Role of Leukocytes in Coronary Vascular Endothelial Injury Due to Ischemia and Reperfusion

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A possible cause of the coronary endothelial injury that occurs with ischemia and reperfusion is the local accumulation of leukocytes during these events. To investigate the role of leukocytes in coronary endothelial injury, we tested the effect of leukocyte removal by filtering on coronary endothelial function in a canine model of regional myocardial ischemia and reperfusion. Blood was supplied to the left anterior descending and circumflex arteries of anesthetized dogs via an extracorporeal circulation. A 60-minute left anterior descending occlusion was followed by 120 minutes of reperfusion either with (n=6) or without (n=6) leukocyte filters in the extracorporeal circuit. Regional myocardial blood flow was measured with radiolabeled microspheres. Radiolabeled autologous transferrin (111In) and erythrocytes (99mTc) were given intravenously during reperfusion for assessment of microvascular permeability. Left anterior descending and circumflex coronary artery rings were assessed in vitro for endothelium-dependent dilation to acetylcholine, ADP, and thrombin. In unfiltered dogs, ischemia and reperfusion increased the protein leak index of ischemic myocardium 2.3-fold compared with that of nonischemic myocardium (2.3±0.5 to 5.2±1.6, p<0.05). In filtered dogs, there was no difference in the protein leak index of nonischemic versus ischemic myocardium (1.5±0.4 versus 1.9±0.5, p=NS). There was impaired left anterior descending coronary artery relaxation (versus circumflex) in response to endothelium-dependent vasodilators in vitro. However, relaxation was not consistently improved by leukocyte filtering. We conclude that leukocytes are responsible for the endothelial injury secondary to ischemia and reperfusion in the coronary microvasculature but have little or no effect on the endothelial injury in epicardial coronary arteries. (Circulation Research 1991;69:1566–1574)

Ischemia and reperfusion cause coronary vascular injury as well as myocardial injury. Increased coronary vascular resistance, impaired vasodilator reserve, and the phenomenon of capillary obstruction, often termed “no-reflow,” are manifestations of coronary vascular injury in this setting.1–7 A primary target of this vascular injury appears to be the coronary endothelium in both the large epicardial vessels and the microcirculation. In the epicardial coronary arteries, endothelial injury causes impaired endothelium-dependent dilation,2–6 whereas in the coronary microcirculation it causes increased permeability and leakage of plasma proteins into the extravascular space.8–10

Although the mechanism by which endothelial damage from ischemia and reperfusion occurs has been unclear, there is reason to suspect that leukocytes play a role in the process. Activated leukocytes are toxic to endothelial cells in vitro.11 It has been proposed that leukocytes cause increased coronary vascular resistance during reperfusion through mechanical obstruction, production of vasoactive substances, or release of agents cytotoxic to endothelium.12,13 Increased myocardial edema after reperfusion, an indirect indication of vascular damage, has been prevented by leukocyte depletion.14 In addition, myocardial infarct size in models of cardiac ischemia and reperfusion has been reduced by antileukocyte interventions.15–18 However, it has been unknown whether leukocytes contribute to the coronary endothelial injury seen after ischemia and reperfusion.
In this study, the role that leukocytes play in coronary endothelial injury was assessed by excluding leukocytes from the coronary circulation in a canine model of ischemia and reperfusion. We used extracorporeal perfusion of the major coronary arteries with and without filters that prevented entry of leukocytes. Two separate assessments of endothelial function were used to quantitate injury: microvascular permeability in vivo and capacity for endothelium-dependent dilation of epicardial arteries in vitro. We found that leukocyte filtration prevents the coronary microvascular injury evident as increased protein permeability during ischemia and reperfusion but was ineffective in reducing the impaired endothelium-dependent dilation of the epicardial arteries.

**Materials and Methods**

Twenty-four male mongrel dogs were anesthetized by induction with thiamylal (20 mg/kg i.v.) followed by α-chloralose (100 mg/kg i.v.), intubated with an endotracheal tube, and mechanically ventilated. Electrocardiographic lead II was continuously monitored throughout the experiments. Heparinized catheters were placed in the aorta via the right femoral artery for continuous pressure monitoring and in the left carotid artery and left external jugular vein for later coronary perfusion through an extracorporeal circulation. A left thoracotomy was performed and the heart suspended in a pericardial cradle. The proximal circumflex coronary artery (CX) and the left anterior descending coronary artery (LAD), just distal to the first diagonal branch, were carefully isolated. Heparin (175 units/kg i.v.) was given as a bolus. The dosage of heparin was determined by monitoring the activated clotting time in pilot studies. The extracorporeal apparatus (described below) was primed with blood by connecting flow through it from the carotid to the jugular catheters. The LAD and CX were ligated proximally and cannulated through arteriotomies just distal to the ligatures with tubing from the extracorporeal apparatus. From ligation to restoration of blood flow to each coronary artery required from 30 seconds to 2 minutes. Twenty minutes of stabilization, during which time arterial blood gases were optimized by ventilator adjustment and bicarbonate administration, was completed with the extracorporeal circulation functioning before proceeding with the experimental protocol.

The extracorporeal circuit used to perfuse the LAD and CX was a modification of one used by Engler et al. and is depicted in Figure 1. Silastic tubing (0.125 in. i.d.) connected the left carotid artery to the LAD and CX through separately controlled lines. Blood was pumped through the tubing by two roller pumps (Masterflex model 7524-10) in parallel, one to each artery. The pump flows were continuously monitored and frequently adjusted to keep the coronary pressures, measured near the coronary cannula tips, at a mean of 80–90 mm Hg. The blood was pumped through two Sepacell R-500 leukocyte removal filters (Baxter) placed in parallel in each line (four total per experiment) in 11 dogs and, by appropriate placement of clamps, through an equal distance of the Silastic tubing without filters in 12 animals (randomly dispersed). Inverted 50-ml syringes placed in line served as pulse dampers and bubble traps. Stopcocks were placed proximal and distal to the filter/control tubing segments for withdrawal of blood samples and proximal to the cannulation sites of each coronary artery as injection ports.

After the stabilization period, the LAD cannula was clamped for 60 minutes. During this time, the extracorporeal line to the LAD was bypassed into the jugular vein at 25 ml/min to prevent stasis and desaturation of blood in the line. At 50 minutes of ischemia, radioactive microspheres were vortexed and injected into the distal port of the tubing supplying the CX to measure ischemic (collateral) blood flow. After 1 hour of ischemia, reperfusion of the LAD circulation was initiated, starting with low flow to provide 25 mm Hg pressure and...
proceeding with stepwise increases in flow to achieve the original 80–90 mm Hg over 10 minutes. Reperfusion was maintained for 120 minutes. At 60 minutes of reperfusion, autologous radiolabeled protein (113mIn transferrin) and erythrocytes (99mTc) were injected into the femoral artery line and allowed to circulate for 60 minutes for measurement of microvascular permeability by the protein leak method (see specific methods below). Blood was drawn for white cell and platelet counts proximal and distal to the filter/control tubing segments of the extracorporeal line at 60 minutes of reperfusion to assess adequacy of leukocyte filtration and effects of the process on platelets.

At the end of 120 minutes of reperfusion, the animals were killed by exsanguination and the hearts were excised. The LAD and CX were carefully dissected free, removed from the heart, and placed in physiological salt solution for immediate preparation for in vitro dilation studies (see below). The heart was cleaned of excess blood, the right ventricle removed, and the remainder of the heart placed in a watertight bag and frozen in isopropanol and ice to facilitate cutting. Below the perfusion sites of the LAD and CX, the left ventricle was sectioned into four rings cut perpendicular to its long axis. Each ring was sectioned into quadrants, and each quadrant was cut into endocardial and epicardial halves. Final tissue samples were approximately 1.0 g in size. The tissue samples were counted for radiolabeled protein, erythrocyte, and microsphere activity in a well counter (model 8000, Packard Instrument Co., Inc., Meriden, Conn.). One animal in which LAD occlusion did not produce a region with blood flow of less than 20 ml/100 g/min myocardium was excluded from analysis because of insufficient ischemia.

**Determination of Coronary Microvascular Protein Leak**

Vascular permeability was assessed as a protein leak index by using methods previously described. Briefly, the rate of extravascular accumulation of radiolabeled protein, normalized for perfused vascular surface area, is used as an index of vascular permeability. This method is relatively insensitive to changes in convective forces, and the effects of low blood flow on tracer delivery to ischemic regions are minimized by allowing an extended time period (60 minutes) for tracer delivery. However, any nonperfused tissue regions may not be assessed by this method. Radiolabeled protein (transferrin) and erythrocytes were injected intravenously after 60 minutes of reperfusion. Sixty minutes after injection (at the end of the 120 minutes of reperfusion), a reference blood sample was drawn from the aorta, the heart excised, and myocardial tissue sections taken. The radiolabeled protein and erythrocyte activity of each tissue sample and of the reference blood sample were then determined. The amount of radiolabeled extravascular protein that accumulated during the 60 minutes after intravascular injection was then calculated, normalized for the concentration of radiolabeled protein in the blood (blood protein activity [cpm/g]) and for vascular surface area (represented by the weight of blood in the tissue) and expressed as a protein leak index (PLI)

\[
\text{PLI} = \frac{\text{extravascular protein activity}}{\text{blood protein activity/tissue blood weight}}
\]

**Calculation of Myocardial Blood Flow**

Regional myocardial blood flow was measured using radiolabeled microspheres. Radiolabeled microspheres (15±3 μm diameter, Du Pont/New England Nuclear, Boston, Mass.) suspended in Tween 80 and dextran were diluted in normal saline. Before injection the total amount of injected microsphere activity was determined. After vigorous mechanical agitation to prevent clumping, the spheres were injected into the coronary cannula port and the port rapidly flushed with normal saline. The injection syringe was later counted for residual radioactivity. Coronary flow at the time of injection was recorded from the calibrated roller pumps' digital displays. Myocardial tissue and blood samples were then counted for microsphere content. By using a modification of the reference sample method as previously reported, the blood flow of each tissue sample was determined. Specific microsphere radiolabels were chosen to minimize peak radioactivity overlap with the tracers used for protein leak determinations.

**Myeloperoxidase Assay**

Tissue samples from ischemic regions were kept frozen at −70°C before assay. Myocardial myeloperoxidase activity was measured using a modification of the methods of Bradley et al. Myocardial samples (approximately 250 mg each) from the ischemic (myocardial blood flow <20 ml/100 g/min) zones were homogenized on ice after suspension in a 10× volume of 20 mM potassium phosphate buffer (pH 7.4). After centrifugation at 21,000 rpm for 30 minutes, the supernatant was discarded, the pellet resuspended in a 10× volume of 50 mM potassium phosphate with 0.5% hexadecyltrimethylammonium bromide, sonicated, frozen at −70°C, thawed, sonicated again, and then heated at 60°C for 2 hours. The suspension was then centrifuged at 9,300 rpm for 15 minutes. One hundred microliters of the supernatant was added to 2.9 ml of 0.0005% hydrogen peroxide in o-dianisidine/potassium phosphate buffer. The rate of change in absorbance at 460 nm was measured and compared with that of 3.0 ml of the hydrogen peroxide/o-dianisidine buffer solution and used to calculate the myeloperoxidase activity of the supernatant.

**In Vitro Epicardial Ring Studies**

The 3–4-cm segments of LAD and CX excised beyond the cannulation sites were immediately cleaned of adherent connective tissue and fat and prepared as described previously. After being cut transversely into 3-mm-wide rings, half of the rings were gently rotated over a tapered wooden stick to remove endothelium. The rings were suspended in
baths between a stationary wire and a wire attached to a Grass FT03 force-displacement transducer. The bathing medium was Earle's physiological salt solution (Sigma Chemical Co., St. Louis, Mo.) at 37°C, pH 7.4, continuously aerated with 95% O2-5% CO2. Rings were stretched to 5 g of passive force (found to give maximum contractions in previous experiments) and equilibrated for 1 hour. Outputs from the transducers were displayed on a multichannel oscillograph (model R-611, Beckman Instruments, Inc., Fullerton, Calif.).

Maximal constriction to 80 mM KCl was initially recorded in each ring. The baths were then repeatedly flushed with fresh medium to allow dilation to proceed. Rings were submaximally precontracted with the thromboxane mimetic U46619 (10 nM) to compare responses of the ischemic LAD and nonischemic CX to three endothelium-dependent vasodilators: acetylcholine (0.0001–10 μM), ADP (0.01–10 μM), and thrombin (0.006–0.6 units/ml). At each successive concentration, relaxations were allowed to plateau before the addition of the next higher concentration. Dilations were expressed as a percentage of the initial U46619 contraction. No endothelium-independent vasodilators were administered because previous work has demonstrated that ischemia and reperfusion does not alter responses to these agents by epicardial coronary rings.3,6

Statistics

Comparisons of PLI between filtered and unfiltered groups at each level of flow were made using unpaired t tests. Comparisons of PLI between flows within each group (filtered or unfiltered) were made using paired t tests.20 Comparison of percent relaxation of rings and E&C50 between LAD and CX were made using analysis of variance. Total concentration–response (dilation) response was compared by analysis of variance with replication.20 A value of p<0.05 was considered statistically significant. Results are expressed as mean±SEM.

Drugs

The drugs used included acetylcholine chloride, ADP, A23187, α-chloralose (all from Sigma), thiamylal sodium (Bio-Ceutic), thrombin (U.S. Biochemical), and U46619 (Upjohn Co., Kalamazoo, Mich.).

Results

Twenty-four dogs were studied, but 12 were excluded from analysis. Six unfiltered (control) and five leukocyte-filtered dogs died of ventricular fibrillation before completion of the study. One control dog was excluded because, despite ligation of the LAD, there was no regional ischemia by microsphere flow measurement. Thus, six dogs in each group were included in the data analysis.

Indexes of Leukocyte Depletion

In blood samples drawn from the extracorporeal tubing after 60 minutes of reperfusion, there was a 79% decrease in leukocyte counts per microliter distal to the Sepacell filters versus samples obtained proximal to the filters (proximal 2.217±0.469 versus distal 467±99, p<0.05). Samples drawn on each side of the control tubing segment in the nonfiltered animals showed no change in leukocyte count (proximal 3.029±0.818 versus distal 2.957±0.776, p=NS). As in previous studies,14,18 there was a decrease in platelet counts in the filtered dogs (43%), but values were in the normal range (>75,000/μl).

The leukocyte filters in the extracorporeal apparatus reduced the myeloperoxidase activity of ischemic myocardium to very low levels. A significant difference was present when ischemic zones were compared between filtered and nonfiltered animals (0.09±0.05 versus 0.88±0.43 units/g, p<0.05). Therefore, cardiac myeloperoxidase levels in ischemic zones obtained at 120 minutes of reperfusion were reduced by 90% by the leukocyte filters.

Microvascular Protein Leak

In each dog, myocardial tissue samples were stratified by blood flow measurements for assessment of PLI. Typical relations between regional myocardial blood flow and regional PLI in one filtered and one unfiltered dog are shown in Figure 2.

In the unfiltered animals, the PLI of the severely ischemic myocardium (<20 ml/100 g/min) was increased more than twofold (2.3±0.5 to 5.2±1.6, p<0.05) compared with the PLI of the nonischemic myocardium (>80 ml/100 g/min) of the same hearts (Figure 3). In the animals perfused with white blood cell–depleted blood, the PLI was consistently low regardless of the level of ischemia. Specifically, there was no difference in PLI in the filtered group between nonischemic and ischemic tissue (1.5±0.4 versus 1.9±0.5, p=NS). There were significant differences when filtered and nonfiltered myocardium were compared at both the severely ischemic (<20 ml/100 g/min) and moderately ischemic (20–50 ml/100 g/min) ranges (p<0.05). However, there was no difference when only mild ischemia (50–80 ml/100 g/min) was present. There was also no difference between the two groups in PLI in the nonischemic tissues (>80 ml/100 g/min). Therefore, the increase in PLI in severely ischemic or moderately ischemic cardiac tissue exposed to reperfusion, which is indicative of abnormal microvascular protein leak, was completely prevented by leukocyte filtering.

In Vitro Endothelium-Dependent Dilation

It is unlikely that cannulation of the coronary arteries altered reactivity, because both the ischemic-reperfused and “normal” vessel responses in unfiltered dogs resembled results in our previous studies.3,6 In control (unfiltered) dogs ischemic, reperfused LAD rings responded to ADP with a reduction in maximum percent relaxation (LAD 84±8% versus CX 101±2%, p<0.05), an increase in EC50 (LAD 0.85±0.31 versus CX 0.16±0.03 μM, p<0.05), and a
significant reduction in relaxation of the total concentration–response curve of the LAD versus CX ($p<0.01$) (Figure 4, top left panel). In the filtered dogs there were no significant differences between LAD and CX in maximum percent relaxation or $EC_{50}$ with ADP. However, there was a small but significant difference between LAD and CX when the total concentration–response curves were compared in the filtered dogs ($p<0.01$) (Figure 4, top right panel).

In response to acetylcholine, the LAD rings in control dogs exhibited a significant increase in $EC_{50}$ (LAD 99±44 versus CX 46±22 nM, $p<0.05$) and a significant reduction in relaxation by comparison of the total concentration–response curves of the LAD and CX ($p<0.01$), but there was not a significant decrease in maximum percent relaxation (Figure 4, middle left panel). In the filtered dogs there were no significant differences between LAD and CX in $EC_{50}$ or maximum percent relaxation, but there was a significant impairment of relaxation in the LAD by comparison of the total concentration–response curves ($p<0.01$) (Figure 4, middle right panel).

In response to thrombin (Figure 4, bottom panels) there was abnormal dilation in the LAD rings in maximum percent relaxation and $EC_{50}$ and by comparison of the total concentration–response curves in both the control and filtered groups. The magnitude of the differences between LAD and CX was similar in both groups of dogs.
Therefore, the effect of leukocyte filtering on dilation of epicardial rings in vitro was inconsistent and depended on which vasodilator was used. Depletion of leukocytes attenuated the impaired dilation in the LAD with ADP but lacked consistent effects with acetylcholine and had no discernible effect on the impaired dilation to thrombin.

Hemodynamics and Coronary Blood Flow

Hemodynamic data are summarized in Table 1. No differences were noted between filtered and nonfiltered animals except that heart rate was higher at baseline in the nonfiltered animals. There were no differences in heart rates during ischemia or reperfusion. Heart rate/blood pressure products and systolic blood pressures were also not different at any time point. Mean coronary blood flow during ischemia was 10.3±4.7 in the control and 23.4±6.4 ml/100 g/min in the filtered group in the LAD distribution of the anterior wall (p=NS). There were also no significant differences in flows in the CX distribution of the posterior and lateral walls between the control and filtered groups.

Discussion

Since the discovery that the reintroduction of blood to ischemic tissue can destroy or further damage still viable cells, the mechanism of reperfusion injury has been a subject of intense investigation. Both myocardial and coronary vascular dysfunction have been documented after ischemia and reperfusion, and white blood cells have been implicated as potentially toxic in this setting. In this study, we present evidence that leukocytes play a major role in the coronary vascular endothelial injury that occurs with cardiac ischemia and reperfusion.

Leukocytes can generate reactive oxygen metabolites that are capable of creating substantial cellular injury.\(^1\)\(^2\)\(^3\) Additionally, leukocytes produce other potent substances that may have deleterious effects (e.g., proteases, leukotrienes, and thromboxane A₂). Inhibiting leukocyte activity by various methods has decreased myocardial necrosis from ischemia and reperfusion\(^1\)\(^5\)\(^-\)\(^5\)\(^1\)\(^8\)\(^,\)\(^2\)\(^1\) and attenuated myocardial dysfunction in models of myocardial “stunning.”\(^2\)\(^4\) Although conflicting results have been reported concerning the latter,\(^2\)\(^5\) However, whether leukocytes cause the coronary vascular changes that occur with ischemia and reperfusion has not been clear.

Neutrophil attachment to vascular endothelial cells is one of the earliest events documented in models of ischemia and reperfusion.\(^2\)\(^6\) This precedes entrance of neutrophils into the myocardium by diapedesis through vessel walls. Vascular injury has been attributed to leukocyte-endothelium interactions in the lungs,\(^2\)\(^7\) skeletal muscle,\(^2\)\(^8\) and mesentery.\(^2\)\(^9\)\(^,\)\(^3\)\(^0\) In other regional circulations, these interactions appear to be less important. Conflicting results have been reported regarding the role of leukocytes in injury caused by ischemia and reperfusion in the kidney,\(^3\)\(^1\)\(^-\)\(^3\)\(^3\) while in the cerebral

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**TABLE 1. Summary of Hemodynamic Data at Baseline and During Ischemia and Reperfusion for Control and Filtered Dogs**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ischemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (beats/min)</td>
<td>SBP (mm Hg)</td>
<td>RPP</td>
</tr>
<tr>
<td>Controls</td>
<td>151±7</td>
<td>98±8</td>
<td>14,747±2,717</td>
</tr>
<tr>
<td>Filtered</td>
<td>128±9(<em>)</em></td>
<td>88±7</td>
<td>11,370±1,238</td>
</tr>
</tbody>
</table>

Values are mean±SEM. HR, heart rate; SBP, systolic blood pressure; RPP, rate · pressure product.

\(*p<0.05\) compared with control by unpaired \(t\) test.
FIGURE 4. Comparisons of endothelium-dependent dilation by ADP, acetylcholine (ACH), and thrombin after ischemia and reperfusion between the left anterior descending coronary artery (LAD, ischemic) and the proximal circumflex coronary artery (CX, nonischemic) in canine hearts perfused with blood either filtered or unfiltered (controls) for leukocytes. *p<0.05, #p=0.05 for LAD compared with CX at same drug concentration. Values are mean±SEM.
circulation, no-reflow does not appear to be mediated by leukocytes.34

Several recent studies have focused on possible participation of leukocytes in reperfusion injury to the coronary vasculature. Engler and coworkers35 have shown that the accumulation of radiolabeled granulocytes in ischemic endocardium in dogs is markedly enhanced by only 5 minutes of reperfusion and correlates with increases in coronary vascular resistance and tissue water content. By filtering leukocytes, they were able to decrease no-reflow assessed histologically and prevent an increase in edema.14 Reynolds and McDonagh36 have reported that while leukocytes are not requisite for the development of no-reflow in the rat heart in vitro, their presence exacerbates this effect and increases coronary vascular resistance. In a dog model, Kofsky and colleagues37 prevented the rise in coronary vascular resistance during reperfusion by filtering leukocytes from the coronary perfusate. Although mechanical obstruction of the microcirculation by leukocytes has been proposed as an explanation for the increased vascular resistance, there is evidence that leukocytes may increase the reactivity of the coronary vasculature. Ito et al32 have recently presented data that implicate leukocytes in vasoconstriction rather than as physical impediments to flow. These findings quite possibly reflect leukocyte-induced dysfunction of the vascular endothelium. Indeed, inhibition of neutrophil function by perfluorochemicals has been shown by Bajaj et al38 to prevent reperfusion damage to canine coronary endothelial cells studied by electron microscopy.

Our study helps delineate the role of the leukocyte in endothelial damage to the coronary circulation from ischemia and reperfusion. From our results it is apparent that injury to the endothelium of the coronary microvasculature, as reflected in loss of its barrier function, is totally due to the effects of leukocytes. Leakage of proteins into the myocardial interstitium, a process that occurs secondary to capillary and venule endothelial cell dysfunction, was prevented when leukocytes were filtered from the blood perfusing the major coronary arteries. Permeability in zones of the heart that were severely ischemic more than doubled with reperfusion by unfiltered blood but did not increase when leukocytes were removed by filtering.

As we and others have reported previously,2,3,9,40 ischemia and reperfusion alter responses of epicardial coronary rings to endothelium-dependent vasodilators. However, the degree of impairment generally has been quite small compared with the substantial abnormality in microvascular permeability.2,3,6,10 Indeed, Quillen et al31 recently reported marked changes in microvascular endothelium-dependent relaxation caused by ischemia and reperfusion in a preparation in which no significant alteration was observed in epicardial coronary artery endothelium-dependent relaxation. We observed little or no improvement resulting from leukocyte fil-

tering on the responses of ischemic-reperfused epicardial coronary artery rings to several endothelium-dependent vasodilators.

There are several possible explanations for the different results in the microvascular endothelial leak assessment versus the epicardial coronary ring vasodilation experiments. In view of the limited degree of the injury in the epicardial arteries, improvement may be more difficult to demonstrate there. Our in vitro vasodilator studies may not be sensitive enough to detect mild to moderate degrees of injury or protection in the epicardial arteries, whereas the PLI appears to be a very sensitive and specific technique for evaluating the microcirculation.1,10 Neutrophils may find the endothelium of the microvasculature a more favorable site for attachment and subsequent damage than the larger epicardial arteries. Histological studies of heart and other organs support the hypothesis that leukocytes are most likely to adhere to the endothelium in the arterioles, capillaries, and venules during ischemia and reperfusion.26,35 This may be a rheological phenomenon related to the size of leukocytes versus the surface area of the vessels or to the lower flow velocities in the smaller vessel beds. Differences in chemotactic or adhesion factors could promote differential leukocyte accumulation.29 It is also plausible that the microvasculature is more sensitive to leukocyte-induced injury than are the epicardial arteries. Alternatively, the epicardial endothelium may sustain injury by another mechanism, such as self-generated reactive oxygen species.22,29

In summary, ischemia and reperfusion of canine hearts cause endothelial injury in the microvasculature, which is mediated by leukocytes. The interaction of neutrophils with the microvascular endothelium could influence myocardial perfusion and cell survival after periods of ischemia followed by reperfusion.

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