Regulation of E-selectin expression in postischemic intestinal microvasculature


Regulation of E-selectin expression in postischemic intestinal microvasculature. Am J Physiol Gastrointest Liver Physiol 278: G878–G885, 2000.—Monolayers of cultured endothelial cells exposed to hypoxia-reoxygenation exhibit a transcription-dependent increase in E-selectin expression and E-selectin-dependent neutrophil-endothelial cell adhesion. The overall objectives of this study were 1) to determine whether ischemia-reperfusion (I/R) promotes upregulation of E-selectin in vivo; 2) if so, to define the mediators of this response; and 3) to assess the contribution of E-selectin to I/R-induced neutrophil recruitment. The dual-radiolabeled monoclonal antibody (MAb) technique was used to measure E-selectin expression in the intestinal vasculature. Ischemia was induced by complete occlusion (30–60 min) of the superior mesenteric artery followed by 3–24 h of reperfusion. Increasing durations of ischemia elicited progressively increasing (2- to 5-fold) levels of E-selectin expression, with the peak response noted after 45 min of ischemia and 5 h of reperfusion. Subsequent experiments revealed that I/R-induced increase in E-selectin expression (at 5 h) is significantly blunted in transgenic mice that overexpress Cu,Zn-superoxide dismutase or by treatment of wild-type mice with either a blocking antibody against tumor necrosis factor (TNF)-α or an inhibitor of nuclear factor-κB (NF-κB) activation (PS341). Administration of an E-selectin-specific MAb dramatically reduced I/R-induced recruitment of neutrophils in the intestine. These findings suggest that superoxide and TNF-α mediate gut I/R-induced E-selectin expression via an NF-κB-dependent mechanism; this upregulation of E-selectin contributes significantly to I/R-induced neutrophil recruitment.

superoxide dismutase; tumor necrosis factor; neutrophils; nuclear factor-kappaB; interferon-gamma

LEUKOCYTE-ENDOTHELIAL CELL adhesion has been implicated as a key initiating step in the pathogenesis of ischemia-reperfusion (I/R) injury. As a consequence, both in vitro and in vivo models of I/R have been used to identify and characterize the specific leukocyte and endothelial cell adhesion molecules (CAMs) that contribute to I/R-induced leukocyte recruitment and tissue injury (13, 23). Monolayers of cultured endothelial cells exposed to hypoxia-reoxygenation (H/R) have proven to be particularly useful for defining the time course and magnitude of endothelial CAM expression after H/R as well as the chemical and molecular processes that underlie these changes. For example, we (17) recently reported that cultured human umbilical vein endothelial cells (HUVECs) exposed to H/R become hyperadhesive to human neutrophils and exhibit a biphasic response, with peak adhesion occurring at 30 min (phase 1) and 240 min (phase 2) after reoxygenation. Blocking monoclonal antibodies (MAbs) were used to reveal a dominant role for P-selectin and intercellular adhesion molecule 1 (ICAM-1) in mediating the phase 1 neutrophil adhesion, whereas E-selectin had a dominant role in mediating the phase 2 adhesion response. The relative contribution of these CAMs to H/R-induced neutrophil adhesion was corroborated by the kinetics of surface expression of ICAM-1 and P- and E-selectin on posthypoxic HUVECs (17).

In addition to revealing an important contribution of E-selectin to the neutrophil-endothelial cell adhesion observed several hours after reoxygenation, in vitro models of I/R have provided evidence that implicates enhanced oxygen radical production as a key event that initiates the increased transcription of E-selectin. HUVECs exposed to H/R exhibit a profound increase in the ratio of oxidized (GSSG) to reduced (GSH) glutathione, and pharmacological agents that produce comparable changes in GSSG/GSH in normoxic HUVEC monolayers also elicit a biphasic increase in neutrophil adhesion and increased endothelial CAM expression similar to those observed in posthypoxic monolayers (20). The increased endothelial expression of E-selectin that accompanies the H/R- or drug-induced oxidant stress (increased GSSG-to-GSH ratio) is greatly attenuated by inhibitors of the nuclear transcription factor nuclear factor-κB (NF-κB) (17, 20). Hence, the results derived from posthypoxic HUVECs suggest that an oxidant stress results in the activation of NF-κB, which in turn stimulates the transcription-dependent expression of E-selectin that subsequently mediates the neutrophil adhesion observed 4 h after reoxygenation.

Although the in vitro models of I/R clearly implicate a role for E-selectin in the recruitment of neutrophils after reperfusion (reoxygenation), relatively little is known about the magnitude and kinetics of expression of this endothelial CAM in the postischemic microvascu-
MATERIALS AND METHODS

Monoclonal antibodies The MAbs used for the in vivo characterization of E-selectin expression were 10E9.6, a rat IgG2a directed against mouse E-selectin (4), and P-23, a nonbinding murine IgG1 directed against human P-selectin (24). 10E9.6 was purchased from Pharmingen (San Diego, CA) and P-23 was provided by Dr Donald C. Anderson (Pharmacia & Upjohn, Kalamazoo, MI). Although we showed previously (10) that MAb 10E9.6 is an excellent binding antibody to vascular endothelial cells in C57BL/6 mice, the ability of this MAb to functionally block neutrophil-endothelial cell interactions in vivo is somewhat controversial. Bosse and Vestweber (4) originally demonstrated that MAB 10E9.6 can completely block the adhesion of HL-60 promyelocytes to TNF-activated murine endothelial cells in culture and that it reduces thioglycolate-induced neutrophil migration into the peritoneum of BALB/c mice by 63%. Ramos et al. (29), on the other hand, reported that MAB 10E9.6 (and another E-selectin-specific MAb, 9A9) does not block leukocyte rolling in TNF-stimulated venules of BALB/c mice and that MAB 10E9.6 does not affect the adhesion of myeloid cells to E-selectin transfectants. To determine whether MAB 10E9.6 possesses the potential to inhibit neutrophil-endothelial cell interactions in our murine model of I/R, we examined the influence of this MAb on the adhesion of freshly isolated murine (C57BL/6) neutrophils to TNF-activated endothelial cells cultured from C57BL/6 mice. The mouse lung microvascular endothelial cells were stimulated for 4 h with TNF-α. Murine neutrophils isolated using a commercial Ficol-Hypaque gradient (NIM-2, Cardinal Associates, Santa Fe, NM) were exposed to the TNF-activated endothelial cell monolayers for 30 min at a neutrophil-to-endothelial cell ratio of 1:1. These experiments were performed either in the presence or in the absence of 40 μg/ml (which corresponds to an in vivo MAb dose of 2 mg/ml, assuming a plasma volume equivalent to 5% of total body mass) of either MAB 10E9.6 or P-23. Neutrophil adhesion was determined from the residual myeloperoxidase (MPO) activity (see Tissue MPO activity for method used to measure MPO activity) associated with the monolayers after washing. The experiments completed using this protocol revealed that 40 μg/ml of 10E9.6 inhibited neutrophil adhesion by 75–100% whereas the nonbinding MAB P-23 had no effect.

Radioiodination of monoclonal antibodies. The binding (10E9.6) and nonbinding (P-23) MAbs were labeled with 125I and 131I (NEN, Boston, MA), respectively, using the iodogen method. In brief, iodogen (Sigma T-0656) was dissolved in chloroform at a concentration of 0.5 mg/ml and 250 μl of this solution was placed in glass tubes and evaporated under nitrogen. A 250-μg sample of MAb was added to each iodogen-coated tube, and either 125I or 131I with a total activity of 250 μCi was added. The mixture was incubated on ice, with periodic stirring for 10 min. The total volume was brought to 2.5 ml by adding PBS (pH = 7.4). After radioiodination, the coupled MAb was separated from free 125I or 131I by gel filtration on a SephadeX PD-10 column (Pharmacia Biotech). The column was equilibrated with PBS containing 1% bovine serum albumin and eluted with the same buffer. Two 2.5-ml fractions were collected, the second of which contained the radioiodinated antibody. Absence of free 125I or 131I was ensured by extensive dialysis of the protein-containing fraction. Less than 1% of the activity of the protein fraction was recovered from the dialysis fluid. Labeled MAbs were stored at 4°C.

Animal procedures. All experimental protocols were applied to either C57BL/6 (wild type) mice (Jackson Laboratories, Bar Harbor, ME), interferon-γ [C57Bl/6-Ifg-KO]-deficient mice (Jackson Laboratories), or transgenic mice with the human gene for Cu/Zn-superoxide dismutase (SOD) [Tgf(SOD1)3Cje]. These Cu/Zn-SOD transgenic mice (11), with 1.5- to 3.0-fold increases in Cu/Zn-SOD activity in different tissues, were developed on a C57BL/6 background and bred by our animal care facility. Both hemizygous positive (SOD-Tg) and negative (SOD-nonTg) mice were used in this study. The C57BL/6-Ifg-KO mice were generated by replacing the one normal Ig-γ gene in mouse embryonic stem cells with a defective allele (6). A total of 111 mice were used in the study. The experimental procedures described were reviewed and approved by the Institutional Animal Care and Use Committee of Louisiana State University Medical Center.

Mice were anesthetized intramuscularly (im) with a mixture of ketamine (100 mg/kg) and xylazine (5 mg/kg). The left jugular vein and right carotid artery were cannulated with polyethylene tubing (PE-10, PE-50). An abdominal incision was made to expose the superior mesenteric artery (SMA), which was then occluded with a microoccluder (occlusion pressure = 85 g) to produce ischemia of the small intestine. The small intestine was exposed to ischemia for periods of 0 (sham control), 30, 45, or 60 min, followed by 3–24 h of reperfusion. Before the abdominal incision was closed, 2 ml of saline was placed in the peritoneal cavity. Mice subjected to 3 h of reperfusion were permitted to recover from the anesthesia before they were reanesthetized (im) with a mixture of ketamine (150 mg/kg) and xylazine (7.5 mg/kg). The sham group was exposed to an identical surgical procedure, with the exception that the SMA was exposed but not occluded.

E-selectin expression. A mixture of 10 μg of 125I-labeled E-selectin MAb (10E6) and a dose 0.5–5.0 μg of 131I-labeled nonbinding MAb (P-23) was injected through the jugular vein catheter. A blood sample was obtained through the carotid artery catheter 5 min after injection of the MAb mixture. The animals were then heparinized (30 units heparin sodium) and rapidly exsanguinated by perfusion of bicarbonate-buffered saline (BBS) through the jugular vein catheter with simultaneous blood withdrawal through the carotid artery catheter. This was followed by perfusion of 10 ml of BBS through the carotid artery catheter after the inferior vena cava was severed at the thoracic level. The small intestine (from ligament of Treitz to ileocecal junction), liver, and lung were harvested and weighed.

The method for calculating E-selectin expression has been described previously (8, 26). In brief, the 125I (binding MAb) and 131I (nonbinding MAb) activities in different tissues and in 50-μl samples of cell-free plasma were counted in a 14800 Wizard 3 gamma-counter (Wallac, Turku, Finland) with automatic correction for background activity and spillover. A
2-µl aliquot of the radiolabeled MAb mixture was assayed to determine total injected activity of each labeled MAb. The radioactivities remaining in the tube used to mix the MAbs and the syringe used to inject the mixture were subtracted from the total injected activity. The accumulated activity of each MAb in an organ was expressed as the percentage of the injected activity per gram of tissue. E-selectin expression was calculated by subtracting the accumulated activity per gram of tissue of the nonbinding MAbs $^{[32P]}$-P-23 from the activity of the binding anti-E-selectin MAb $^{[125I]}$-10E9.6. This value, expressed as percent injected dose per gram of tissue, was converted to nanograms of MAb per gram of tissue by multiplying the above value by the total injected binding activity of each MAb. Previous studies have shown that MAbs retain their functional activity after radiiodination, as evidenced by a similar effectiveness of labeled and nonlabeled MAbs to block white blood cell adherence in rat mesenteric venules (27). In addition, we have shown (10) that constitutive and endotoxin-induced expression of E-selectin is not detectable in the small intestine and other organs of E-selectin-deficient mice, unlike their wild-type counterparts.

Tissue MPO activity. Samples of small intestine were obtained either in the control period or after reperfusion, rinsed with cold PBS, blotted dry and immediately frozen with liquid nitrogen. The samples were stored at −80°C until being thawed for MPO activity determination using methods previously described (15). In brief, the tissues were homogenized in 20 mM phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 20 min at 4°C. The pellet was rehomogenized with an equivalent volume of 50 mM phosphate buffer (pH 6.0) containing 10 mM EDTA and 0.5% hexadecyltrimethylammonium bromide. MPO (an index of neutrophil infiltration into tissues) was determined by measuring the H$_2$O$_2$-dependent oxidation of 3,3′,5,5′-tetramethylbenzidine and expressed as units per gram of wet weight.

Electrophoretic mobility shift assay. Changes in intestinal DNA binding activity of NF-κB activity were determined using electrophoretic mobility shift assays (EMSAs). Nuclear extracts were prepared from isolated nuclei as previously described (35). Double-stranded DNA nucleotides containing the consensus κB motif (underlined), 5′-AGC- TTAGCTCCCTCA-G3′ and 5′-GATCCGGGAAAGTCCCTCTA-3′ were labeled with $^{[32P]}$dATP in the presence of the Klenow fragment of DNA polymerase I. The assay was performed in 15 µl containing 10 µg of nuclear extract protein, binding buffer (10 mM Tris pH 7.5, 20 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 12% glycerol, 1 µg poly dl-dC, and 1 µg denatured salmon testis DNA), and 1 × 10$^5$ counts/min $^{32P}$-labeled DNA, as described by Read et al. (30). The mixture was incubated at room temperature for 10 min and analyzed by electrophoresis on a 5% nondenaturing polyacrylamide gel. After electrophoresis, gels were dried and visualized by a PhosphorImager and quantitated with ImageQuant software (Molecular Dynamics, Sunnyvale, CA). For supershift analysis, nuclear extracts were incubated with 1 µl of antiserum against the NF-κB subunit of p50, p65, or c-Rel at 4°C for 1 h before the addition of binding buffer and labeled DNA. Antibodies for p50 and c-Rel were obtained from Santa Cruz Biotech (Santa Cruz, CA), and anti-p65 was purchased from Rockland Chemicals (Gilbertsville, PA).

Experimental protocols. To evaluate the magnitude and kinetics of I/R-induced E-selectin expression, the small intestine was exposed to variable durations of both ischemia (0–60 min) and reperfusion (3–24 h) as described above. On the basis of these experiments, optimal periods of ischemia (45 min) and reperfusion (5 h) were chosen for the subsequent mechanistic studies. Hence, E-selectin expression was measured in wild-type, 1f$^g$-KO, SOD-Tg, and SOD-nonTg (genotype-negative littermates of Cu,Zn-SOD transgenic mice) mice exposed to 45 min of intestinal ischemia followed by 5 h of reperfusion. To define the contributions of TNF-α to the I/R-induced expression of E-selectin, wild-type mice (n = 5) received a blocking antibody against murine TNF-α (TN3; 20 mg/kg iv immediately before ischemia) (7). The role of NF-κB was assessed by treatment with either a proteasome inhibitor (PS341; 0.3 mg/kg iv, 1 h before ischemia; n = 5) or the antioxidant pyrrolidine dithiocarbamate (PDTC; 250 mg/kg ip, 1 h before ischemia; n = 5) (5). In those studies designed to address the relative contribution of E- and P-selectin to the neutrophil recruitment (MPO) elicited by intestinal I/R, wild-type mice received an intravenous dose (2 mg/kg) of a blocking MAb directed against either E-selectin (10E9.6) or P-selectin (RB40.34) or a combination of the two MAbs. The MAb(s) was administered 5 min before the induction of ischemia, and samples for MPO determination were obtained at 5 h after reperfusion.

Statistical evaluation. All values are expressed as means ± SE. Data were compared using an ANOVA with Fisher's protected least significant difference post hoc test. Statistical significance was set at P < 0.05.
a TNF-α-blocking MAb significantly (43%) attenuated I/R-induced E-selectin expression. These observations suggest that TNF-α, but not interferon, contributes to the increased E-selectin expression elicited by intestinal I/R.

Role of NF-κB. In control intestine, NF-κB activity was very low (Fig. 4). Ischemia (45 min) without reperfusion did not alter NF-κB activity; however, within 30 min after reperfusion of the ischemic intesti-
assays showed that anti-p50 antibodies produced a supershift band that corresponded with the disappearance of the original protein-DNA complex, indicating that p50 subunits, either as homodimers or heterodimers with other members of the Rel proteins, were activated. Both anti-p65 and anti-c-Rel antibodies failed to produce a supershift band (Fig. 4), suggesting that p65 and c-Rel subunits are not part of the activation complex.

Figure 5 illustrates how two agents that have been shown to interfere with H/R-induced NF-κB activation in HUVECs influence the E-selectin responses to I/R. The proteasome inhibitor MG341 attenuated I/R-induced E-selectin expression by 36%, whereas the antioxidant PDTC did not alter the E-selectin response to I/R. To assess the contribution of selectins to the recruitment of granulocytes in postischemic mouse intestine, MPO activity was measured in intestinal samples obtained at 5 hr of reperfusion in wild-type mice not receiving a MAb against E-selectin or P-selectin or a combination of the two MAbs. In wild-type mice not receiving a MAb against E-selectin, a fivefold increase in MPO was elicited by I/R (Fig. 6). Although a trend for reduced neutrophil accumulation was noted in mice treated with the proteasome inhibitor MG341, this did not reach statistical significance.
with the P-selectin MAb, this did not achieve statistical significance. However, the E-selectin MAb reduced the I/R-induced MPO value by 60%. Treatment with a combination of the two MAbs tended to lower the MPO response below that observed for E-selectin immunoneutralization alone; however, this did not achieve statistical significance. These findings indicate that E-selectin is a major molecular determinant of the granulocyte recruitment that is observed at 5 h after reperfusion of the ischemic intestine.

**DISCUSSION**

A major objective of this study was to determine the kinetics and magnitude of E-selectin expression in the intestinal microvasculature after I/R. Although H/R-induced upregulation of E-selectin on HUVECs has been extensively characterized (17, 25), there is relatively little quantitative information concerning how I/R affects the expression of this endothelial CAM in vivo. Several lines of evidence in the literature support the possibility that I/R elicits a significant increase in the expression of E-selectin in different regional vascular beds subjected to an ischemic insult. 1) Plasma levels of circulating soluble E-selectin, which is shed from the surface of activated endothelium, are elevated after myocardial I/R (28). 2) E-selectin mRNA levels are increased in postischemic tissues (33). 3) Immunohistochemical detection of E-selectin reveals an increased expression that is confined to endothelial cells lining postcapillary venules (34, 36). Although these findings suggest that E-selectin biosynthesis and its expression on the surface of endothelial cells are likely increased after I/R, the precision of these approaches does not allow for meaningful quantitative estimates of the changes in E-selectin expression that occur in intact vessels. In the present study, the dual-radiolabeled MAb technique (19, 27) was used to quantify the expression of E-selectin in the postischemic intestinal vasculature. This method has been shown to provide accurate and reproducible estimates of selectin expression in different regional vascular beds (2, 8, 10).

Our estimates of E-selectin expression using the dual-radiolabeled MAb method indicate that I/R elicits an increased expression that is dependent on the duration of both the ischemic insult and the reperfusion period. For a given period of reperfusion (5 h), the magnitude of the E-selectin upregulation was significantly greater after a 45-min ischemic insult compared with a 30-min insult. However, increasing the ischemic duration to 60 min did not yield a larger response, suggesting that the stimulus for upregulation of this endothelial CAM in murine small intestine is maximal at 45 min of ischemia. Our effort to define the kinetics of E-selectin expression after I/R revealed that peak expression is achieved at 5 h, with a return to basal values at 24 h. This time course of E-selectin expression in the postischemic intestine is very similar to the kinetics of E-selectin expression recently described for HUVECs exposed to I/R (17). Furthermore, the time of peak I/R-induced E-selectin expression in mouse intestine is identical to that previously reported (using the dual-radiolabeled MAb method) for P-selectin in postischemic mouse intestine (8). However, in contrast to E-selectin, there is an early (10–30 min), significant increase in P-selectin expression after I/R.

The second major objective of this study was to define the contributions of superoxide, NF-κB, and certain cytokines (TNF-α, interferon-γ) to I/R-induced E-selectin expression. Studies on HUVEC monolayers exposed to H/R suggest that the increased E-selectin expression elicited by I/R is linked to oxidant-mediated activation of the nuclear transcription factor NF-κB (17). Using transgenic mice that overexpress Cu,Zn-SOD (11), we have provided evidence that the enhanced superoxide production that is associated with reperfusion of the ischemic intestine (22) is an important signal for the increased biosynthesis and expression of E-selectin in the postischemic intestinal microvasculature. E-selectin expression in the gut of SOD-Tg mice was 55% of the value measured in wild-type mice. We previously reported (8) that the rapid (10–30 min) I/R-induced upregulation of P-selectin does not differ between SOD-Tg and wild-type mice. Hence, our findings, coupled with previously published observations, suggest that superoxide (or a superoxide-derived oxidant) is a more potent stimulus for transcription-dependent upregulation of E-selectin than for mobilization of preformed P-selectin to the endothelial cell surface.

A variety of cytokines, including TNF-α and interferon-γ, are released from postischemic tissues (16, 32). Many of these cytokines elicit the translocation of NF-κB into the nucleus, where it binds to the promoter region of genes for inflammatory molecules such as E-selectin (30, 31). In the present study, we assessed the contribution of TNF-α and interferon-γ to I/R-induced E-selectin expression in the intestinal vasculature. Administration of a TNF-α-blocking MAb, which was previously shown to be effective in attenuating endotoxin-induced E-selectin expression in murine intestine (9), reduced I/R-induced E-selectin expression by ~45%, suggesting that this cytokine is another important stimulus for E-selectin biosynthesis and expression in postischemic intestine. The comparable E-selectin responses of wild-type and Ifg-KO mice to I/R suggests that interferon-γ does not contribute significantly to the E-selectin response.

Both oxidants and TNF-α are known to promote the biosynthesis of E-selectin in HUVECs by promoting the nuclear translocation of an active, binding heterodimeric form (p50/p65) of NF-κB (30, 31). HUVECs exposed to H/R exhibit nuclear translocation of p50/p65 with a corresponding increase in E-selectin expression (17). Furthermore, experimental strategies directed toward interfering with the activation and/or upregulation of p50/p65, such as treatment with antioxidants (PDT), proteasome inhibitors (PS341) or ds-oligonucleotides containing the NF-κB cognate sequence, effectively attenuate the increased E-selectin expression observed on HUVECs exposed to H/R (17) or TNF-α (18). In the present study, we have demonstrated significant nuclear translocation of NF-κB...
within 1 h after reperfusion of ischemic murine intestine. However, unlike posthypoxic HUVECs, which exhibit nuclear translation of p50/p65, the dominant dimeric form of NF-κB that was detected in postischemic intestine is p50/p50. The contribution of the p50/p50 homodimer to activation of the E-selectin gene remains unclear; the available evidence favors a role for p50/p50 in this response (30, 31). It should be noted, however, that our inability to detect p50/p50 in whole homogenates of postischemic intestine does not exclude the possibility that this heterodimer is activated and upregulated in vascular endothelial cells, which comprise >5% of total intestinal mass.

To further address the possible involvement NF-κB in I/R-induced E-selectin expression, we treated some mice with agents that have previously been shown to interfere with NF-κB-mediated E-selectin expression on posthypoxic HUVECs. Ichikawa et al. (17) reported that HUVEC monolayers pretreated with either a proteasome inhibitor (MG132) or an antioxidant (PDTC) significantly attenuated H/R-induced E-selectin expression. In the present study, we observed that the proteasome inhibitor PS341 reduced I/R-induced E-selectin expression in mouse intestine by ~36%, which compares with the 58% reduction in E-selectin expression observed in posthypoxic HUVECs (17). These findings with the proteasome inhibitor suggest that NF-κB may well provide a linkage between superoxide and TNF-α and the increased E-selectin expression induced by I/R. However, in contrast to the previously published in vitro data, the dithiocarbamate PDTC was ineffective in blunting the upregulation of E-selectin observed in our in vivo model of I/R. A definitive explanation for the ineffectiveness of PDTC in vivo is not readily available, but it may reflect a limited bioavailability of the compound to vascular endothelial cells in vivo or the fact that PDTC can act as a prooxidant because of its ability to chelate and transport certain transition metals (e.g., copper) into cells (14). Furthermore, PDTC has been shown to inactivate intracellular antioxidant enzymes such as SOD and glutathione peroxidase, thereby facilitating the intracellular accumulation of reactive oxygen species (14).

Another major objective of this study was to assess the importance of E-selectin in mediating I/R-induced neutrophil recruitment. Although there are several published reports that implicate P-selectin as a mediator of I/R-induced leukocyte recruitment (13, 26), there are relatively few data that support a role for E-selectin in the same recruitment process (1). The latter situation likely results from two factors: 1) a focus of most I/R studies on the inflammatory events that occur within the first hour after reperfusion, and 2) the use of anti-human MAbs with limited binding affinity to E-selectin expressed in rodent microvessels (21). In the present study, we determined whether an anti-murine E-selectin MAb that blocks the adhesion of murine neutrophils to monolayers of TNF-activated murine microvascular endothelial cells alters the accumulation of neutrophils (MPO) observed in murine small intestine at 5 h after reperfusion, i.e., at the time of peak E-selectin expression. We found that immunoneutralization of E-selectin reduced I/R-induced neutrophil accumulation by 60%, whereas administration of a blocking MAb to murine P-selectin did not significantly alter this response. These observations indicate that the time-dependent increase in E-selectin expression observed in postischemic intestine is a functionally significant response that accounts for much of the I/R-induced neutrophil accumulation at 5 h after reperfusion. However, our findings do not necessarily implicate E-selectin as the principal rolling receptor in postischemic intestinal microvasculature because there is published evidence that suggests that E-selectin serves a function, other than rolling, that appears to be important for neutrophil recruitment to inflammatory sites in mice (29).

In conclusion, this study demonstrates that intestinal I/R is accompanied by an increased E-selectin expression that is dependent on both the duration of the ischemic insult and period of reperfusion. Superoxide and TNF-α contribute to the I/R-induced E-selectin upregulation, in part because of the activation of NF-κB. The increased E-selectin expression accounts for most of the neutrophil accumulation that occurs several hours after reperfusion.

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