Genetic Disruption of Poly (ADP-Ribose) Synthetase Inhibits the Expression of P-Selectin and Intercellular Adhesion Molecule-1 in Myocardial Ischemia/Reperfusion Injury

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Abstract—The nuclear enzyme poly (ADP-ribose) synthetase (PARS) has been shown to play an important role in the pathogenesis of ischemia/reperfusion injury and circulatory shock. The aim of this study was to investigate whether PARS activity may modulate endothelial-neutrophil interaction. We present evidence that genetic disruption of PARS provides protection against myocardial ischemia and reperfusion injury by inhibiting the expression of P-selectin and intercellular adhesion molecule-1 (ICAM-1) and, consequently, by inhibiting the recruitment of neutrophils into the jeopardized tissue. Furthermore, using in vitro studies, we demonstrate that in fibroblasts lacking a functional gene for PARS, cytokine-stimulated expression of ICAM-1 is significantly reduced compared with fibroblasts from animals with a normal genotype. Similarly, in cultured human endothelial cells, oxidative- or cytokine-dependent expression of P-selectin and ICAM-1 is reduced by pharmacological inhibition of PARS by 3-aminobenzamide. These findings provide the first direct evidence that PARS activation participates in neutrophil-mediated myocardial damage by regulating the expression of P-selectin and ICAM-1 in ischemic and reperfused myocardium, and they also provide the basis for a novel therapeutic approach for the treatment of reperfusion injury. (Circ Res. 1998;83:85-94.)

Key Words: nitric oxide ■ peroxynitrite ■ cell adhesion molecule ■ neutrophil ■ 3-aminobenzamide

Endothelial dysfunction is an important early-recurring phenomenon in virtually all forms of ischemia and reperfusion injury. The dysfunction appears to be triggered by the endothelial generation of a large burst of oxidant molecules and amplified by the accumulation of neutrophils into injured tissue.1,2 Neutrophil recruitment into the reperfused tissue, which involves leukocyte rolling along the endothelium, leukocyte activation, and, finally, adhesion to the endothelium and transmigration into the tissue, is importantly regulated by adhesion molecules on the surface of activated endothelial cells.3,4 Leukocyte rolling is promoted by the selectin family of adhesion glycoproteins.5-8 P-Selectin, located within Weibel-Palade bodies in the vascular endothelium and platelets, is rapidly translocated to the cell surface after hypoxia/reoxygenation stimulus.5-10 ICAM-1 on endothelial cells is then responsible for firm adherence of neutrophils by interaction with the leukocyte CD11/CD18 receptors.5,11-14 The pathogenetic relevance of adhesion molecule expression in ischemia and reperfusion injury has been confirmed by using monoclonal antibodies and genetically engineered animals lacking adhesion receptors in myocardial, splanchic, cerebral, and liver reperfusion damage.9,13-15 However, the mechanism of adhesion molecule expression during reperfusion is incompletely understood.

A candidate pathway of oxidant-induced injury involves the nuclear enzyme PARS. Once activated, in response to single-strand DNA breaks, PARS initiates an energy-consuming inefficient repair cycle by transferring ADP ribose units to nuclear proteins, which rapidly depletes the intracellular NAD1 and ATP energetic pools and thus slows the rate of glycolysis and mitochondrial respiration, leading to cellular dysfunction and death.16,17 In vitro studies have demonstrated that such a cellular suicide mechanism is responsible for cellular injury in response to oxygen-derived free radicals, NO, and peroxynitrite (a cytotoxic oxidant produced by NO and superoxide).18-23 Recent studies established that activation of PARS plays a crucial role in mediating vascular and energetic failure in shock and reperfusion injury.21-25 Notably, in a rat model of myocardial ischemia and reperfusion, we have shown that 3-aminobenzamide treatment reduces infarct size and neutrophil accumulation into myocardial tissue.23 Eliasson et al26 have demonstrated that neural damage following vascular stroke is diminished in mice lacking PARS.26 Genetic disruption of PARS also prevents neutrophil tissue infiltration and organ injury during local or systemic inflammation.27

The present report extends these previous findings and demonstrates that mice lacking a functional gene for PARS exhibit reduced endothelial expression of P-selectin and ICAM-1 and diminished neutrophil infiltration into the reperfused myocardium. Furthermore, we provide evidence that PARS regulates the expression of these adhesion molecules.
in fibroblasts and endothelial cells in vitro. The present data demonstrate a novel mechanism whereby PARS inhibition exerts beneficial effects in reperfusion.

Materials and Methods

Myocardial Ischemia and Reperfusion
The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85-23, revised 1985) and with the approval of the Institutional Animal Care and Use Committee. PARS+/− mice and littermate wild-type control mice (PARS−/−) (129/Sv×C57BL6, 20 to 22 g) were kindly provided by Dr Z.Q. Wang of the Institute of Molecular Pathology, Vienna, Austria. Animals were anesthetized with thiopentone sodium (4 mg/mL, 10 μL/g body wt of mouse). The animals were placed in a supine position with their paws taped to the operating table. After a midline cervical skin incision, the trachea was cannulated with a PE-90 catheter, and paws taped to the operating table. After a midline cervical skin incision, the trachea was cannulated with a PE-90 catheter, and a dissection microscope. After occlusion for a 60-minute period, hearts were rapidly removed for quantification of cardiac cellular damage by using a commercial kit (Sigma Chemical Co).

Immunohistochemical Staining for Nitrotyrosine
Tyrosine nitration was detected in cardiac sections by immunohistochemistry. Frozen sections (5 μm thick) were fixed in 4% paraformaldehyde and incubated for 2 hours with a blocking 0.1 mol/L phosphate-buffered saline containing 0.1% Triton X-100 and 2% normal goat serum) in order to minimize nonspecific adsorption. Sections were then incubated overnight with 1:500 dilution of primary anti-nitrotyrosine antibody or with control solutions. Controls included buffer alone or nonspecific purified rabbit IgG. Specific labeling was detected by incubation for 30 minutes with a biotin-conjugated goat anti-rabbit IgG and amplified with avidin-biotin peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories) after quenching endogenous peroxidase with 3% H2O2 in 100% methanol for 15 minutes. Diaminobenzidine was used as a chromogen. To quantify the degree of nitrotyrosine staining, a grading system of 0 to 4 was used: 0, no staining; 1 to 3, increasing degrees of intermediate staining; and 4, extensive staining.

Histopathological Analysis and Damage Score
Tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin for histological evaluation of tissue damage. In order to have a quantitative estimation of cardiac damage, sections (n=6 for each animal) were scored by 2 independent observers blinded to the experimental protocol. The following morphological criteria were considered: score 0, no damage; score 1 (mild), interstitial edema and focal necrosis; score 2 (moderate), diffuse myocardial cell swelling and necrosis; score 3 (severe), necrosis with the presence of contraction bands and neutrophil infiltrate; and score 4 (highly severe), widespread necrosis with the presence of contraction bands, neutrophil infiltrate, and hemorrhage.

Myeloperoxidase Activity
Myeloperoxidase activity was determined as an index of neutrophil accumulation, as previously described. Cardiac tissues, collected 60 minutes after reperfusion, were homogenized in a solution containing 0.5% hexadecyltrimethylammonium bromide dissolved in 10 mmol/L potassium phosphate buffer (pH 7) and centrifuged for 30 minutes at 20 000g at 4°C. An aliquot of the supernatant was allowed to react with a solution of tetramethylbenzidine (1.6 mmol/L) and 0.1 mmol/L H2O2. The rate of change in absorbance was measured by spectrophotometry at 650 nm. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 μmol of hydrogen peroxide per minute at 37°C and expressed in milliunits per milligram protein.

Serum Creatine Phosphokinase Activity
Serum levels of creatine phosphokinase were evaluated as an index of cardiac cellular damage by using a commercial kit (Sigma Chemical Co).

Figure 1. Injury of myocytes after coronary occlusion and reperfusion is reduced in PARS−/− mice. Myocardial injury was induced in PARS−/− mice and their littermate PARS+/− control mice by 1-hour occlusion of the LAD, followed by 1-hour reperfusion (LAD-IR). Serum creatine phosphokinase, an enzyme present in viable myocytes but lost in the blood stream during myocardial injury, was measured as an index of myocyte cytotoxicity. Serum levels of creatine phosphokinase were significantly enhanced in PARS+/− mice subjected to LAD-IR compared with sham-operated PARS+/− mice (P<0.001). In contrast, the increase of serum levels of creatine phosphokinase was significantly reduced in PARS−/− mice subjected to LAD-IR compared with PARS+/− mice subjected to LAD-IR (#P<0.001). Each data point is the mean±SEM of 6 animals for each group.
Immunohistochemical Staining for P-Selectin and ICAM-1

P-Selectin and ICAM-1 expression was evaluated in cardiac sections by immunohistochemistry. Frozen sections (5 μm thick) were fixed in 4% paraformaldehyde and incubated in 2% normal rat serum (for P-selectin evaluation) or hamster serum (for ICAM-1) for 2 hours in order to minimize nonspecific adsorption. Sections were then incubated overnight at 4°C with monoclonal biotinylated antibodies directed at P-selectin (rat anti-mouse CD62P) or ICAM-1 (hamster anti-mouse CD54) at a dilution 1:500. Controls included buffer alone or nonspecific purified IgG. Antibody binding sites were visualized with an avidin-biotin peroxidase complex immunoperoxidase technique (Vector Laboratories) using diaminobenzidine. To quantify the degree of P-selectin staining on the endothelial wall, a grading system of 0 to 4 was used: 0, no staining; 1 to 3, increasing degrees of intermediate staining; and 4, extensive staining. To quantify the degree of ICAM-1 staining, a grading system of 0 to 4 was used: 0, no staining; 1, constitutive presence of staining along the endothelial wall; 2 to 3, increasing degrees of intermediate staining along the endothelial wall; and 4, increased staining along the endothelial wall and presence of staining on myocytes. In each experimental group, 5 or 6 sections were evaluated by 2 independent observers blinded to the experimental protocol.

Cell Culture

HUVECs were obtained from the American Type Culture Collection. HUVECs were cultured in F12K nutrient medium containing 10% FCS, heparin (100 μg/mL), and endothelial cell growth supplement (30 μg/mL). Mouse fibroblasts from a strain genetically deficient in PARS and fibroblasts from the corresponding wild-type controls were grown in DMEM with 10% FBS under standard cell culture conditions. When cells reached 90% to 100% confluence in 96-well plates, the culture medium was replaced by fresh medium, and the cells were incubated with peroxynitrite (100 nmol/L to 1000 μmol/L), human recombinant interleukin-1, or TNF-α (1 to 1000 U/mL) in the presence or absence of the PARS inhibitor 3-aminobenzamide (1 mmol/L).

Measurement of Cell Viability

Mitochondrial respiration, as an indicator of cell viability, was assessed by the mitochondria-dependent reduction of MTT to formazan. Cells in 96-well plates were incubated at 37°C with MTT (0.2 mg/mL) for 1 hour. Culture medium was removed by aspiration, and the cells were solubilized in dimethyl sulfoxide (100 μL). The extent of reduction of MTT to formazan within cells was quantified by the measurement of optical density at 550 nm.

Figure 2. Myocardial architecture after coronary occlusion and reperfusion is preserved in PARS+/+ mice. Representative cardiac sections from sham-operated PARS+/+ (a) or PARS+/+ (b) mice revealed a normal tissue structure. After 1-hour occlusion and 1-hour reperfusion of the LAD (LAD-IR), a marked disruption of the myocardial structure characterized by the appearance of extensive necrosis and contraction bands (arrows) was demonstrated in cardiac sections from PARS+/+ mice (c). In the PARS+/+ mice subjected to LAD-IR, myocardial architecture appeared normal (d). Magnification ×400. A similar pattern was seen in the 5 or 6 different tissue sections in each experimental group.
Neutrophil infiltration after coronary occlusion and reperfusion is reduced in PARS−/− mice. Myeloperoxidase, an enzyme present in neutrophils, was measured as an index of neutrophil infiltration into the injured tissue. Tissue myeloperoxidase activity was significantly enhanced in PARS+/+ mice subjected to myocardial injury (ischemia and reperfusion of the LAD [LAD-IR]) compared with sham-operated PARS−/− mice (P<0.001). In contrast, the increase of myeloperoxidase was significantly reduced in PARS−/− mice subjected to LAD-IR compared with PARS+/+ mice subjected to LAD-IR (P<0.001). Each data point is the mean±SEM of 6 animals for each group.

ELISA for P-Selectin and ICAM-1 Expression

The expression of P-selectin and ICAM-1 was detected in HUVECs and in fibroblasts by an ELISA method. Briefly, after stimulation with peroxynitrite or cytokines, cells in 96-well plates were washed with HBSS (pH 7.4), fixed with 1% paraformaldehyde for 15 minutes, and incubated for 1 hour with a 2% BSA-HBSS solution. The primary monoclonal antibodies directed at P-selectin or ICAM-1 (1:750 dilution) were then added for 2 hours. Thereafter, a secondary developing antibody (peroxidase conjugate) was applied for 1 hour, followed by incubation with the substrate 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (1 mg/mL in 0.2 mol/L citrate/phosphate buffer, pH 5, containing 0.1% hydrogen peroxide) for 30 minutes. All incubations were carried out at room temperature. Chromophore development was determined by measuring optical density at 405 nm with a Spectramax microplate reader. Background absorbance was determined from cells incubated without primary antibodies.

Materials

Cell culture medium and FCS were obtained from GIBCO. Primary anti-nitrotyrosine antibody was purchased by Upstate Biotech. Primary monoclonal ICAM-1 (CD54) or P-selectin (CD62P) antibodies for immunohistochemistry and cytokines were purchased by Pharmingen. The primary monoclonal antibodies directed at ICAM-1 or P-selectin for ELISA were obtained from R & D Systems. Reagents and secondary and nonspecific IgG antibodies for immunohistochemical analysis were from Vector Laboratories Inc. Peroxynitrite was synthesized and kindly provided by Dr H. Ischiropoulos (University of Pennsylvania, Philadelphia). All other chemicals were from Sigma/Aldrich.

Data Analysis

All values in the figures and text are expressed as mean±SEM of n observations, where n represents the number of mice (n=6 animals for each group) in the in vivo experiments or wells in the in vitro cell culture experiments (n=9 wells from 3 independent experiments). The results were examined by ANOVA, followed by the Bonferroni correction post hoc t test. A value P<0.05 was considered significant.

Results

Myocardial Injury After Coronary Occlusion and Reperfusion Is Reduced in PARS−/− Mice

In wild-type mice, 1-hour occlusion of the LAD followed by 1-hour reperfusion resulted in a marked myocardial injury. Serum creatine phosphokinase, an index of myocyte injury, increased to 6082±550 U/L over a control level of 233±102 U/L (P<0.001, Figure 1). At histological examination of the reperfused hearts, a marked necrosis of the tissue with development of contraction bands was observed (see Figure 2 for representative section). The degree of the damage (on a scale of 0 to 4) averaged 2.83±0.09.

Absence of a functional PARS gene in PARS−/− mice resulted in a significant prevention of reperfusion injury of previously ischemic hearts. Serum creatine phosphokinase was reduced by ≈40% in PARS−/− mice compared with their wild-type PARS+/+ littermates (P<0.001, Figure 1). The histological features were typical of normal cardiac structure (see Figure 2 for representative section) or mild architectural alterations, characterized by interstitial edema and localized necrotic areas. The damage score was significantly reduced (1.47±0.16) in PARS−/− mice compared with their PARS+/+ littermates (P<0.001).

Neutrophil Infiltration and Peroxynitrite Formation Is Reduced in PARS−/− Mice Subjected to Myocardial Ischemia and Reperfusion

A hallmark of reperfusion injury is the accumulation into the injured tissue of neutrophils, which augments the damage to vascular and parenchymal cellular elements. Therefore, we next evaluated the extent of neutrophil infiltration and peroxynitrite production in myocardial reperfusion injury in PARS−/− mice compared with PARS+/+ wild-type control mice. Assessment of neutrophil infiltration in myocardial tissue was performed by measurement of the activity of myeloperoxidase, an enzyme specific to granulocyte lysosomes, and, therefore, directly correlated to the number of neutrophils. Myeloperoxidase activity was significantly elevated after myocardial ischemia and reperfusion in PARS+/+ mice (Figure 3). The elevation of the enzyme correlated well with the appearance of a positive immunohistochemical staining for nitrotyrosine, a tyrosine nitration product of peroxynitrite, in the injured cardiac tissue (Figure 4). Tissue myeloperoxidase activity was significantly reduced in PARS−/− mice compared with PARS+/+ mice. Moreover, nitrotyrosine staining was virtually abolished in the PARS−/− mice after reperfusion. On a scale of 0 to 4, the intensity of staining was 0.54±0.10 in cardiac sections of PARS+/+ mice, which was significantly lower than the intensity of staining of cardiac sections of PARS−/− mice (3.04±0.14, P<0.001).

Expression of P-Selectin and ICAM-1 Is Reduced in PARS−/− Mice Subjected to Myocardial Ischemia and Reperfusion

One of the early endothelial events in the process of neutrophil recruitment during myocardial ischemia and reperfusion
is the release of P-selectin from preformed storage pools within Weibel-Palade body membranes. This adhesion glycoprotein is not constitutively expressed on unstimulated endothelium in noninflamed tissues. However, when endothelial cells are exposed to certain stimuli, such as hydrogen peroxide, thrombin, histamine, or complement, P-selectin is rapidly mobilized (1 to 2 minutes) to the cell surface, allowing the leukocytes to roll along the endothelium.4–10 Thereafter, ICAM-1, constitutively expressed at low levels on the surface of endothelial cells, is upregulated and is responsible for the firm adhesion of neutrophils.4,11–15 Considering these pathogenic events, we next compared the expression on endothelial cells of P-selectin and ICAM-1 in PARS2/2 and PARS1/1 mice subjected to myocardial ischemia and reperfusion injury. Myocardial tissue sections obtained from PARS2/2 mice undergoing 1 hour of coronary artery occlusion followed by 1 hour of reperfusion showed positive staining for P-selectin localized in the vascular endothelial cells of microvessels. No staining was observed in sham-operated control wild-type mice (Figure 5). These data are consistent with previous in vivo studies demonstrating that P-selectin is translocated on the cell surface only after cellular activation by the reperfusion inflammatory process.4–10 In tissue obtained from PARS2/2 mice, no upregulation of P-selectin was found during ischemia and reperfusion (Figure 5). The intensity of staining was 2.62±0.14 for cardiac sections of PARS1/1 mice and 0.15±0.09 for cardiac sections of PARS2/2 mice (P<0.001).

Staining of myocardial tissue sections obtained from sham operated wild-type mice with anti–ICAM-1 antibody showed a specific staining along cardiac vessels, demonstrating that ICAM-1 is constitutively expressed in endothelial cells. After 1 hour of ischemia followed by 1 hour of reperfusion, the staining intensity substantially increased in the area of early necrosis. Immunohistochemical staining was mainly localized in endothelial vascular wall, but a diffuse staining was also localized in myocytes within the necrotic lesion (the degree of staining was scored 3.17±0.16). Sections from PARS-deficient mice did not reveal any upregulation of the constitutive ICAM-1, which was normally expressed in the endothelium along the vascular wall (the degree of staining was scored 1.12±0.07; P<0.001) (Figure 6).
Inhibition of PARS Inhibits Expression of P-Selectin and ICAM-1 in Human Endothelial Cells and Murine Fibroblasts

From the above in vivo experiments, the hypothesis emerges that PARS activation is a crucial determinant of the changes of adhesive capability of endothelium. However, the in vivo experiments cannot prove a direct correlation between PARS activation and cellular surface expression of adhesion molecules. Therefore, in additional in vitro experiments, we sought to investigate whether inhibition of PARS may directly affect endothelial expression of adhesion molecules. Therefore, in additional in vitro experiments, we sought to investigate whether inhibition of PARS may directly affect endothelial expression of adhesion molecules. Incubation of HUVECs with peroxynitrite or immunostimulation with TNF-α induced the expression of P-selectin and upregulation of ICAM-1, respectively. Inhibition of PARS by 3-aminobenzamide inhibited the oxidant-dependent expression of P-selectin and the cytokine-mediated upregulation of ICAM-1 (Figure 7).

To obtain unequivocal evidence of the involvement of PARS in adhesion molecule expression, experiments were carried out using murine fibroblasts lacking the gene for PARS (PARS⁺/⁻) and control fibroblasts with a normal genotype (PARS⁺/+). Immunostimulation of the cells with TNF-α or interleukin-1 for 24 hours induced a significant expression of ICAM-1 in a concentration-dependent manner in PARS⁺/+ cells, whereas it elicited no ICAM-1 expression in PARS⁺/- cells. Pharmacological inhibition of PARS by 3-aminobenzamide in normal fibroblasts reduced the cytokine-mediated expression of ICAM-1 (Figure 8).

Discussion

The present studies provide evidence that activation of PARS mediates leukocyte-endothelial interaction by regulating the expression of P-selectin and ICAM-1 during the acute inflammatory response of myocardial ischemia and reperfusion. In PARS-deficient mice subjected to occlusion and reperfusion of the coronary artery, the upregulation of P-selectin and ICAM-1 in the infarcted myocardium was completely abolished.

Endothelial cells appear to be major regulators of neutrophil traffic, regulating the process of neutrophil chemotraction, adhesion, and emigration from the vasculature to the tissue. During the early phase of reperfusion, P-selectin is rapidly released to the cell surface from preformed storage pools after exposure to certain stimuli, such as hydrogen peroxide.
peroxide, thrombin, histamine, or complement, and allows the leukocytes to roll along the endothelium. ICAM-1, constitutively expressed on the surface of endothelial cells, is then involved in the neutrophil adhesion. Hypoxic endothelial cells synthesize proinflammatory cytokines, which can upregulate endothelial expression of the constitutive adhesion molecule ICAM-1 in an autocrine fashion. Significant expression of ICAM-1 in microvessels of previously ischemic tissues occurs within 1 hour after reperfusion. In addition to endothelial cells, as early as within the first hour of reperfusion a significant induction of ICAM-1 mRNA occurs in vivo in previously ischemic myocytes. The expression of P-selectin and ICAM-1 corresponds with the induction of neutrophil recruitment, which is maximal within the first hour of reperfusion, and persists, at a lower rate, in the late phase of reperfusion. In accordance with these findings, we observed that a 1-hour occlusion of coronary artery followed by a 1-hour reperfusion induced the appearance of P-selectin on the endothelial vascular wall and upregulated the surface expression of ICAM-1 on endothelial cells and myocytes. Genetic disruption of PARS abolished the expression of P-selectin and the upregulation of ICAM-1, while maintaining unaffected the constitutive levels of ICAM-1 on endothelial cells. These results demonstrate that inhibition of PARS activity can interrupt the interaction between neutrophils and endothelial cells both at the early rolling phase mediated by P-selectin and at the late firm adhesion phase mediated by ICAM-1. The absence of an increased expression of the adhesion molecule in the cardiac tissue of PARS mice correlated with the reduction of leukocyte infiltration as assessed by the specific granulocyte enzyme myeloperoxidase and with the moderation of postreperfusion tissue damage, as evaluated by reduction of serum creatine phosphokinase levels and by histological examination. It is noteworthy, however, that tissue myeloperoxidase activity was not completely abolished. This result is consistent with previous studies demonstrating that constitutive levels of ICAM-1 appear to be sufficient to support a lower degree of CD11/CD18-dependent transendothelial migration of activated neutrophils.

Reduction of neutrophil infiltration was also paralleled with the inhibition of nitrotyrosine immunoreactivity. Nitro-
tyrosine formation, along with its detection by immunostaining, was initially proposed as a relatively specific means for detection of the “footprint” of peroxynitrite. Recent evidence indicates, however, that certain other reactions can also induce tyrosine nitration; eg, the reaction of nitrite with hypochlorous acid and the reaction of myeloperoxidase (and certain other peroxidases) with hydrogen peroxide can lead to the formation of nitrotyrosine. Increased nitrotyrosine staining is considered, therefore, as an indication of “increased nitrosative stress” rather than a specific marker of peroxynitrite. Our finding (ie, that nitrotyrosine staining is reduced in the PARS \(^{-/-}\) animals) coupled with the cardioprotective effects of PARS gene ablation proves the existence of a self-amplifying suicide cycle in which early oxidant production by endothelium activates PARS; the consequent endothelial injury with activation of neutrophil-attractive factors and neutrophil infiltration leads to further production of oxidants, which ultimately are responsible for the myocardial injury.

Several in vitro and in vivo studies have demonstrated that the catalytic activity of the nuclear enzyme PARS, induced by single DNA strand breakage, is a direct result of oxidant injury in conjunction with a variety of immunological stimuli, including bacterial endotoxin and cytokines. More specifically, it has been previously reported that oxidant injury by NO, peroxynitrite, and/or hydrogen peroxide induces metabolic changes and cytotoxicity in association with the intracellular elevation of PARS activity in macrophages and in pulmonary epithelial, smooth muscle, and endothelial cells. In the present in vitro experiments with HUVEC cells, we have demonstrated that oxidant injury by peroxynitrite or TNF-\(\alpha\) stimulation induces an upregulation of P-selectin and ICAM-1 surface expression, a process that is prevented by the PARS inhibitor 3-aminobenzamide. On the
basis of these results, we conclude that enhanced adhesion molecule expression may be mediated by an increase in intracellular PARS activity.

The specific mechanism of PARS activation in regulating adhesion molecule expression, a process that may also occur in nonendothelial cells, remains to be elucidated. PARS-related changes in cellular energetics and the related processes involving calcium sequestration, biosynthetic processes, and maintenance of the normal cell shape and adherence may be involved. Alternatively, or in addition to the energetic changes, poly(ADP-ribosyl)ation may directly play a role in gene expression. Poly(ADP-ribosyl)ation may lead to the relaxation of chromatin, with the consequence that genes become more accessible to RNA polymerase. PARS regulates the expression of a variety of genes, including the gene for the inducible NO synthase, major histocompatibility class II, and DNA methyltransferase. PARS has been shown to regulate, directly or indirectly, promoter activation: in human endothelial cells, inhibition of PARS reduces oxidant-induced binding activity of the transcription factor activator protein-1 to the promoter of ICAM-1, whereas in immunostimulated murine macrophages, inhibition of PARS reduces the binding of nuclear factor κB to its target sequence.

In conclusion, the data presented here, as a whole, demonstrate that PARS is directly involved in the regulation of the expression of adhesion molecules and that, consequently, PARS plays a role in the tissue infiltration of neutrophils. The mode of action of PARS inhibition, as it relates to inhibition of neutrophil infiltration, in the present model is different from the mode of action of PARS inhibitors in the mesenteric microcirculation inflamed with zymosan: in the latter model, PARS appeared to modulate a postadhesion phenomenon. Taken together, the data presented in the present study and in another recent report demonstrate that PARS regulates the infiltration of neutrophils into the inflamed tissues via a number of distinct mechanisms. The discovery of the concept that PARS regulates neutrophil trafficking may provide new insights in the interpretation of recent reports demonstrating the protective effect of PARS inhibition in experimental models of endotoxic shock, various forms of ischemia and reperfusion injury, and stroke. In addition to its effect on preserving the cellular energetic status and protecting against oxidant-induced cell necrosis, regulation of neutrophil recruitment may represent a novel important anti-inflammatory mode of action of PARS inhibition.

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Pars Modulates P-Selectin and ICAM-1 Expression

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