

Activation of Poly(ADP-Ribose) Polymerase-1 Is a Central Mechanism of Lipopolysaccharide-Induced Acute Lung Inflammation

LUCAS LIAUDET, PÁL PACHER, JON G. MABLEY, LÁSZLÓ VIRÁG, FRANCISCO G. SORIANO, GYÖRGY HASKÓ, and CSABA SZABÓ

Inotek Corporation, Beverly, Massachusetts; and Department of Surgery, New Jersey Medical School, UMDNJ, Newark, New Jersey

Recent studies demonstrated that activation of the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) by oxidant-mediated DNA damage is an important pathway of tissue injury in conditions associated with oxidative stress. Using a dual approach of PARP-1 suppression, by genetic deletion or pharmacological inhibition with the phenanthridinone PARP inhibitor PJ-34, we now demonstrate an essential role of PARP-1 in the development of pulmonary inflammation induced by lipopolysaccharide (LPS). PARP-1^{+/+} and PARP-1^{-/-} mice received an intratracheal instillation of LPS (50 µg), followed after 24 h by bronchoalveolar lavage to measure the cytokines TNF-α, IL-1β, and IL-6, the chemokines MIP-1α and MIP-2, leukocyte counts and myeloperoxidase activity (neutrophil accumulation), protein content (high permeability edema), and nitrite/nitrate (nitric oxide production). Malondialdehyde (an index of lipid peroxidation) was measured in lung tissue. Similar experiments were conducted in BALB/c mice treated with PJ-34 or vehicle. The absence of functional PARP-1 reduced LPS-induced increases of cytokines and chemokines, alveolar neutrophil accumulation, lung hyperpermeability, NO production, and lipid peroxidation. Histological analysis revealed attenuated lung damage after PARP inhibition. Our findings support a mechanistic role of PARP-1 in the regulation of LPS-induced lung inflammation. Pharmacological inhibition of PARP may be useful in clinical conditions associated with overwhelming lung inflammation.

Keywords: lung; ARDS; lipopolysaccharide; poly(ADP-ribose) polymerase; chemokines

Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear DNA-binding enzyme present throughout the phylogenetic spectrum that participates in DNA repair in response to genotoxic stress (1, 2). DNA single-strand breakage is the obligatory trigger of PARP-1 activation, which, in turn, cleaves its substrate nicotinamide adenine dinucleotide (NAD⁺) into nicotinamide and ADP-ribose, and covalently attaches ADP-ribose units to various nuclear proteins (3). This process results in a reduced cellular content in NAD⁺, slowing the rate of glycolysis, mitochondrial respiration, and high-energy phosphate gen-

eration, ultimately leading to cell death via the necrotic pathway (4). In addition, PARP-1 has been recently shown to be involved in the regulation of inflammatory processes, being functionally associated with important transcription factors, notably nuclear factor (NF)-κB (5, 6).

Acute respiratory distress syndrome (ARDS) is a severe form of lung inflammation most commonly complicating gram-negative septic shock. Endotoxin released from the bacterial cell wall is considered to be an important eliciting factor in the development of ARDS in this setting, which triggers the intrapulmonary production of various proinflammatory mediators (7–9). We hypothesized that PARP-1 activation resulting from this overwhelming oxidative stress might participate in the development of acute lung injury induced by intratracheally administered lipopolysaccharide (LPS). Using a dual approach of both genetic suppression of PARP-1 and pharmacological PARP inhibition with the novel, phenanthridinone compound PJ-34 (10, 11), we demonstrate that activation of PARP-1 is a pivotal mechanism of LPS-induced pulmonary inflammation.

METHODS

In vivo studies were performed in accordance with National Institutes of Health guidelines and with the approval of the local institutional animal care and use committee.

Intratracheal LPS Administration

The experiments were conducted both in male BALB/c mice, 8–10 wk old, and in PARP-1-deficient (PARP-1^{-/-}) and wild-type (PARP-1^{+/+}) littermates (genetic background: 129/Sv × C57BL6), as previously described (12). The animals were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (30 mg/kg), given intraperitoneally. A 1-cm midline cervical incision was made to expose the trachea. Intratracheal administration of LPS (*Escherichia coli*, O127:B8, 50 µg/mouse) or vehicle (phosphate-buffered saline [PBS] pH 7.4), in a volume of 100 µl, was performed with a bent 27-gauge tuberculin syringe (13, 14). The cervical incision was closed with 5.0 silk suture and the mice returned to their cage. The animals recovered rapidly after surgery. In preliminary studies, a time course of the pulmonary inflammatory response was investigated. We found that 24 h after LPS injection there was a marked degree of pulmonary inflammatory response, which largely resolved by 3 d. Therefore, in all subsequent studies, measurements were taken at 1-d post-LPS, which was considered the peak time of the pulmonary inflammatory alterations.

Bronchoalveolar Lavage

Twenty-four hours after the surgery, the mice were reanesthetized with ketamine/xylazine intraperitoneally. A laparotomy was performed and the animals were bled by transection of the inferior vena cava to reduce hemorrhage in the lungs. Bronchoalveolar lavage was performed by the intratracheal instillation of 1 ml PBS (pH 7.4) into the exposed lungs (maintained within the thoracic cavity). The lavage fluid was reinfused three times into the lungs before final collection. The bronchoalveolar lavage fluid (BALF) was then centrifuged at 5,000 rpm for 10 min and the cell-free supernatant was frozen at -70° C until further analysis. The volume of cell-free supernatants was measured for each animal. In addition, a cell count was performed in the BALF

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Correspondence and requests for reprints should be addressed to Csaba Szabo, M.D., Ph.D., Inotek Corporation, Suite 419 E, 100 Cummings Center, Beverly, MA 01915. E-mail: szabocsaba@aol.com

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taken from PARP-1^{+/+} and PARP-1^{-/-} animals. The cells were resuspended in a volume of 0.5 ml (0.4 ml PBS and 0.1 ml 0.4% Trypan blue) and total leukocytes were counted in a hemocytometer. Differential cell counts were also performed and were found, in all experimental groups, to be approximately 90% neutrophilic and approximately 5% lymphocytic; there was no significant difference between experimental groups. Lungs were then removed and either processed for wet-to-dry ratio determinations or immediately frozen in liquid nitrogen and stored at -70° C.

Treatment Groups

BALB/c mice challenged with intratracheal LPS were treated with PJ-34 (20 mg/kg, *n* = 12) or vehicle (isotonic saline, 0.5 ml, *n* = 12), administered intraperitoneally, 1 h after surgery. The treatment was repeated after 6 and 12 h. A group of mice (*n* = 8) was challenged with intratracheal PBS instead of LPS (sham mice). In the experiments using genetically engineered mice, seven PARP-1^{+/+} and seven PARP-1^{-/-} mice were challenged with LPS, whereas five PARP-1^{+/+} and five PARP-1^{-/-} mice received PBS (sham animals).

Measurements

Cytokines and chemokines. The production of the proinflammatory cytokines tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6, as well as of the chemokines macrophage inflammatory protein (MIP)-1 α (a CC chemokine) and MIP-2 (a CXC chemokine), was determined in the BALF using commercially available enzyme-linked immunosorbent assay (ELISA), following the protocol provided by the manufacturer (R&D Systems, Minneapolis, MN). BALFs were diluted (1:2 to 1:5) in animals challenged with LPS, whereas they were assayed undiluted in sham animals. Results are expressed as picogram (or nanogram) per milliliter BALF.

Myeloperoxidase activity. The activity of myeloperoxidase (MPO), an indicator of neutrophil accumulation, was determined directly in the BALF or in whole lung homogenates. Pieces of lung tissue or an aliquot of the BALF (20 μ l) were mixed with a solution of 1.6 mM tetramethylbenzidine and 1 mM hydrogen peroxide. Activity was measured spectrophotometrically as the change in absorbance at 650 nm at 37° C, using a Spectramax microplate reader (Molecular Devices, Sunnyvale, CA). Results are expressed as milliunits of MPO activity per milliliter BALF or per milligram tissue protein.

Nitrate/nitrite concentration. The pulmonary production of nitric oxide (NO) was determined by the measurement of nitrate and nitrite, the stable end products of NO metabolism, in the BALF. First, nitrate was reduced to nitrite by incubation with nitrate reductase (610 mU/ml) and NADPH (170 mM) at room temperature for 3 h. After 3 h, nitrite concentration in the samples was measured by the Griess reaction, by adding 100 μ l of Griess reagent (0.1% naphthalenediamine dihydrochloride in H₂O and 1% sulfanilamide in 5% concentrated H₃PO₄; vol 1:1). The optical density at 550 nm (OD 550, corrected for absorbance at 650 nm) was measured in a Spectramax microplate reader. Nitrite concentrations were calculated by comparison of OD 550 of standard solutions of sodium nitrite prepared in PBS. Nitrate/nitrite concentrations are expressed in nanomoles per milliliter BALF.

Protein assay. The amount of proteins in the BALF was assayed using the Bradford assay and is expressed in milligrams protein per milliliter BALF.

Malondialdehyde formation in lung homogenates. Malondialdehyde formation was utilized to quantify the lipid peroxidation in the lung and measured as thiobarbituric acid-reactive material. Lung samples were homogenized (50 mg/ml) in 1.15% KCl buffer. Homogenates (200 μ l) were then added to a reaction mixture consisting of 1.5 ml 0.8% thiobarbituric acid, 200 μ l 8.1% sodium dodecyl sulfate, 1.5 ml 20% acetic acid (pH 3.5), and 600 μ l distilled H₂O and heated at 90° C for 45 min. After cooling to room temperature, the samples were cleared by centrifugation (10,000 \times g, 10 min) and their absorbance measured at 532 nm, using 1,1,3,3-tetramethoxypropane as an external standard. The level of lipid peroxides was expressed as nanomoles MDA per milligram protein, measured by the Bradford assay.

Lung Histology and Immunohistochemistry

Histopathological changes induced by LPS were evaluated in BALB/c mice treated or not with PJ-34 (*n* = 4 mice/group). Twenty-four hours

after surgery, the animals were anesthetized and killed by exsanguination and the lungs were inflated-fixed with 4% paraformaldehyde. Paraffin-embedded lungs were sectioned at 3 μ m and stained with hematoxylin and eosin for morphological analysis.

To confirm the PARP inhibitory effect of PJ-34, PARP activity in tissues was measured by using an immunohistochemical method of PARP activity utilizing biotinylated NAD (11, 15). Briefly, cryosections (10 μ m) were fixed for 10 min in 95% ethanol at -20° C and then rinsed in PBS. Sections were permeabilized by 1% Triton X-100 in 100 mM Tris pH 8.0, for 15 min. Reaction mixture (10 mM MgCl₂, 1 mM dithiothreitol, 30 μ M biotinylated NAD⁺, in 100 mM Tris, pH 8.0) was then applied to the sections for 30 min at 37° C. Reaction mixes containing 5 mM 3-aminobenzamide or biotinyl-NAD⁺ free reaction mix were used as controls. After three washes in PBS, incorporated biotin was detected by peroxidase-conjugated streptavidine (1:100, 30 min, room temperature). After 3 \times 10 min washes in PBS, color was developed with cobalt-enhanced nickel-DAB substrate. Sections were counterstained in Nuclear Fast Red, dehydrated, and mounted in Vectamount.

Data Analysis

All the data were statistically evaluated using analysis of variance, followed by post-hoc analysis with Tukey's test when appropriate. Significance was assigned to *p* < 0.05.

Chemicals

Unless otherwise specified, all chemicals were purchased from Sigma Chemicals (St. Louis MO). PJ-34 was synthesized as previously described (11).

RESULTS

PARP-1 Suppression Reduces the Production of Proinflammatory Cytokines

Figure 1 shows the results of the measurements of the proinflammatory cytokines TNF- α (Figure 1A) and IL-1 β (Figure 1B) in PARP-1^{+/+} and PARP-1^{-/-} mice. Baseline cytokines were undetectable in the BALF from sham animals. A massive production of cytokines was measured in PARP-1^{+/+} mice instilled with LPS, which was largely reduced in PARP-1^{-/-} animals. In BALB/c mice, treatment with PJ-34 also resulted in a significant downregulation of the production of TNF- α (Figure 1D). However, contrasting with the beneficial effect of the genetic suppression of PARP-1 on IL-1 β , the effect of PJ-34 was only partial and did not reach significance (Figure 1E). PARP deficiency and pharmacological inhibition of PARP with PJ-34 also tended to reduce LPS-induced IL-6 production (*see* Figure E1A and E1C in the online data supplement).

Reduction of Chemokine Expression in the Absence of Functional PARP-1

In the absence of LPS exposure, there were no detectable levels of the chemokines MIP-1 α and MIP-2 in the BALF. In PARP-1^{+/+} mice challenged with LPS, both chemokines increased to 442 \pm 37 pg/ml BALF (MIP-1 α , Figure 1C) and 1203 \pm 152 pg/ml BALF (MIP-2; *see* Figure E1B in the online data supplement). These levels were significantly lower in PARP-1^{-/-} mice, reaching 244 \pm 26 pg/ml BALF for MIP-1 α and 310 \pm 36 pg/ml BALF for MIP-2 (*p* < 0.05 for both chemokines). In BALB/c mice, although PJ-34 significantly reduced the expression of MIP-1 α (Figure 1F), it did not significantly attenuate that of MIP-2 (*see* Figure E1D in the online data supplement).

Effect of PARP-1 Suppression on Neutrophil Accumulation

As illustrated in Figure 2, a large increase in MPO activity, indicative of the accumulation of polymorphonuclear cells, was detected in the BALF of PARP-1^{+/+} mice (Figure 2A) and

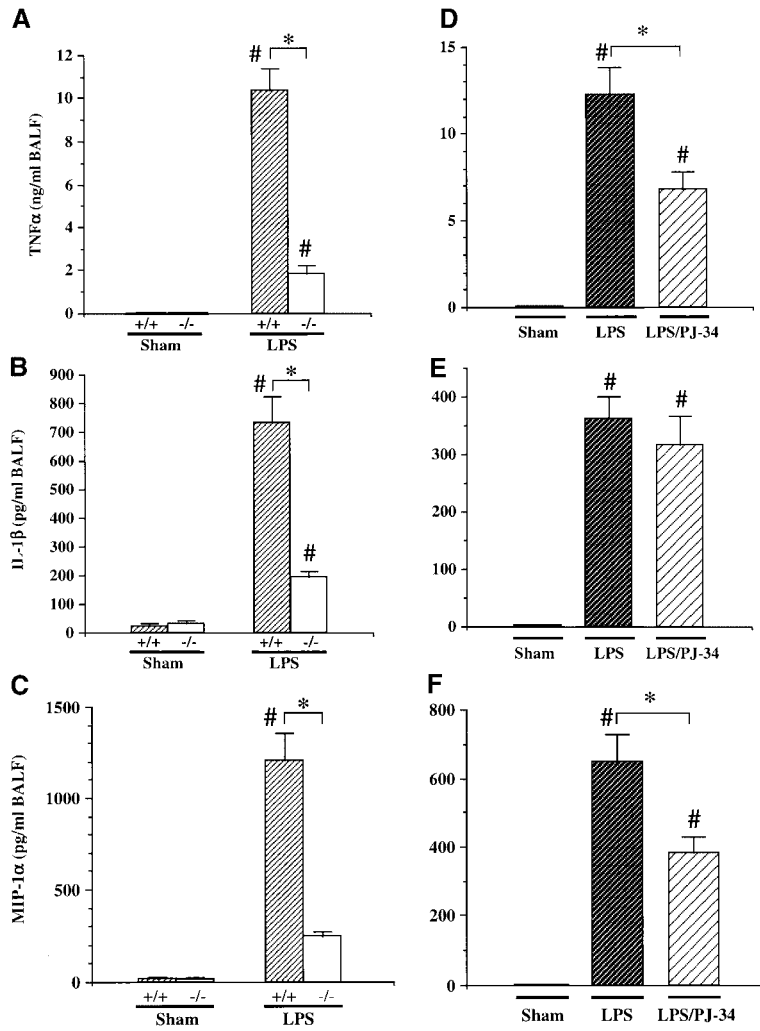


Figure 1. Levels of proinflammatory cytokines and chemokines in the bronchoalveolar lavage fluid (BALF). PARP-1^{+/+} and PARP-1^{-/-} mice (left panel) as well as BALB/c mice (right panel) received an intratracheal instillation of phosphate-buffered saline (sham animals) or lipopolysaccharide (LPS). BALB/c mice challenged with LPS were treated with PJ-34 (20 mg/kg intraperitoneally 1, 6, and 12 h after LPS) or vehicle. Proinflammatory mediators were measured in BALF obtained after 24 h. In PARP-1^{+/+} mice, LPS induced a significant increase in TNF- α (A), IL-1 β (B), and MIP-1 α (C) levels that were suppressed in PARP-1^{-/-} mice. In BALB/c mice, PJ-34 significantly reduced TNF- α (D) and MIP-1 α (F), but not IL-1 β (E) levels. Means \pm SEM. #p < 0.05 LPS versus sham. *p < 0.05 PARP-1^{-/-} versus PARP-1^{+/+} and PJ-34 versus vehicle-treated BALB/c mice.

BALB/c mice (Figure 2B) after LPS administration. Genetic deletion of PARP-1 was associated with a significant reduction in MPO activity in the BALF, an observation that was reproduced in BALB/c mice treated with PJ-34. Similarly, a significant increase in whole lung MPO activity was noted after LPS injection, which was markedly reduced in the absence of functional PARP (not shown). In addition, the massive leukocyte infiltration (mainly neutrophilic) of the alveolar spaces noted in the PARP-1^{+/+} mice (from $5 \pm 1 \times 10^4$ to $149 \pm 15 \times 10^4$ cells/

BALF, $p < 0.01$) was significantly prevented in PARP-1^{-/-} animals ($80 \pm 15 \times 10^4$ cells/BALF, $p < 0.01$).

Reduction of High Permeability Pulmonary Edema by Suppression of PARP-1 Activation

We have measured the protein content of the BALF as an indicator of high permeability pulmonary edema. In sham mice from the PARP-1 colony, a small amount of proteins was recovered in the BALF. Following LPS, the protein content in-

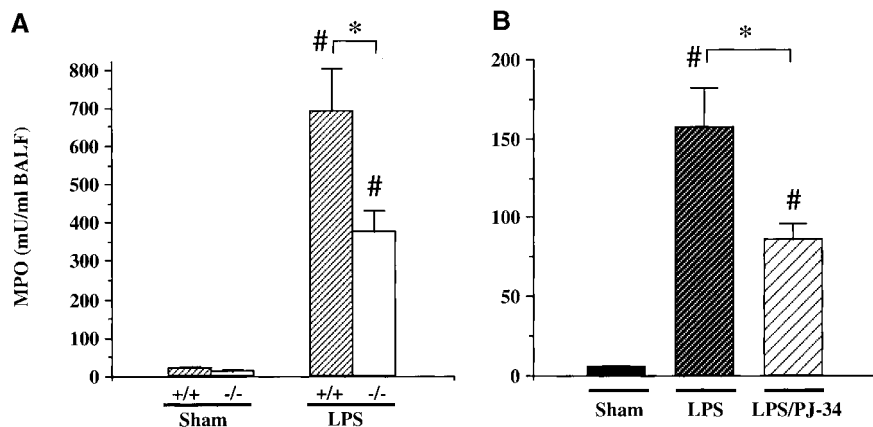


Figure 2. Myeloperoxidase (MPO) activity in the BALF. MPO activity was measured in the BALF obtained 24 h after the intratracheal administration of phosphate-buffered saline (sham animals) or LPS in PARP-1^{+/+} and PARP-1^{-/-} mice (A) and in BALB/c mice (B), treated with PJ-34 or vehicle. The increase in MPO induced by LPS was significantly reduced in the absence of functional PARP-1. Means \pm SEM. #p < 0.05 LPS versus sham. *p < 0.05 PARP-1^{-/-} versus PARP-1^{+/+} and PJ-34 versus vehicle-treated BALB/c mice.

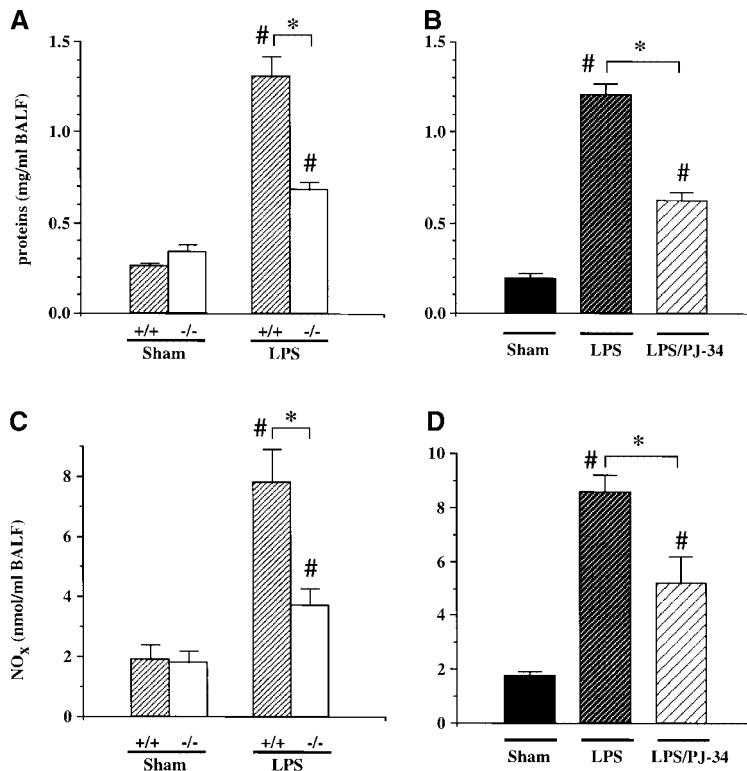


Figure 3. Protein content in the BALF and pulmonary nitric oxide (NO) production. The protein content (A and B), an index of high permeability pulmonary edema, and the concentrations of the NO metabolites nitrate and nitrite (NO_x, C and D) were determined in BALF obtained 24 h after an intratracheal challenge with LPS or vehicle (phosphate-buffered saline, sham animals), both in PARP-1^{+/+} and PARP-1^{-/-} mice (left panel) and in BALB/c mice treated or not treated with PJ-34 (right panel). The protein content as well as the levels of NO_x increased after LPS administration, but these effects were significantly attenuated in mice genetically deficient in PARP-1 (A and B) as well as in BALB/c mice treated with PJ-34 (C and D). Means ± SEM. #p < 0.05 LPS versus sham. *p < 0.05 PARP-1^{-/-} versus PARP-1^{+/+} and PJ-34 versus vehicle-treated BALB/c mice.

creased largely in the PARP-1^{+/+} mice, but less so in the PARP-1^{-/-} mice. Similarly, in BALB/c mice, PJ-34 provided a significant protection against the increase in BAL protein content (Figure 3A and 3B). Similar findings were obtained when measuring lung wet-to-dry ratios. For instance, LPS increased wet-to-dry ratios from 4.4 ± 0.1 to 5.4 ± 0.2 ($p < 0.01$) in the BALB/c mice, and treatment with PJ-34 reduced this value to 4.8 ± 0.1 ($p < 0.05$, $n = 5$). Similarly, in PARP^{+/+} animals LPS induced a significant increase in wet-to-dry ratio (from 4.7 ± 0.1 to 6.3 ± 0.6 , $n = 5$, $p < 0.01$), but no significant increase was seen after LPS in the PARP-deficient mice (4.6 ± 0.3 to 4.7 ± 0.3 , $n = 5$).

Decreased Production of NO in the Absence of Functional PARP-1

Increased NO production due to the induced expression of type II NO synthase is a typical consequence of LPS administration (17). LPS induced a significant increase of NO_x in the BALF of PARP-1^{+/+} animals and BALB/c mice, which was significantly ($p < 0.01$) attenuated in PARP-1^{-/-} mice as well as in BALB/c mice treated with PJ-34 (Figure 3C and 3D).

Genetic Deletion or Pharmacological Inhibition of PARP-1 Decreases Lipid Peroxidation

An increased formation of MDA, attesting of enhanced lipid peroxidation, was observed in PARP-1^{+/+} and BALB/c mice after LPS administration. In contrast, MDA increased significantly less in lungs from PARP-1^{-/-} mice and BALB/c mice receiving PJ-34 (data not shown).

Confirmation of the PARP Inhibitory Effect of PJ-34 *in Vivo*

PJ-34 treatment abolished the activity of poly(ADP-ribose) polymerase, as measured by an immunohistochemical functional assay (see Figure E2 in the online data supplement), confirming that the current pharmacological dosing regimen with PJ-34 was sufficient to block PARP activation *in vivo*.

PARP Inhibition with PJ-34 Reduces the Morphological Alterations Induced by LPS in the Lung

In animals treated with vehicle, the morphological alterations were characterized by an increased thickness of the alveolar septa and diffuse alveolar flooding with both leukocytes and erythrocytes (see Figure E3A in the online data supplement). Treatment with PJ-34 reduced the damage induced by LPS, notably by decreasing the extent of alveolar hemorrhages and the leukocyte extravasation into the alveolar spaces (see Figure E3B in the online data supplement).

DISCUSSION

The data presented in this study support the proposal that PARP-1 activation plays a central role in the development of lung inflammation following the intratracheal administration of LPS. Suppression of PARP-1 either by genetic deletion or pharmacological inhibition markedly downregulated the expression of proinflammatory mediators and reduced the amount of extravasated leukocytes (notably polymorphonuclear [PMN] cells) in the alveolar spaces. These effects were associated with a significant suppression of high permeability pulmonary edema and lipid peroxidation and an improvement of lung morphology.

Background and Previous Work

The obligatory trigger of PARP-1 activation is the formation of DNA breaks induced by free radicals and oxidants, most notably peroxynitrite, formed from the rapid reaction of NO with the superoxide radical (O₂⁻) (18). The enhanced production of both NO and O₂⁻ during acute lung inflammation also favors the formation of peroxynitrite, as attested by the formation of 3-nitrotyrosine, an indirect marker of peroxynitrite generation, in the lungs of endotoxin-treated rats (8) and in autopsy specimens of sepsis-induced diffuse alveolar damage (9). Several earlier reports have implicated a role of PARP activation in experimental models of pulmonary inflammation. Pulido and co-

workers reported that pharmacological inhibition of PARP suppressed the decrease in lung ATP levels and attenuated the dysfunction of pulmonary vasorelaxation induced by the systemic administration of LPS (19). In a similar model, we found that 3-aminobenzamide, a prototypical PARP inhibitor, reduced pulmonary microvascular leakage (20). In a nonseptic model of lung inflammation, PARP inhibition prevented edema formation in isolated rat lungs exposed to the excitatory amino acid *N*-methyl-D-aspartate (21). Finally, we have demonstrated that peroxynitrite-dependent activation of PARP caused energy depletion and increased permeability in human pulmonary epithelial cells *in vitro* (22). Our present data further extend these findings, by demonstrating a pivotal role of PARP-1 in the regulation of the inflammatory response in a clinically relevant model of acute lung injury.

Suppression of Proinflammatory Cytokine Expression and NO Overproduction by Genetic Deletion of PARP-1

The upregulation of proinflammatory cytokine expression and of nitric oxide production induced by LPS in PARP-1^{+/+} mice was largely abolished in mice genetically deficient in PARP-1, an effect that may be ascribed to at least two distinct mechanisms. First, overactivation of PARP-1, by depleting the cellular stores in NAD⁺ and ATP, has been shown to mediate necrotic cell death in conditions of overwhelming oxidative stress (4, 23). In such conditions, the absence of functional PARP-1 shifts the necrotic cell population toward the normal and apoptotic cell populations, which is considered beneficial, as necrotic but not apoptotic cells release their content into the extracellular space, resulting in a progressive amplification of the initial inflammatory process (4, 23, 24).

The second mechanism linking PARP-1 to the regulation of the inflammatory response relies in the direct interaction of PARP-1 with several distinct proinflammatory signaling pathways. Two recent studies have demonstrated a functional association between PARP-1 and the transcription factor nuclear factor-kappa B (NF-κB), which is upstream of the synthesis of acute phase proinflammatory mediators (5, 6). The defective NF-κB-dependent transcription activation resulting from PARP-1 suppression has been associated *in vivo* with a reduced production of TNF-α and interferon (IFN)-γ, a decreased expression of iNOS, and an improved survival rate in response to endotoxin (6). Furthermore, the absence of PARP-1 or its pharmacological inhibition has been shown to suppress LPS-mediated induction of mitogen-activated protein (MAP) kinase activity (25) and oxidant-induced activation of the transcription factor AP-1 (26), which both play a major role in the pleiotropic transduction of intracellular inflammatory cascades. In addition to NF-κB, MAP kinase, and AP-1, a number of other signaling pathways may be functionally regulated by PARP-1, in view of the growing list of transcription factors or transcription coactivators shown to be associated with PARP-1, including AP-2 (27), TEF-1 (28), oct-1 (29), and YY1 (30). Because PARP inhibition and PARP deficiency resulted in a general downregulation of the inflammatory response, it is not possible to define the relative contribution of the various affected mediators to the pathogenesis of LPS-induced lung injury. For instance, the fact that NO production was downregulated does not necessarily mean that the NO production by iNOS has significantly contributed to the pathogenesis of the disease in the current model.

Regulation of Leukocyte Trafficking by PARP-1 during Acute Lung Inflammation

It is widely acknowledged that polymorphonuclear neutrophils are important cellular effectors of the diffuse alveolar damage

characterizing ARDS (31, 32). Here, we report that PARP-1-deficient mice are protected against the flooding of alveolar spaces by leukocytes, notably PMN (as attested by decreased MPO activity in the BALF) induced by LPS. These observations are consistent with previous findings supporting a role of PARP-1 in the regulation of leukocyte trafficking in conditions such as inflammation, shock, and reperfusion injury (12, 13, 33). Both *in vitro* and *in vivo* data support the proposal that this effect could be ascribed, at least in part, to a reduced expression of the adhesion molecules ICAM-1 and P-selectin by suppression of PARP-1 activity (34). Here, we propose an additional mechanism related to the downregulation of chemokine production in the absence of functional PARP-1.

Chemokines (and other chemoattractant molecules), play a central role in the pathophysiology of ARDS by directing the migration of inflammatory cells through chemotactic gradients (35). In rodents, the CXC chemokines MIP-2 and KC, and the CC chemokine MIP-1α represent powerful granulocyte-activating chemokines (36, 37). The extensive release of both MIP-1α and MIP-2 into the BALF of PARP-1^{+/+} mice exposed to LPS was largely reduced in PARP-1^{-/-} animals. This is consistent with recent work showing a reduced production of MIP-1α in PJ-34-treated or in PARP-1-deficient macrophages and fibroblasts stimulated with LPS *in vitro* (38). The functional association between PARP-1 and the transcription factors NF-κB and AP-1 is a likely mechanism underlying the effect of PARP-1 suppression on chemokine expression. These observations may be clinically relevant, as a direct correlation has been established between the BALF levels of the CXC chemokine IL-8, the severity of lung inflammation, and mortality in patients with ARDS (39–41). PARP-1 appears to play a critical role in regulating leukocyte trafficking, acting both at the level of adhesion molecules and at the level of chemokine expression.

Suppression of PARP-1 Reduces High Permeability Pulmonary Edema and Lipid Peroxidation

The typical lesion in ARDS is characterized by widespread destruction of the alveolar epithelium and flooding of the alveolar spaces with proteinaceous exudates containing large amounts of neutrophils (42). The reduced production of proinflammatory mediators and leukocyte infiltration in the lungs of PARP-1^{-/-} mice translated into a significant reduction of high permeability pulmonary edema, evidenced by a decreased protein content in the BALF. This finding may have clinical implications, as the formation of high permeability edema eventually results in alveolar collapse, reduced lung compliance, and severe gas exchange abnormalities (43), alterations that are further exacerbated by the initiation of mechanical ventilation in the clinical setting (44). An additional effect of PARP-1 suppression was a reduced formation of malondialdehyde, indicative of decreased oxidant stress and lipid peroxidation. Because the formation of oxidants is a prerequisite for the activation of PARP-1, these data indicate that PARP-1 may enhance its own activation by favoring the formation of oxidants.

The beneficial effects were obtained while PJ-34 administration was delayed after the instillation of LPS, which is relevant to the clinical scenario. Thus, although part of the effects attributed to PARP-1 suppression are related to an interference with early phenomena, such as the transcriptional activation of proinflammatory genes, our data indicate that pharmacological inhibition of PARP remains a valid therapeutic option in the posttreatment paradigm. PARP-1 inhibition may represent a novel, useful adjunct for the therapy of clinical conditions associated with severe pulmonary inflammation, most notably ARDS.

References

- De Murcia G, Shall S, editors. From DNA damage and stress signaling to cell death; poly ADP-ribosylation reactions. Oxford, England: Oxford University Press; 2000.
- Szabo C, editor. Cell death: The role of PARP. Boca Raton, FL: CRC Press; 2000.
- Szabo C, Dawson VL. Role of poly(ADP-ribose) synthetase in inflammation and ischaemia-reperfusion. *Trends Pharmacol Sci* 1998;19:287-298.
- Ha HC, Snyder SH. Poly(ADP-ribose) polymerase is a mediator of necrotic cell death by ATP depletion. *Proc Natl Acad Sci USA* 1999;96:13978-13982.
- Hassa PO, Hottiger MO. A role of poly(ADP-ribose) polymerase in NF-kappaB transcriptional activation. *Biol Chem* 1999;380:953-959.
- Oliver FJ, Menissier-de Murcia J, Nacci C, Decker P, Andriantsitohaina R, Muller S, de la Rubia G, Stoclet JC, de Murcia G. Resistance to endotoxic shock as a consequence of defective NF-kappaB activation in poly(ADP-ribose) polymerase-1 deficient mice. *EMBO J* 1999;18:4446-4454.
- Kollef MH, Schuster DP. The acute respiratory distress syndrome. *N Engl J Med* 1995;332:27-37.
- Wizemann TM, Gardner CR, Laskin JD, Quinones S, Durham SK, Goller NL, Ohnishi ST, Laskin DL. Production of nitric oxide and peroxynitrite in the lung during acute endotoxemia. *J Leukocyte Biol* 1994;56:759-768.
- Kooy NW, Royall JA, Ye YZ, Kelly DR, Beckman JS. Evidence for in vivo peroxynitrite production in human acute lung injury. *Am J Respir Crit Care Med* 1995;151:1250-1254.
- Abdelkarim GE, Gertz K, Harms C, Katchanov J, Dirnagl U, Szabo C, Endres M. Protective effects of PJ34, a novel, potent inhibitor of poly(ADP-ribose) polymerase (PARP) in vitro and in vivo models of stroke. *Int J Mol Med* 2001;7:255-260.
- Garcia Soriano F, Virag L, Jagtap P, Szabo E, Mabley JG, Liaudet L, Marton A, Hoyt DG, Murthy KG, Salzman AL, et al. Diabetic endothelial dysfunction: the role of poly(ADP-ribose) polymerase activation. *Nat Med* 2001;7:108-113.
- Liaudet L, Soriano FG, Szabo E, Virag L, Mabley JG, Salzman AL, Szabo C. Protection against hemorrhagic shock in mice genetically deficient in poly(ADP-ribose) polymerase. *Proc Natl Acad Sci USA* 2000;97:10203-10208.
- Harrod KS, Mounday AD, Whitsett JA. Adenoviral E3-14.7K protein in LPS-induced lung inflammation. *Am J Physiol Lung Cell Mol Physiol* 2000;278:L631-L639.
- Borron P, McIntosh JC, Korfhagen TR, Whitsett JA, Taylor J, Wright JR. Surfactant-associated protein A inhibits LPS-induced cytokine and nitric oxide production in vivo. *Am J Physiol Lung Cell Mol Physiol* 2000;278:L840-L847.
- Bakondi E, Bai P, Szabó É, Hunyadi J, Gergely P, Szabó C, Virág L. Detection of poly(ADP-ribose) polymerase activation in oxidatively stressed cells and tissues by using biotinylated NAD substrate. *J Histochem Cytochem*. (In press)
- Martin TR. Cytokines and the acute respiratory distress syndrome (ARDS): a question of balance. *Nat Med* 1997;3:272-273.
- Liaudet L, Soriano FG, Szabo C. Biology of nitric oxide signaling. *Crit Care Med* 2000;28:N37-N52.
- Szabo C. DNA strand breakage and activation of poly-ADP ribosyltransferase: a cytotoxic pathway triggered by peroxynitrite. *Free Radic Biol Med* 1996;21:855-869.
- Pulido EJ, Shames BD, Selzman CH, Barton HA, Banerjee A, Bensard DD, McIntyre RC Jr. Inhibition of PARS attenuates endotoxin-induced dysfunction of pulmonary vasorelaxation. *Am J Physiol* 1999;277:L769-L776.
- Szabo A, Salzman AL, Szabo C. Poly(ADP-ribose) synthetase activation mediates pulmonary microvascular and intestinal mucosal dysfunction in endotoxin shock. *Life Sci* 1998;63:2133-2139.
- Said SI, Berisha HI, Pakbaz H. Excitotoxicity in the lung: N-methyl-D-aspartate-induced, nitric oxide-dependent, pulmonary edema is attenuated by vasoactive intestinal peptide and by inhibitors of poly(ADP-ribose) polymerase. *Proc Natl Acad Sci USA* 1996;93:4688-4692.
- Szabo C, Saunders C, O'Connor M, Salzman AL. Peroxynitrite causes energy depletion and increases permeability via activation of poly(ADP-ribose) synthetase in pulmonary epithelial cells. *Am J Respir Cell Mol Biol* 1997;16:105-109.
- Virag L, Salzman AL, Szabo C. Poly(ADP-ribose) synthetase activation mediates mitochondrial injury during oxidant-induced cell death. *J Immunol* 1998;161:3753-3759.
- Virag L, Scott GS, Cuzzocrea S, Marmer D, Salzman AL, Gzabo C. Peroxynitrite-induced thymocyte apoptosis: the role of caspases and poly(ADP-ribose) synthetase (PARS) activation. *Immunology* 1998;94:345-355.
- Szabo C, Wong H, Bauer P, Kirsten E, O'Connor M, Zingarelli B, Mendelejev J, Hasko G, Vizi E, Salzman A, et al. Regulation of components of the inflammatory response by 5-iodo-6-amino-1,2-benzopyrone, an inhibitor of poly(ADP-ribose) synthetase and pleiotropic modifier of cellular signal pathways. *Int J Oncol* 1997;1093-1101.
- Roebuck KA, Rahman A, Lakshminarayanan V, Janakidevi K, Malik AB. H2O2 and tumor necrosis factor-alpha activate intercellular adhesion molecule 1 (ICAM-1) gene transcription through distinct cis-regulatory elements within the ICAM-1 promoter. *J Biol Chem* 1995;270:18966-18974.
- Kannan P, Yu Y, Wankhade S, Tainsky MA. PolyADP-ribose polymerase is a coactivator for AP-2-mediated transcriptional activation. *Nucleic Acids Res* 1999;27:866-874.
- Butler AJ, Ordahl CP. Poly(ADP-ribose) polymerase binds with transcription enhancer factor 1 to MCAT1 elements to regulate muscle-specific transcription. *Mol Cell Biol* 1999;19:296-306.
- Nie J, Sakamoto S, Song D, Qu Z, Ota K, Taniguchi T. Interaction of OCT-1 and automodification domain of poly(ADP-ribose) synthetase. *FEBS Lett* 1998;424:27-32.
- Oei SL, Griesenbeck J, Schweiger M, Babich V, Kropotov A, Tomilin N. Interaction of the transcription factor YY1 with human poly(ADP-ribose) transferase. *Biochem Biophys Res Commun* 1997;240:108-111.
- Abraham E, Carmody A, Shenkar R, Arcaroli J. Neutrophils as early immunologic effectors in hemorrhage- or endotoxemia-induced acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L1137-L1145.
- Heflin AC Jr, Brigham KL. Prevention by granulocyte depletion of increased vascular permeability of sheep lung following endotoxemia. *J Clin Invest* 1981;68:1253-1260.
- Zingarelli B, Szabo C, Salzman AL. Blockade of poly(ADP-ribose) synthetase inhibits neutrophil recruitment, oxidant generation, and mucosal injury in murine colitis. *Gastroenterology* 1999;116:335-345.
- Zingarelli B, Salzman AL, Szabo C. Genetic disruption of poly(ADP-ribose) synthetase inhibits the expression of P-selectin and intercellular adhesion molecule-1 in myocardial ischemia/reperfusion injury. *Circ Res* 1998;83:85-94.
- Strieter RM, Kunkel SL, Keane MP, Standiford TJ. Chemokines in lung injury: Thomas A. Neff Lecture. *Chest* 1999;116:103S-110S.
- Gerard C, Frossard JL, Bhatia M, Saluja A, Gerard NP, Lu B, Steer M. Targeted disruption of the beta-chemokine receptor CCR1 protects against pancreaticitis-associated lung injury. *J Clin Invest* 2002;110:2022-2027.
- Standiford TJ, Kunkel SL, Lukacs NW, Greenberger MJ, Danforth JM, Kunkel RG, Strieter RM. Macrophage inflammatory protein-1 alpha mediates lung leukocyte recruitment, lung capillary leak, and early mortality in murine endotoxemia. *J Immunol* 1995;155:1515-1524.
- Virag L, Jagtap P, Mabley J, Szabo E, Hasko G, Hoyt D, Salzman AL, Southan G, Soriano FG, Liaudet L, et al. Cytoprotective effects of novel phenanthridinone inhibitors of poly(ADP-ribose) polymerase (Abstract). *FASEB J* 2001;15:A567.
- Donnelly SC, Strieter RM, Kunkel SL, Walz A, Robertson CR, Carter DC, Grant IS, Pollok AJ, Haslett C. Interleukin-8 and development of adult respiratory distress syndrome in at-risk patient groups. *Lancet* 2001;341:643-647.
- Donnelly TJ, Meade P, Jagels M, Cryer HG, Law MM, Hugli TE, Shoemaker WC, Abraham E. Cytokine, complement, and endotoxin profiles associated with the development of the adult respiratory distress syndrome after severe injury. *Crit Care Med* 1994;22:768-776.
- Aggarwal A, Baker CS, Evans TW, Haslam PL. G-CSF and IL-8 but not GM-CSF correlate with severity of pulmonary neutrophilia in acute respiratory distress syndrome. *Eur Respir J* 2000;15:895-901.
- Bachofen M, Weibel ER. Structural alterations of lung parenchyma in the adult respiratory distress syndrome. *Clin Chest Med* 1982;3:35-56.
- Artigas A, Bernard GR, Carlet J, Dreyfuss D, Gattinoni L, Hudson L, Lamy M, Marini JJ, Matthay MA, Pinsky MR, et al. The American-European Consensus Conference on ARDS. Part 2: Ventilatory, pharmacologic, supportive therapy, study design strategies, and issues related to recovery and remodeling. Acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1998;157:1332-1347.
- Brochard L, Roudot-Thoraval F, Roupie E, Delclaux C, Chastre J, Fernandez-Mondejar E, Clementi E, Mancebo J, Factor P, Matamis D, et al. Tidal volume reduction for prevention of ventilator-induced lung injury in acute respiratory distress syndrome. The Multicenter Trial Group on Tidal Volume Reduction in ARDS. *Am J Respir Crit Care Med* 1998;158:1831-1838.