronary microcirculation, measured as ΔCBF-ACh, correlates significantly with the extent of endothelial activation, measured as increased expression of HLA-1, HLA-Dr, and ICAM-1 (sum score) (r = -0.37, P = 0.001) (Figure 3), and with the extent of endothelial expression of HLA-1 (r = -0.42, P < 0.001) and ICAM-1 (r = -0.36, P = 0.002) alone. For HLA-Dr, only a tendency was observed (r = -0.21, P = 0.075). Endothelial function of the coronary microcirculation (ΔCBF-ACh) correlates significantly with the intensity of lymphocyte infiltrates, considering CD2+ (r = -0.34, P = 0.005), CD3+ (r = -0.34, P = 0.004), CD4+ (r = -0.27, P = 0.025), CD8+ (r = -0.31, P = 0.011), and
CD45RO+ lymphocytes ($r=-0.29$, $P=0.017$) and tendentially with macrophages ($r=-0.21$, $P=0.085$).

Endothelium-independent vasodilation of the coronary microcirculation ($\Delta$CBF-Ad) correlates with the intensity of CD4+ lymphocyte infiltrates ($r=-0.24$, $P=0.048$) but not with other leukocyte populations or endothelial activation.

**Impact of Other Factors**

All subjects in our study sample were middle-aged, with only small variations. Ejection fraction and other hemodynamic measurements did not vary extensively among the study subjects 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 leukocyte infiltrates.

We performed a multivariate analysis (linear regression ANOVA), considering age, ejection fraction, endothelium-independent vasodilation ($\Delta$CBF-Ad), endothelial activation (sum score), and myocardial virus persistence as potential candidates to influence endothelial function ($\Delta$CBF-ACh) ($r=0.66$, $r^2=0.43$, $P<0.001$). $\Delta$CBF-ACh was found to be significantly influenced by myocardial virus persistence ($\beta=-0.344$, $P=0.001$), endothelial activation ($\beta=-0.299$, $P=0.003$), and $\Delta$CBF-Ad ($\beta=0.389$, $P<0.001$) but not by ejection fraction ($\beta=-0.008$, $P=0.94$) or age ($\beta=0.080$, $P=0.43$).

**Discussion**

In this study, we have differentiated for the first time the effects of myocardial leukocyte infiltrates, endothelial activation, and myocardial virus persistence on endothelial function of the coronary microcirculation. According to our observations, discussed in detail below, we consider myocardial virus persistence, myocardial leukocyte infiltrates, and endothelial activation to influence endothelial function of the coronary microcirculation.

The terms used to describe nonischemic heart disease remain controversial. The term “cardiomyopathy” is usually applied if left ventricular systolic function is impaired. Nevertheless, patients with regional wall motion disturbances
that do not lead to an impaired ejection fraction cannot be considered healthy; rather, they may represent a group with a diagnosis made early in the course of the disease when progression may be imminent. In this study, to facilitate the description, we apply “cardiomyopathy” also to patients with only mildly or regionally impaired left ventricular function.

Inflammatory or viral myocardial disease appears to be more common than expected. We recommend establishing a definite diagnosis of myocardial disease by immunohistological and PCR viral evaluation of myocardial biopsies early in the course of the disease when left ventricular function is only mildly impaired. At that stage, through specific immunomodulatory therapies like interferon or glucocorticoids, a cure for the disease is possible, and deterioration of left ventricular function can be prevented. In severe heart failure, left ventricular function is much more difficult to improve, and therapeutic options are only symptomatic.

Regardless of definitions and terms, the main aim of this study was to determine effects of myocardial leukocyte infiltrates, endothelial activation, and myocardial virus persistence on endothelial function of the coronary microcirculation. Endothelial function may explain symptoms, especially in patients with only mild or regional left ventricular dysfunction, and may represent an important predictor of prognosis.

Myocardial inflammation, in terms of endothelial activation and leukocyte infiltrates, is associated with endothelial dysfunction of the coronary microcirculation. Endothelial function of the coronary microcirculation is significantly impaired in patients with myocardial inflammation compared with patients without inflammatory immune response. The severity of endothelial dysfunction correlates significantly with the extent of endothelial activation measured as increased expression of HLA-1, HLA-Dr, and ICAM-1 (sum score), as well as with the extent of endothelial expression of HLA-1 and ICAM-1 and tendentially HLA-Dr alone, which is in line with previous observations in systemic arteries. Endothelial function of the coronary microcirculation also correlates significantly with the intensity of lymphocyte infiltrates, considering CD2+, CD3+, CD4+, CD8+, and CD45RO+ lymphocytes and tendentially macrophages. Myocardial CD4+ leukocyte infiltrates, but not other infiltrates with other leukocyte populations or endothelial activation, are associated with impaired endothelium-independent vasodilation.
Myocardial virus persistence is associated with endothelial dysfunction of the coronary microcirculation. To further elucidate whether endothelial dysfunction in patients with virus persistence is secondary to inflammatory processes induced by the virus or by direct toxic effects of the virus, we differentiated subgroups: one with myocardial inflammation (leukocyte infiltrates or endothelial activation) and one without an inflammatory immune response (no leukocyte infiltrates and no endothelial activation). In both subgroups, endothelial function was significantly impaired in patients with myocardial virus persistence compared with patients without virus. Thus, even in the absence of inflammatory immune responses, myocardial virus persistence is associated with endothelial dysfunction. We therefore conclude that endothelial dysfunction in patients with myocardial virus persistence seems to be mediated by mechanisms other than myocardial inflammatory infiltrates or endothelial activation. Direct toxic effects and circulating cytokines may play a role in this context. This hypothesis is supported by previous work in which endothelial dysfunction in patients with myocardial virus persistence was observed in systemic arteries.11

In a multivariate analysis, we confirmed that myocardial inflammation and myocardial virus persistence are associated independently with endothelial dysfunction of the coronary microcirculation. Patients with myocardial virus persistence only and patients with inflammation only must be considered to have different diseases or to be at different stages of the disease, with a combination of virus persistence and inflammation as a potential link between the disease stages. Patients with inflammation only are likely to have an autoimmune disorder that may have been induced by a previous virus infection with or without myocardial virus persistence. Because different virus types induce different pathophysiological mechanisms, even the patients with myocardial virus persistence must be considered to have different diseases.

The number of patients in the present study was too small and coinfections with different viruses were too common to differentiate endothelial function for the various viruses. The effects of different virus types on endothelial function of the coronary microcirculation warrant further exploration, but large numbers of patients are required.

Because we tried to minimize other factors that affect endothelial function, we conclude that myocardial virus persistence, myocardial leukocyte infiltrates, and endothelial activation are independently associated with endothelial dysfunction of the coronary microcirculation. This finding is clinically important because endothelial dysfunction represents a marker of prognostic relevance14–18 and may influence therapeutic decisions. Endothelial dysfunction of the coronary microcirculation may partly explain the symptoms of patients with myocardial virus persistence or myocardial inflammation.

Conclusions

Endothelial function of the coronary microcirculation is impaired in patients with myocardial virus persistence and in patients with myocardial leukocyte infiltrates and endothelial activation. Because we tried to minimize other factors that affect endothelial function, we consider myocardial virus persistence, myocardial leukocyte infiltrates, and endothelial activation to have direct effects on endothelial function. Endothelial function and endothelial activation are related but can be observed independently. Myocardial virus persistence itself can, even in the absence of myocardial leukocyte infiltrates or endothelial activation, lead to endothelial dysfunction. In patients with myocardial leukocyte infiltrates or endothelial activation and myocardial virus persistence, endothelial dysfunction is more pronounced. Our findings are clinically important because endothelial function may predict prognosis.

References


33. Deleted in proof.


Differential Aspects of Endothelial Function of the Coronary Microcirculation Considering Myocardial Virus Persistence, Endothelial Activation, and Myocardial Leukocyte Infiltrates
Katja B. Vallbracht, Peter L. Schwimmbeck, Uwe Kühl, Ursula Rauch, Bettina Seeberg and Heinz-Peter Schultheiss

_Circulation_. 2005;111:1784-1791; originally published online April 4, 2005;
doi: 10.1161/01.CIR.0000160863.30496.9B
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/111/14/1784

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/