Molecular Determinants of Reperfusion-Induced Leukocyte Adhesion and Vascular Protein Leakage


Abstract The adherence and emigration of leukocytes have been implicated as a rate-limiting step in the microvascular dysfunction associated with reperfusion of ischemic tissues. The objective of the present study was to define the relation between leukocyte–endothelial cell adhesion and albumin leakage in rat mesenteric venules exposed to ischemia and reperfusion (I/R). Leukocyte adherence and emigration as well as albumin extravasation were monitored in single postcapillary venules using intravital fluorescence microscopy. Ischemia (0, 10, 15, or 20 minutes) was induced by complete occlusion of the superior mesenteric artery, and all parameters were monitored for 30 minutes after reperfusion. The magnitude of the leukocyte adherence and emigration and albumin leakage elicited by I/R was positively correlated with the duration of ischemia. The albumin leakage response was also highly correlated with the number of adherent and emigrated leukocytes. Monoclonal antibodies against the adhesion glycoproteins CD18, CD11b, intercellular adhesion molecule-1 (ICAM-1) (at 10 and 30 minutes), and L-selectin (at 10 minutes), but not P- or E-selectin, reduced I/R-induced leukocyte adherence and emigration as well as albumin leakage. Platelet-leukocyte aggregates were formed in postischemic venules; the number of aggregates was reduced by antibodies against P-selectin, CD11b, CD18, and ICAM-1, but not E- or L-selectin. These results indicate that reperfusion-induced albumin leakage is tightly coupled to the adherence and emigration of leukocytes in postcapillary venules. This adhesion-dependent injury response is primarily mediated by CD11b/CD18 on activated neutrophils and ICAM-1 on vascular endothelium and appears to require L-selectin–dependent leukocyte rolling. (Circ Res. 1994;74:336-343.)

Key Words • vascular permeability • selectins • leukocyte integrins • platelet aggregation

Intravital microscopic studies of tissues exposed to ischemia and reperfusion (I/R) have revealed an acute inflammatory response that is characterized by enhanced protein efflux and an increased adherence and emigration of leukocytes in postcapillary venules. A role for leukocytes as mediators of the microvascular dysfunction elicited by I/R is supported by whole-organ studies that demonstrate (1) an accumulation of neutrophils in postischemic tissues, (2) attenuation of I/R-induced vascular injury in animals rendered neutropenic with neutrophil antiserum, and (3) a reduction in reperfusion-induced vascular leakage by monoclonal antibodies that prevent leukocyte adhesion. The latter observation has led to the recognition that leukocyte–endothelial cell adhesion may be a rate-limiting step in the pathogenesis of I/R-induced tissue injury. This, in turn, has heightened the level of interest in defining both leukocyte and endothelial cell adhesion glycoproteins that mediate I/R-induced leukocyte accumulation and in defining more precisely the association between leukocyte–endothelial cell adhesion and vascular dysfunction.

Although there are some published reports that examine the contribution of different adhesion glycoproteins to I/R-induced granulocyte accumulation (using tissue-associated myeloperoxidase activity as an index), most of the detailed information concerning this issue has been derived from in vitro models that allow for quantitation of neutrophil adhesion to endothelial cell monolayers exposed to anoxia (or hypoxia) followed by reoxygenation. These studies, however, have generated conflicting observations, with one study implicating both the leukocyte adhesion glycoprotein CD11/CD18 and intercellular adhesion molecule-1 (ICAM-1) in anoxia/reoxygenation (A/R)-induced neutrophil adhesion, whereas the results of another report support a role for endothelial cell–associated P- and E-selectins in mediating the adhesion. Although these studies have greatly extended our understanding of the contribution of different adhesion molecules to A/R-induced leukocyte–endothelial cell adhesion under static conditions, it remains unclear whether these findings can be extrapolated to postcapillary venules, wherein endothelial cells are exposed to significant shear forces after reperfusion. Other areas of uncertainty include the temporal and spatial relation between I/R-induced leukocyte adhesion/emigration and microvascular dysfunction and the contribution of endothelial cell adhesion molecules to reperfusion-induced albumin leakage in postcapillary venules. These issues are addressed in the present study using a model of rat
mesentery that allows for simultaneous measurement of leukocyte adherence and emigration, as well as albumin leakage, in discrete segments of postcapillary venules exposed to I/R. Monoclonal antibodies directed against specific components of the CD11/CD18 adhesion glycoprotein complex (CD11b and CD18) and L-selectin on leukocytes or endothelial cell adhesion molecules (ICAM-1, P-selectin, and E-selectin) were used to assess the relative contribution of these glycoproteins to the leukocyte–endothelial cell interactions and albumin leakage observed in postcapillary venules exposed to I/R.

Materials and Methods

Surgical Procedure

Male Sprague-Dawley rats (200 to 250 g) were maintained on a purified laboratory diet and fasted for 24 hours before each experiment. The animals were initially anesthetized with pentobarbital (65 mg/kg body weight); then a tracheotomy was performed to facilitate breathing during the experiment. The right carotid artery was cannulated, and systemic arterial pressure was measured with a Statham P23A pressure transducer (Gould, Oxnard, Calif) connected to the carotid artery cannula. Systemic blood pressure and heart rate were continuously recorded with a physiological recorder (Grass Instruments Co, Quincy, Mass). The left jugular vein and superior mesenteric artery were also cannulated for drug administration.

Intravital Microscopy

Rats were placed in a supine position on an adjustable Plexiglas microscope stage, and the mesentery was prepared for microscopic observation as described previously.16,17 Briefly, the mesentery was draped over a nonfluorescent coverslip that allowed for observation of a 2-cm² segment of tissue. The exposed bowel wall was covered with Saran Wrap (Dow Chemical Co, Indianapolis, Ind); then the mesentery was superfused with bicarbonate-buffered saline (BBS, 37°C, pH 7.4) that was bubbled with a mixture of 5% CO₂ and 90% N₂.

An inverted microscope (TMD-2S, Diaphot, Nikon, Tokyo, Japan) with a ×40 objective lens (Fluor, Nikon) was used to observe the mesenteric microcirculation. The mesentery was transilluminated with a 12-V 100-W direct current–stabilized light source. A video camera (VK-C150, Hitachi, Ibaragi, Japan) mounted on the microscope projected the image onto a color monitor (PVM-2030, Sony, Tokyo, Japan), and the images were recorded using a videocassette recorder (NV-8950, Panasonic, Tokyo, Japan). A video time-date generator (WJ810, Panasonic) projected the time, date, and stopwatch function onto the monitor.

Single unbranched venules with diameters ranging between 25 and 35 μm and length of >150 μm were selected for study. Venular diameter was measured either on-line or off-line using a video caliper (Microcirculation Research Institute, Texas A&M University, College Station, Tex). Red blood cell centerline velocity was measured in venules using an optical Doppler velocimeter (Microcirculation Research Institute, Texas A&M University). The velocimeter was calibrated against a rotating glass disk coated with red blood cells. Venular blood flow was calculated from the product of mean red blood cell velocity (V_{mean}=centerline velocity/1.6) and microvascular cross-sectional area, assuming cylindrical geometry. Wall shear rate (γ) was calculated based on the Newtonian definition: γ=8V_{mean}/diameter.

The number of adherent leukocytes was determined off-line during playback of videotape images. A leukocyte was considered to be adherent to venular endothelium if it remained stationary for a period of ≥30 seconds.14 Adherent cells were expressed as the number per 100-μm length of venule. The number of emigrated leukocytes was also determined off-line during playback of videotape images. Any interstitial leukocytes present in the mesentery at the onset of the experiment were subtracted from the total number of leukocytes that accumulated during the course of the experiment. Leukocyte emigration was expressed as the number per field of view surrounding the venule. Platelet-leukocyte aggregates that were visible within postcapillary venules were quantified and expressed as the number of aggregates crossing a fixed point within the venule over a 2-minute period.

To quantify albumin leakage across mesenteric venules, 50 mg/kg of fluorescein isothiocyanate-labeled bovine albumin (Sigma) was administered intravenously to the animals 15 minutes before each experiment.17 Fluorescence intensity (excitation wavelength, 420 to 490 nm; emission wavelength, 520 nm) was detected using a silicon-intensified target camera (C-2400-08, Hamamatsu Photonics, Shizuoka, Japan). The fluorescence intensity of fluorescein isothiocyanate/albumin within three segments of the venule under study (I) and in three contiguous areas of perivascular interstitium (I') area were measured at various times after administration of fluorescein isothiocyanate/albumin using a computer-assisted digital imaging processor (NIH Image 1.35 on a Macintosh computer). An index of vascular albumin leakage was determined from the ratio of I to I', at specific times during the reperfusion phase.

Experimental Protocols

After all parameters measured on-line were in a steady state, images from the mesenteric preparation were recorded on videotape for 10 minutes. Immediately thereafter, the superior mesenteric artery was ligated with a snare created from polyethylene tubing. The mesentery was made ischemic for 0 (sham), 10, 15, or 20 minutes. After the ischemic period, the ligation was gently removed. In some experiments, the animals were pretreated (15 minutes before control measurements) with a monoclonal antibody (MAb) directed against CD18 (CL26, 100 μg per rat),19 CD11b (1B6c, 1.5 mg/kg),20 ICAM-1 (1A29, 2 mg/kg),21,22 L-selectin (HRL3, 1 mg/kg),23 P-selectin (PB1.3, 2 mg/kg),24 E-selectin (CL3, 1.5 mg/kg),25 or a nonbinding antibody (2 mg/kg P6H6 or 1 mg/kg HRL4),24 and the same protocol was used for a 20-minute ischemic period. CL26 and 1A29 were provided by UpJohn Laboratories, Kalamazoo, Mich; ICAM-1, lB6c, and HRL3 and HRL4 were provided by Repligen, Cambridge, Mass; PB1.3 and P6H6 were provided by Cytel Corp, San Diego, Calif; and HRL3 and HRL4 were supplied by Dr M. Miyasaka. Preliminary experiments with inflammatory mediators were used to determine the minimal effective dose of each MAb. At the doses used, none of the MAbs caused leukopenia.

In another set of experiments, phallolidin 50 nmol/L (Sigma), an F-actin stabilizer,16,26 was continuously superfused onto the mesentery during the entire course of the observation period, and the same protocol was performed.

Statistics

The data were analyzed using standard statistical analysis, ie, one-way ANOVA and Scheffe’s (post hoc) test. All values are reported as mean±SEM from five or six rats (five rats per group were used in the initial characterization experiments), and statistical significance was set at P<.05.

Results

In untreated (control) rats, the red blood cell velocity and wall shear rate in mesenteric venules were 2.84±0.18 mm/s and 482±17 s⁻¹ under control conditions. During occlusion of the superior mesenteric artery (SMA), blood flow ceased within mesenteric venules. Ischemic periods up to 20 minutes in duration were associated with significant and sustained reperfusion; ie, red blood cell velocity (2.32±0.14 mm/s) and wall shear rate (388±15 s⁻¹) were restored toward
normal values after release of the SMA occlusion. Longer durations of ischemia (≥30 minutes) were not associated with a consistent reperfusion response, with flow rarely occurring to a significant extent after release of the SMA occlusion. Consequently, measurements of leukocyte–endothelial cell adhesion and albumin leakage were obtained only in mesenteric venules exposed to ≤20 minutes of ischemia.

Fig 1 illustrates the time course of the changes in the number of adherent and emigrated leukocytes and albumin leakage in mesenteric venules after 20 minutes of ischemia and 30 minutes of reperfusion. Significant increases in albumin leakage and adherent leukocytes were noted as early as 5 minutes after reperfusion, with further increases in both parameters noted over the remaining reperfusion period. The number of emigrated leukocytes was significantly elevated at 10 minutes after reperfusion and increased progressively thereafter. In animals subjected to 20 minutes of sham ischemia (manipulation of the SMA) and 30 minutes of “reperfusion,” leukocyte adherence was 3.2±0.6 per 100 μm, with 1.2±0.4 emigrated leukocytes per field and an albumin leakage index of 4.6±1.7%. Corresponding values obtained in mesenteric preparations exposed to 20 minutes of ischemia and 30 minutes of reperfusion were 19±1.7 per 100 μm, 9.2±1.0 per field, and 45.5±2.4%, respectively.

Fig 2 compares the increments in leukocyte adherence and emigration and vascular albumin leakage induced by different durations of ischemia followed by 30 minutes of reperfusion. All three parameters were increased as the duration of ischemia was lengthened, such that adherence, emigration, and permeability increased 6-, 7.6-, and 10-fold, respectively, after 20 minutes of ischemia.

Fig 3 illustrates the dependence of I/R-induced albumin leakage in single venules on the number of adherent (panel A) and emigrated (panel B) leukocytes. All values were derived from the 30-minute values presented in Fig 2. Albumin leakage was highly correlated with both leukocyte adherence ($r=0.84$, $P<0.05$) and leukocyte emigration ($r=0.93$, $P<0.05$). Albumin leakage was greater in regions of the venule that exhibited a high level of leukocyte adherence/emigration than in regions exhibiting little or no adherence/emigration.

Fig 4 summarizes the effects of MAb directed against different leukocyte (CD18, CD11b, and L-selectin) and endothelial cell (ICAM-1, P-selectin, and E-selectin) adhesion molecules on the adherence (panel A) and emigration (panel B) of leukocytes and albumin leakage (panel C) induced by 20 minutes of ischemia followed by reperfusion. MAb against CD18 and CD11b reduced leukocyte adherence by 75%, whereas the ICAM-1 MAb reduced adherence by 50% to 55%. However, no significant changes in leukocyte adherence were noted in animals receiving MAb directed against either P-selectin, E-selectin, or a nonbinding MAb (P6H6 or HRL4). The MAb directed against L-selectin significantly attenuated the leukocyte adherence at 10 minutes (46%) but not at 30 minutes (Fig 4A). A similar pattern of effectiveness in reducing leukocyte emigration (Fig 4B) was observed with the different MAb; i.e., MAb directed against CD18, CD11b, and ICAM-1 reduced the number of emigrated leukocytes by 65% to 82%, 77% to 79%, and 57% to 68%, whereas the nonbinding MAb and MAb against P- and E-selectin had no effect. The MAb against L-selectin attenuated the leukocyte emigration at 10 minutes (77%) but not at 30 minutes. Fig 4C illustrates that the large increase in albumin leakage induced by I/R was significantly attenuated by MAb directed against CD18 (58% to 71%), CD11b (56% to 70%), and ICAM-1 (38% to 55%) at both 10 and 30 minutes after reperfusion. The L-selectin-specific MAb attenuated the albumin leakage observed at 10 minutes (54%), but not at 30 minutes, after reperfusion. The P-selectin MAb significantly reduced albumin leakage (32%) at 30 minutes, but not 10 minutes, after reperfusion. The E-selectin and nonbinding MAb had no effect on I/R-induced albumin leakage.

Exposure of the rat mesentery to I/R was frequently associated with the appearance of large platelet-leukocyte aggregates that filled the venule lumen and rapidly coursed through the vessel with flowing blood. These aggregates have been previously described by other
investigators as “flying thrombi.” Although such aggregates were never observed during control conditions, 10.4 ± 0.9 aggregates per 5 minutes were observed in venules exposed to 20 minutes of ischemia and 30 minutes of reperfusion. Fig 5 summarizes the effects of the different MAbs on the I/R-induced formation of platelet-leukocyte aggregates. The most profound reduction (79%) in aggregate formation was observed in animals receiving the P-selectin MAAb. However, reductions in aggregate formation were also noted in animals treated with either CD18 (58%), CD11b (62%), or ICAM-1 (58%), but not with the E-selectin, L-selectin, or nonbinding MAbs.

Fig 6 illustrates the effects of phallolidin on the enhanced leukocyte adherence (panel A) and emigration (panel B) and albumin leakage (panel C) elicited by I/R. Phallolidin attenuated the I/R-induced leukocyte emigration and albumin leakage but did not affect the leukocyte adherence observed in postcapillary venules after I/R. Phallolidin did not significantly alter the rate of formation of leukocyte-platelet aggregates in postischemic venules.

The values for red blood cell velocity and wall shear rate in postcapillary venules at 30 minutes after a 20-minute ischemic insult were not significantly different between controls and animals receiving either a nonfunctional (nonbinding) or functional MAAb.

**Discussion**

One of the major objectives of the present study was to develop an experimental model that allows for simultaneous measurement of leukocyte adherence and emigration as well as albumin leakage in single postcapillary venules exposed to I/R. The results obtained with this model indicate that ischemia followed by reperfusion promotes leukocyte adherence and emigration and elicits an increased albumin efflux in mesenteric venules. The magnitude of these responses was related to the duration of the ischemic insult, with longer periods of ischemia eliciting larger increments in the adherence and emigration of leukocytes and albumin leakage. These findings suggest that longer durations of ischemia result in a greater production of the inflammatory agents that mediate I/R-induced leukocyte–endothelial cell adhesion and albumin leakage. It is possible that the leukocyte adhesion and albumin leakage responses elicited by I/R are mediated by different agents produced by the tissue. Alternatively, the vascular dysfunction may be a response that is secondary to the enhanced leukocyte adherence and/or emigration.

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**Fig 3.** Graph showing dependence of ischemia/reperfusion (I/R)-induced albumin leakage (permeability index) on the number of adherent (A) and emigrated (B) leukocytes within discrete regions of rat mesenteric venules. I/R 10, I/R 15, and I/R 20 denote the duration (in minutes) of ischemia, which is followed by 30 minutes of reperfusion.

**Fig 4.** Bar graphs showing the effects of different monoclonal antibodies directed against leukocyte (CD18, CD11b, and L-selectin) and endothelial cell (intercellular adhesion molecule-1 [ICAM-1], P-selectin, and E-selectin) adhesion molecules on the increased leukocyte adherence (A), leukocyte emigration (B), and albumin leakage (C) induced by 20 minutes of ischemia and 30 minutes of reperfusion. The effects were assessed at 10 (striped bars) and 30 (solid bars) minutes after reperfusion. I/R 20 indicates the untreated group subjected only to ischemia and reperfusion; NB, nonbinding monoclonal antibody. Data are presented for 10 and 30 minutes after reperfusion. *P < .05 vs corresponding control value; †P < .05 vs I/R 20 untreated group.
The possibility that I/R-induced vascular protein leakage in postcapillary venules is a secondary response to leukocyte adherence and/or emigration is supported by several observations made in our model system: (1) the changes in leukocyte adherence/emigration and albumin leakage were temporally related (Fig 1); (2) the magnitude of the albumin leakage induced by I/R is tightly coupled to the number of adherent (Fig 3A) and emigrated (Fig 3B) leukocytes; and (3) monoclonal antibodies that attenuated leukocyte adherence and emigration also reduced the albumin leakage response to I/R. Although these observations strongly implicate leukocyte-endothelial cell interactions in the loss of barrier function in postischemic venules, the relative contributions of leukocyte adherence and emigration to the leakage response remain unclear. Previous studies in our laboratory suggest that leukocyte emigration, rather than adherence per se, is an important determinant of leukocyte-mediated vascular protein leakage elicited by inflammatory mediators.28 Our new observations with phallolidin tend to support this view in postischemic mesenteric venules (Fig 6). Phallolidin, an F-actin stabilizer,26 significantly attenuated albumin leakage and leukocyte emigration in postischemic venules without affecting leukocyte adherence. This compound has previously been shown to greatly attenuate the emigration, but not the adherence, of leukocytes in mesenteric venules exposed to inflammatory stimuli such as N-formyl-methionyl-leucyl-phenylalanine, leukotriene B4, and platelet-activating factor.16 Although there is little evidence that phallolidin, at the dose used in the present study, impairs neutrophil function or endothelial cell viability,16 these considerations cannot be entirely dismissed.

Although there are several published reports that describe the influence of ischemia and reperfusion on leukocyte adherence in postcapillary venules,1,4,7 relatively little is known about the molecular determinants of this adhesion process. The leukocyte and endothelial cell surface glycoproteins that mediate the adhesive interactions induced by I/R have been characterized using endothelial cell monolayers exposed to A/R.13 A/R elicits an increased neutrophil adherence to endothelial cell monolayers that is inhibited by MAbs directed against either the α (CD11a and CD11b) or β (CD18) subunits of the leukocyte adhesion glycoprotein family CD11/CD18. The inhibitory effects of CD11a- and CD11b-specific MAbs were less than the effect of the CD18 MAb. An ICAM-1–specific MAb reduced the A/R-induced neutrophil adherence to an extent comparable to that observed with MAbs against either CD11a or CD11b. Immune neutralization of either E-selectin, L-selectin, or P-selectin had no effect on the A/R-induced neutrophil adherence in this static adhesion assay.13 Some in vivo studies have shown that MAbs directed against CD18 largely abolish the increased leukocyte adherence observed in cat mesenteric venules exposed to I/R.1,30 However, the contribution of other adhesion glycoproteins to I/R-induced leukocyte adherence has not been systematically assessed before the present study.

The results of the present study indicate that the increased leukocyte adherence and emigration in rat mesenteric venules elicited by I/R are primarily mediated by an interaction between CD11b/CD18 on leukocytes and ICAM-1 on endothelial cells. Administration of a CD18-specific MAb before the induction of ischemia virtually eliminated the leukocyte hyperadhesivity observed at 10 and 30 minutes after reperfusion. Identical results were obtained with a CD11b-specific MAb, suggesting that the CD11b/CD18 heterodimer accounts for nearly all of the adhesion observed in this model.

![Fig 5](http://circres.ahajournals.org/)

Fig 5. Bar graph showing the effects of different monoclonal antibodies directed against leukocyte (CD18, CD11b, and L-selectin) and endothelial cell (intercellular adhesion molecule-1 [ICAM-1], P-selectin, and E-selectin) adhesion molecules on platelet-leukocyte aggregation in rat mesenteric venules induced by 20 minutes of ischemia and 30 minutes of reperfusion. I/R 20 indicates the untreated group subjected only to ischemia and reperfusion; NB, nonbinding monoclonal antibody. Data are presented for 30 minutes after reperfusion. *P<.05 vs corresponding control value; †P<.05 vs I/R 20 untreated group.

![Fig 6](http://circres.ahajournals.org/)

Fig 6. Bar graphs showing effects of phallolidin on leukocyte adherence (A) and emigration (B) and albumin leakage (C) elicited by 20 minutes of ischemia and 30 minutes of reperfusion. I/R 20 indicates the untreated group subjected only to ischemia and reperfusion. Data are presented for 30 minutes after reperfusion. *P<.05 vs corresponding control value; †P<.05 vs I/R 20 untreated group.
system. The contribution of CD11a/CD18 to I/R-induced adhesion could not be assessed because of the inaccessibility of MAbs directed against this epitope on rat leukocytes. However, our findings predict that a CD11a-specific MAb should be ineffective in reducing I/R-induced leukocyte adherence and emigration in rat mesentery.

Three adhesion complexes expressed on the surface of endothelial cells appear to contribute to leukocyte–endothelial cell adhesion during an acute inflammatory response. ICAM-1 is constitutively expressed (at low levels) on the surface of endothelial cells, and it appears to serve as the ligand for both CD11a/CD18 and CD11b/CD18. E-selectin is not expressed on quiescent endothelial cells; however, it can be (cytokine activation) mobilized to the endothelial cell surface, where it interacts with counterreceptors on leukocytes to initiate adhesion. Both ICAM-1 and E-selectin require several hours for maximal surface expression after endothelial cell activation. P-selectin, on the other hand, is rapidly (<10 minutes) mobilized to the endothelial cell surface in response to certain inflammatory stimuli (histamine, thrombin, and H2O2). This lectinlike adhesion molecule interacts with distinct oligosaccharide moieties such as sialyl Lewis X on leukocytes, where it mediates a low-affinity binding that is manifested as leukocyte rolling.

Our results indicate that the only endothelial cell adhesion molecule that contributes to leukocyte adherence and emigration in our I/R model is ICAM-1. An ICAM-1 MAb significantly reduced both the adherence and emigration elicited by 20 minutes of ischemia and 30 minutes of reperfusion, whereas MAbs directed against P- or E-selectin had no effect. Given the short duration of the experiment, the lack of involvement of E-selectin is predictable, since it is not constitutively expressed on endothelial cells and its expression exhibits a slow time course (hours). ICAM-1 is constitutively expressed on endothelial cells; thus, it is likely that CD11b/CD18 on reperfusion-activated leukocytes binds to the ICAM-1 that was already expressed on venular endothelial cells before the induction of ischemia. These observations are consistent with the data obtained from monolayers of human umbilical vein endothelial cells exposed to A/R; enzyme immunoassay experiments indicate that ICAM-1, but not E-selectin, is basally expressed on human umbilical vein endothelial cell monolayers and that 30 minutes of anoxia followed by 30 minutes of reoxygenation does not increase the expression of either adhesion glycoprotein.

The lack of involvement of P-selectin in I/R-induced leukocyte adhesion is somewhat surprising in view of the rapidity of expression of the adhesion molecule in response to inflammatory stimuli, the role of P-selectin in mediating leukocyte rolling, and the protection against myocardial I/R injury afforded by the same P-selectin MAb used in the present study. The P-selectin MAb did not affect the enhanced leukocyte adherence observed either at 10 or 30 minutes after reperfusion, which suggests that significant P-selectin expression on endothelial cells was not elicited by I/R. Since rolling is a prerequisite for firm adhesion (adherence) of leukocytes in postcapillary venules, it is likely that another adhesion molecule, such as L-selectin, mediates the leukocyte rolling that precedes firm adherence in postischemic venules. MAbs directed against L-selectin have been shown to reduce the spontaneous rolling of leukocytes in rat mesenteric venules. In the present study, we provide evidence that also implicates L-selectin in the leukocyte adhesion and albumin leakage observed in the initial moments after reperfusion. We observed that a MAb directed against L-selectin significantly attenuates the enhanced leukocyte adherence/emigration and albumin leakage normally observed at 10 minutes after reperfusion. L-selectin is constitutively present and functional on nonactivated neutrophils but is rapidly shed in response to activation by a variety of stimuli. Furthermore, it has recently been reported that L-selectin function is required for CD11/CD18-mediated neutrophil adhesion in the rabbit mesenteric microvasculature. The sum of this evidence suggests that L-selectin–dependent leukocyte rolling is required for CD11b/CD18-dependent leukocyte adherence elicited by I/R. Our observations that a MAb directed against L-selectin reduces the early increases (at 10 minutes) in leukocyte adherence and emigration observed after I/R and that a P-selectin MAb has no effect suggest that L-selectin on leukocytes is not the principal ligand for P-selectin in postischemic mesenteric venules. A definitive explanation for the lack of effectiveness of the L-selectin MAb at 30 minutes of reperfusion is not available; however, it is possible that the MAb binds to L-selectin that is shed from activated neutrophils, thereby reducing the circulating MAb level below that necessary to neutralize leukocyte-bound L-selectin.

Although P-selectin did not affect I/R-induced leukocyte adhesion to venular endothelial cells, the lectinlike molecule does appear to play a major role in the formation of platelet-leukocyte aggregates in reperfused postcapillary venules. I/R and other experimental perturbations promote the formation of platelet-leukocyte aggregates, which emanate from the initial adhesion of leukocytes to endothelial cells in postcapillary venules. These aggregates progressively increase in size at sites of intense leukocyte adhesion, presumably as a consequence of homotypic leukocyte aggregation and leukocyte–platelet aggregation. Once the aggregate is sufficiently large, it is dislodged from the venular wall by the flowing column of blood and is swept away to enter systemic circulation. The results of the present study indicate that MAbs directed against either CD18, CD11b, or ICAM-1 reduce the appearance of leukocyte-platelet aggregates by 50%, whereas a P-selectin MAb reduces aggregate formation by 80%. Platelet-leukocyte aggregation is a P-selectin–dependent process in which activated platelets rapidly express P-selectin on the cell surface. The P-selectin MAb used in this study (PB1.3) blocks in vitro adherence of rat platelets to neutrophils by reacting with platelets but not neutrophils. Consequently, the effectiveness of the P-selectin MAb in reducing the appearance of platelet-leukocyte aggregates in postischemic venules can be attributed entirely to its reactivity with P-selectin expressed on platelets rather than endothelial cells. The contributions of CD11b/CD18 and ICAM-1 to platelet-leukocyte aggregation presumably stem from their role in mediating I/R-induced leukocyte–endothelial cell adherence, which initiates the entire aggregation process. The β2 integrin may also contribute to the homotypic aggrega-
tion of neutrophils20 associated with the formation of the platelet-containing aggregates. Although the pathophysiological significance of these aggregates remains undefined, it is conceivable that platelet-leukocyte aggregates make an important contribution to the distant organ injury that is observed after reperfusion of the ischemic bowel.42

Increased microvascular permeability is a well-described consequence of I/R.2-9,11 The results of the present study provide direct evidence that I/R increases the leakage of albumin in postcapillary venules. Our data with monoclonal antibodies directed against different leukocyte and endothelial cell adhesion molecules provide new insight concerning the role of leukocyte adhesion in I/R-induced microvascular dysfunction. As predicted from several whole-organ studies,9-11 CD18 immunoneutralization is very effective in attenuating the albumin leakage in mesenteric venules elicited by I/R. However, we have also demonstrated that the vascular leakage response is reduced by MAbs directed against either CD11b, L-selectin, or ICAM-1, but not E-selectin. The magnitude of the attenuation in albumin leakage observed with these MAbs closely paralleled the ability of the respective MAbs to reduce I/R-induced leukocyte adherence and emigration, suggesting that the protective effect of the MAbs is largely a result of their ability to reduce leukocyte-endothelial cell adhesion. An unexpected finding was the attenuation of I/R-induced albumin leakage by the P-selectin MAb, despite its lack of effect on leukocyte-endothelial cell adhesion. The protective effect of the P-selectin MAb may be related to its influence on platelet-leuko- cyte aggregation. Platelets are known to release substances that can increase vascular permeability.44 Consequently, by limiting the accumulation of platelets at sites of leukocyte-endothelial cell adhesion within postcapillary venules, the P-selectin MAb could reduce I/R-induced vascular albumin leakage without affecting the number of adherent and emigrated leukocytes. Nonetheless, the protective effect of the P-selectin MAb was small relative to those afforded by the MAbs that also attenuate leukocyte adherence and emigration, i.e., MAbs against CD11b, CD18, ICAM-1, and L-selectin. Thus, overall, our findings indicate that the microvascular dysfunction elicited by I/R is mediated by activated neutrophils that adhere to venular endothelium via interactions involving the leukocyte adhesion glycoprotein CD11b/CD18, which preferentially recognizes ICAM-1, and L-selectin.

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