Deletion of peroxiredoxin 6 potentiates lipopolysaccharide-induced acute lung injury in mice*

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Objective: To investigate the role and signaling pathway of peroxiredoxin 6, a newly identified peroxidase, in lipopolysaccharide-induced acute lung injury.

Design: Prospective, randomized, controlled study.

Setting: Research laboratory.

Subjects: Peroxiredoxin 6 (−/−) and wild-type C57BL/6 mice.

Interventions: Wild-type or peroxiredoxin 6 (−/−) mice were challenged by intratracheal instillation of lipopolysaccharide (5 mg/kg) for 4 hrs or 24 hrs for lung injury measurement. In other studies, peritoneal macrophages, isolated from wild-type and peroxiredoxin 6 (−/−) mice, were preincubated in presence or absence of mitogen-activated protein kinases inhibitors for 30 mins before being stimulated with lipopolysaccharide (1 μg/ml) for 4 hrs.

Measurements and Main Results: Bronchoalveolar lavage myeloperoxidase activity and the lung injury score were significantly increased in peroxiredoxin 6 (−/−) mice compared with wild-type mice after lipopolysaccharide instillation at both 4 hrs and 24 hrs. Hydrogen peroxide and malondialdehyde levels, as well as nuclear factor-κB activities, tumor necrosis factor-α, interleukin-1β, and matrix metalloproteinase-9 messenger RNA, protein concentration, and activities were significantly increased whereas total antioxidative capability was markedly decreased in lungs of peroxiredoxin 6 (−/−) mice compared with wild-type mice. In vitro studies showed intracellular reactive oxygen species levels and release of tumor necrosis factor-α, interleukin-1, and matrix metalloproteinase-9 were significantly increased in macrophages from peroxiredoxin 6 (−/−) mice compared with that from wild-type mice after lipopolysaccharide stimulation. Cytokines release was partially suppressed by extracellular signal-regulated kinase and c-Jun N-terminal kinase inhibitors, but not by the p38 mitogen-activated protein kinase inhibitor.

Conclusions: Deletion of peroxiredoxin 6 exagerrates lipopolysaccharide-induced acute lung injury and inflammation with increased oxidative stress, inflammatory responses, and matrix degradation, all of which were partially dependent on nuclear factor-κB, extracellular signal-regulated kinase, and c-Jun N-terminal kinase pathways. (Crit Care Med 2011; 39:756–764)

Key Words: peroxiredoxin 6; acute lung injury; reactive oxygen species; inflammation; matrix metalloproteinase

Lung infection is a major risk factor of acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) development with high mortality and morbidity in critically ill patients (1). Lipopolysaccharide (LPS), an important virulence factor of Gram-negative bacteria, has been shown to increase capillary permeability, interstitial and alveolar edema, and a buildup of circulating inflammatory cells in lungs (2, 3) in vivo through several mechanisms (4–8). LPS recruits activated neutrophils into the lungs and activates nuclear factor-κB (NF-κB), which leads to cytokine production (7). LPS also increases production of reactive oxygen species (ROS) to cause specific oxidative damage to deoxyribonucleic acid and protein (4) and also serve as intracellular signaling molecules to control the activation of NF-κB (6, 9) and also serve as intracellular signaling pathways such as mitogen-activated protein kinases (MAPKs) (10).

ROS are usually scavenged by peroxidases. Peroxiredoxins (Prdx’s) represent a novel superfamily of nonselelo peroxi-
dases that catalyze the reduction of a broad spectrum of peroxides (11). In con-
trast to the other five members of this family with two reactive cysteines, Prdx 6 has a single redox-active cysteine and is highly expressed in the lungs especially in Clara cells and alveolar epithelial type 2 cells. Prdx 6 acts as a scavenger to catalyze reduction of hydrogen peroxide (H₂O₂), fatty acid hydroperoxides, and phospholipid hydroperoxides (12). Like other Prdx family members (13, 14), Prdx 6 has been studied intensively for its anti-
oxidant effects (15), but the role of Prdx 6 in regulation of inflammatory re-
sponses in lungs is poorly understood. Recently, it has been reported that Prdx 3 (−/−) mice were more susceptible to LPS-induced ALI (16) and that Prdx 2 is protective in LPS-induced lethal shock (17). However, the specific functions of Prdx 6 in the regulation of endotoxin-
induced inflammatory responses and the possible mechanisms in ALI and ARDS have not been reported.

The aim of this study was to explore the roles of Prdx 6 in LPS-induced ALI and inflammation. By studying Prdx 6 (−/−) cells and mice, we found that Prdx 6 deficiency exacerbated LPS-induced ALI...
and inflammation by modulating inflammatory gene expression through endogenous ROS-dependant activation of the NF-κB, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (c-JNK) pathways.

**MATERIALS AND METHODS**

*Animals and LPS-induced ALI Model.* Prdx 6 (−/−) and littermate C57BL/6J male mice at age from 8 to 12 wks and body weight 22 ± 2 g, were used in this study. Breeding and genotyping have been described previously (18). ALI was induced by intratracheal instillation of 5 mg/kg O55:B5 Escherichia coli LPS (Sigma, St. Louis, MO) by a gavage feeding needle (24 G) as done previously (19) after anesthesia with ketamine (80 mg/kg) and angesia with xylazine (30 mg/kg), both injected intraperitoneally. Mice were euthanized 4 hrs or 24 hrs after LPS or vehicle administration, and mice without any treatment were served as negative controls. The animal protocol was approved by the Animal Care Committee of Fudan University.

**Lung Morphology.** Lung tissue slides were stained with hematoxylin and eosin for histologic evaluation. Severity of lung injury was scored as follows: no injury = 1; injury to 25% of the field = 2; injury to 50% of the field = 3; injury to 75% of the field = 4; and diffuse injury = 5. All samples were reviewed by three independent pathologists who were blinded to the sample identity.

**Bronchoalveolar Lavage.** Bronchoalveolar lavage was done by intratracheal instillation of 1.0 mL of PBS into the lungs in situ with gentle and repeated flushing three times. The bronchoalveolar lavage fluid (BALF) was then centrifuged at 1,800 rpm for 10 min, and the supernant was frozen at −80°C for subsequent analysis. The concentration of protein in the BALF was measured by using the Micro BCA Protein Assay Kit (Pierce, MA).

**Peritoneal Macrophage Isolation and Experimental Groups.** Cells collected from peritoneal cavity lavage were suspended in Dulbecco’s modified eagle medium (Invitrogen, Carlsbad, CA) medium with 10% fetal bovine serum (Invitrogen, Carlsbad, CA), in the presence of penicillin (100 units/mL) and streptomycin (100 μg/mL) for 4–6 hrs. Approximately 90% adherent cells after washing with PBS were mainly peritoneal macrophages by morphology examination. The macrophages were then harvested and resuspended at density of 5 × 10^6 cells/well in six-well plates and 1 × 10^5 cells/well in 24-well plates for 4 hrs. Some cells were treated with 1 μg/mL LPS whereas untreated cells were served as control. The ERK1/2 inhibitor PD98059 (5 μM/mL; catalog no. 9900, Cell Signaling Technology, Danvers, MA), p38 MAPK inhibitor ML3404 (5 μM/mL; catalog no. 506121Calbiochem, La Jolla, CA) and c-JNK inhibitor SP600125 (5 μM/mL; cat- log no. 420119; Calbiochem, La Jolla, CA) were added to culture medium in wells 30 mins before LPS stimulation. Four hours later, cultured supernatants in each well were collected for tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and matrix metalloproteinase-9 (MMP-9) quantity test, and cells were lysed by using Trizol reagent for gene expression measurement.

**Determination of Intracellular ROS.** Cells from six-well plates were harvested for measurement of intracellular ROS levels by using probe dichlorodihydrofluorescein diacetate (DCFH-DA) (Gemed Sciences, Arlington, MA) according to reported methods (20) for flow cytometry analysis.

**Neutrophil Infiltration and Oxidative Stress.** Activity of lung myeloperoxidase (MPO) has been used to show pulmonary neutrophil infiltration and activation (21). Levels of ROS in the lungs were quantified by measurement of H₂O₂. Lipid and protein peroxidation were measured as levels of malondialdehyde and protein carbonylation. MPO, H₂O₂, malondialdehyde, protein carbonyl, total superoxide dismutase (SOD) activity, and total antioxidative capacity in lungs were measured by using assay kits from the manufacture (Jiancheng, Nanjing, China) (22, 23).

**NF-κB Activity Measurement.** Nuclear protein was extracted by using Nuclear Extract kit (Active Motif, Carlsbad, CA). P65 deoxyribonucleic acid-binding activity was measured by TransAM kit according to the manufacturer’s instructions (Active Motif, Carlsbad, CA).

*Real-Time Reverse Transcription-Polymerase Chain Reaction.* Total RNA from lungs and cells were extracted with Trizol reagent (Invitrogen, Carlsbad, CA). RNA (2 μg) was reversely transcribed to complementary deoxyribonucleic acid and 40 ng of complementary deoxyribonucleic acid was used as a template for real-time reverse transcription-polymerase chain reaction in ABI PRISM 7900. Gene-specific oligonucleotides were designed by using Applied Biosystems (Carlsbad, CA) Primer Express 2.0 software as previously described (24). The primer sequences of β-actin, TNF-α, IL-1β, and MMP-2 and -9 are shown in Table 1. The amplification cycling reactions (40 cycles) were performed as follows: 15 secs at 95°C and 1 min at 60°C. Relative quantification values of the target genes were standardized according to the comparative threshold cycle (2^−ΔΔCt). Enzyme-Linked Immunosorbent Assay. TNF-α, IL-1β, and MMP-9 concentrations in BALF or cell culture supernatant were determined by using commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) according to the manufacturer’s protocol.

**Zymography.** MMP-9 and MMP-2 activities in mice lungs were assayed by zymography as previously described (22). The activated forms were identified at 83 kDa for MMP-9 and 62 kDa for MMP-2. Quantification of MMP-9 and MMP-2 activities was done by using the Alphalager 2200 software.

**Statistical Analyses.** Data were shown as mean ± so. Statistical significance was analyzed by SPSS software (IBM, Somers, NY). Group differences were evaluated by one-way analysis of variance or by Student’s t test. The histologic semiquantitative analysis was done by the nonparametric Mann-Whitney U test. Differences between mean values were considered statistically significant at p < .05.

**RESULTS**

*LPS Induced Significantly More Lung Injury in Prdx 6 (−/−) Mice.* To determine the roles of Prdx 6 in LPS-induced lung injury, we evaluated the responses of Prdx 6 (−/−) mice and wild-type (WT) mice after intratracheal LPS exposure. Increased histopathology damage, as defined by increased protein (fibrin) and polymorphonuclear cells in alveoli, bronchial wall thickening was observed in the lungs of Prdx 6 (−/−) mice compared with WT mice (Fig. 1A). Both WT and Prdx 6 (−/−) mice showed more severe lung injury at 24 hrs than that at 4 hrs. Inflammation scores in Prdx 6 (−/−) mice were significantly higher than that in WT mice (Fig. 1B). LPS instillation induced an elevated protein leakage in

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**Table 1.** Primer sets used for quantitative reverse transcriptase-polymerase chain reaction

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence (Forward/Reverse)</th>
<th>Primer Positions</th>
<th>Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>ACGGCCAGCTCATCAGCTATGT/ TGGATGGCAGCAGCATGTCCTAT</td>
<td>811–904</td>
<td>X03672</td>
</tr>
<tr>
<td>IL-1β</td>
<td>CACGCGGCTCCGGAGATATGCAC/ AGCTGGAGAGCTTTCAGCTA</td>
<td>372–443</td>
<td>NM_008361</td>
</tr>
<tr>
<td>TNF-α</td>
<td>ATCCGGACCTGGAACCT/ ACACGGCTGAGCTTTCCAGTATA</td>
<td>19–88</td>
<td>NM_013693</td>
</tr>
<tr>
<td>MMP-2</td>
<td>CACCTCAAGAAGTGACAGGAT/ GGATGCCGATGGTCCTGAA</td>
<td>218–298</td>
<td>NM_008610</td>
</tr>
<tr>
<td>MMP-9</td>
<td>CACAGGTCACCGCCCTTCTACT/ CACAGGTCACCGCTTACTACG</td>
<td>1170–1243</td>
<td>NM_013599</td>
</tr>
</tbody>
</table>
BALF, which is a key hallmark of ARDS and reflects a loss of epithelial basement membrane integrity and an increased vascular leakage. Furthermore, significantly increased protein levels in BALF were observed in Prdx 6 (-/-) mice at 24 hrs compared with WT mice (Fig. 1C).

To study LPS-induced lung inflammatory responses, lung MPO activity, which is a marker of pulmonary neutrophil infiltration and activation, was measured in both WT and Prdx 6 (-/-) mice. LPS instillation caused a significant increase of MPO activity in Prdx 6 (-/-) mice compared with WT mice both at 4 hrs and 24 hrs (Fig. 1D).

Prdx 6 Deficiency Markedly Enhanced LPS-Induced Oxidative Stress in Lungs. Oxidative stress is one main mechanism of ALI. Prdx 6 is a peroxidase; therefore, we tested oxidative stress parameters to determine Prdx 6 antioxidative roles in protecting LPS-induced ALI. Total SOD activity was decreased after LPS instillation although without statistical difference between Prdx 6 (-/-) mice and WT mice (Fig. 2A). However, H2O2, a reduced product from superoxide anion by SOD, was significantly higher in Prdx 6 (-/-) mice compared with WT mice (Fig. 2B). Markedly increased ROS, shown as H2O2 level in Prdx 6 (-/-) mice, resulted in more lipid and protein peroxidation compared with WT mice, as indicated by higher levels of malondialdehyde (Fig. 2C) and protein carbonylation (Fig. 2D). Therefore, lungs of Prdx 6 (-/-) mice exhibited a significant decrease in total antioxidative capacity as compared with WT mice after LPS stimulation (Fig. 2E). These results demonstrate that Prdx 6 contributes to LPS-induced oxidative stress in lungs.

Enhanced NF-κB Activity and Cytokine Expression in Lungs and BALF of Prdx 6 (-/-) Mice. In ALI, activation of transcription factors such as NF-κB and enhanced production of proinflammatory cytokines such as TNF-α and IL-1β are ROS dependent (9, 25–27). Of note, NF-κB activation and TNF-α and IL-1β production is a cascade reaction. NF-κB activity in lungs was significantly increased after LPS challenge, reaching a maximal point at 4 hrs and was much higher in Prdx 6 (-/-) mice compared with WT mice (Fig. 3A).

Because absence of Prdx 6 increased LPS-induced production of ROS and NF-κB, we further tested inflammatory cytokine expression. Consistent with increased activity of NF-κB in LPS-challenged Prdx 6 (-/-) mice, lung TNF-α and IL-1β messenger ribonucleic acid (mRNA) levels were significantly increased in a time-dependent manner after LPS challenge compared with vehicle administration, which were significantly increased in Prdx 6 (-/-) mice compared with WT mice (Fig. 3B and C). Similarly, LPS instillation resulted in temporarily increased TNF-α and IL-1β protein expression compared with mice with vehicle treatment at 4 hrs, returning to nearly baseline at 24 hrs (Fig. 3D and E). Increased protein levels were also observed in Prdx 6 (-/-) mice at both time points, suggesting increased lung inflammation in these mice.
Figure 2. Enhanced oxidative stress markers in peroxiredoxin 6 [Prdx 6 (−/−)] mice. Prdx 6 (−/−) and wild-type (WT) mice were subjected to lipopolysaccharide (LPS) 5 mg/kg intratracheal instillation for 4 hrs and 24 hrs, and lung superoxide dismutase (SOD) activity (A), lung hydrogen peroxide (H2O2) (B), lipid peroxidation indicated as malondialdehyde (MDA) (C), protein peroxidation indicated as protein carbonyls (D), and levels of total antioxidative capacity (TAOC) (E) in Prdx 6 (−/−) mice and WT mice were determined. Values are expressed as means ± SD of n = 6 per group. *p < .05, **p < .01 as compared with vehicle-challenged mice; †p < .05, ††p < .01 as compared with LPS-challenged WT mice, and ‡‡p < .01 as compared with corresponding genotype mice at 4 hrs after LPS instillation.

Figure 3. Peroxiredoxin 6 (Prdx 6) deficiency increased lipopolysaccharide (LPS)-induced nuclear factor-κB (NF-κB) activity and cytokine production. Prdx 6 (−/−) and wild-type (WT) mice were subjected to LPS 5 mg/kg intratracheal instillation for 4 hrs and 24 hrs. A. NF-κB activity in Prdx 6 (−/−) and WT mice. B, C. Time course of messenger ribonucleic acid (mRNA) levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in lungs in Prdx 6 (−/−) mice and WT mice. D, E. Concentrations of TNF-α and IL-1β in bronchoalveolar lavage (BAL). Data were expressed as means ± SD of n = 6 per group. *p < .05, **p < .01 as compared with vehicle-challenged mice; †p < .05, ††p < .01 as compared with LPS-challenged WT mice, and ‡‡p < .01 as compared with corresponding genotype mice at 4 hrs after LPS instillation. OD, optical density.
**Prdx 6 (−/−) Mice Have Increased Alveolar-Vascular Permeability in Response to LPS.** As has been previously described, ALI/ARDS is associated with pronounced matrix degradation, which is responsible for increased alveolar-vascular permeability. Matrix degradation is mainly mediated by MMPs (28), which has been reported to be up-regulated in ALI/ARDS (22). We found that LPS challenge rapidly and significantly increased MMP-9 and MMP-2 mRNA expression in Prdx 6 (−/−) mice compared with WT mice at both 4 hrs and 24 hrs, whereas mRNA expression of MMP-9 in WT mice was only significantly increased at 24 hrs compared with vehicle-treated mice (Fig. 4A and B). Zymography showed that activities of MMP-9 were increased significantly during LPS exposure with a peak level at 4 hrs in both Prdx 6 (−/−) mice and WT mice compared with control, with significantly higher levels in Prdx 6 (−/−) mice compared with WT mice. In addition, there was no significant change of MMP-2 activity in both groups of mice (Fig. 4C–E). Thus, these data suggest that LPS-induced matrix degradation was significantly enhanced in absence of Prdx 6 in mice.

**Prdx 6 Deficiency Markedly Increased ROS Levels and LPS-induced Cytokine Expression in Macrophages.** ROS production was measured to determine the contribution of Prdx 6 in superoxide formation in macrophages. Intracellular ROS levels at baseline in macrophages from Prdx 6 (−/−) mice was 1.5- to 2.0-fold higher than that from WT mice (Fig. 5A), suggesting that lack of Prdx 6 gene reduces scavenging of ROS and leads to increased ROS production in macrophages from Prdx 6 (−/−) mice.

Activation of the MAPKs pathways plays an essential role in the mediation of macrophage responses to LPS (29). Therefore, we investigated whether MAPKs-signaling cascades could be affected by Prdx 6 during LPS exposure. Notably, LPS stimulation significantly increased mRNA and protein levels of TNF-α and IL-1β compared with vehicle-treated cells, with much higher levels in macrophages from Prdx 6 (−/−) mice than that in WT mice. The increased mRNA level of TNF-α was substantially inhibited by pretreatment with ERK1/2 inhibitor PD98059 or c-JNK inhibitor SP600125 in Prdx 6 (−/−) group and by pretreatment with c-JNK inhibitor SP600125 in WT group (Fig. 5C). The increased protein levels of TNF-α and IL-1β were abolished by pretreatment with PD98059, SP600125, or ML3403 in Prdx 6 (−/−) group while attenuated by pretreatment with PD98059 in WT group (Fig. 5D and E). Hence, these data demonstrate that Prdx 6 regulates LPS-induced cytokine expression in macrophages through MAPKs pathways.

**MMP-9 Expression Was Increased in LPS-stimulated Prdx 6 (−/−) Macrophages Through ERK and c-JNK Pathways.** The *in vitro* data indicated that Prdx 6 plays a regulatory role in LPS-activated MMP expression. The above data (Fig. 5) showed that MAPKs pathways are involved in Prdx 6-regulated inflammation. Whether Prdx 6 regulated LPS-induced MMP-9 activation through MAPKs pathways or not was not known in primary mouse macrophages. LPS stimulation significantly increased mRNA and protein levels of MMP-9 compared with vehicle-treated cells, with a significantly higher level in macrophages from Prdx 6 (−/−) mice compared with WT mice. Pretreatment with ERK1/2 inhibitor PD98059 or c-JNK inhibitor SP600125 abolished an LPS-induced increase of MMP-9 mRNA and protein levels in macrophages from Prdx 6 (−/−) mice and WT mice, but pretreatment with SP600125 did not
Figure 5. Peroxiredoxin 6 (Prdx 6) deficiency increased reactive oxygen species (ROS) levels and lipopolysaccharide (LPS)-induced cytokine production by macrophages. A, Intracellular ROS levels in macrophages were measured by dichlorodihydrofluorescein diacetate (DCFH-DA) for flow cytometry analysis. B, C, Messenger ribonucleic acid (mRNA) levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) in macrophages from Prdx 6 (-/-) mice, and wild-type (WT) mice were quantified before or after extracellular signal-regulated kinase 1/2 (PD), mitogen-activated protein kinase (ML), and Jun N-terminal kinase (SP) inhibitor application for 30 min followed by LPS (0 or 1 μg/mL) for 4 hrs. D, E, TNF-α and IL-1β protein concentrations in the culture media. Data were expressed as means ± SD of n = 6 per group. *p < .05, **p < .01 as compared with vehicle-challenged mice; †p < .05, ††p < .01 as compared with LPS-challenged macrophages from WT mice; ‡p < .05, ‡‡p < .01 as compared with macrophages from corresponding genotype mice at 4 hrs after LPS instillation.
change MMP-9 protein expression in macrophages from WT mice (Fig. 6A, B).

**DISCUSSION**

Our results support the initial hypothesis that deficiency of Prdx 6 causes increased susceptibility to LPS-induced ALI, as evidenced by more severe lung edema, increased protein leakage, and enhanced neutrophil accumulation in alveolar space. MPO activity was also significantly increased in Prdx 6 (−/−) mice after LPS challenge. In addition, we also found that, after LPS instillation, Prdx 6 (−/−) mice had intensified oxidative stress, elevated inflammatory gene expression, and increased matrix degradation compared with results in WT mice. *In vitro*, LPS stimulation of macrophages markedly increased inflammatory cytokine expression in Prdx 6 (−/−) macrophages compared with WT macrophages. The increased expression of MMP-9 induced by LPS was abolished in presence of inhibitors of ERK and c-JNK. Overall, these results appear to be related to loss of the Prdx 6 gene because the expression of other antioxidant enzymes was unchanged in Prdx 6 (−/−) mice (30).

These results demonstrate that Prdx 6 may protect LPS-induced lung injury by reducing inflammatory mediators and inflammatory cells infiltration.

It has been widely accepted that LPS-induced lung injury is partially through oxidative stress (6). Similar to our data, a decrease of SOD activity levels and increase of H₂O₂, lipid peroxidation and protein oxidation in injured lungs occurred in response to LPS stimulation (23). In general, H₂O₂ is mainly derived from reduction of superoxide anion by SOD followed by further reduction to H₂O by catalase, Prdx’s, etc. H₂O₂ mediates lipid and protein oxidation. In our study, no differences of the activity of SOD between Prdx 6 (−/−) and WT mice were found, similar to a previous report (30), suggesting that the significantly elevated level of H₂O₂ in Prdx 6 (−/−) mice might not be due to over production but due to limited reduction by Prdx 6 deficiency. The higher level of H₂O₂ thus resulted in more lipid peroxidation and protein oxidation of lung tissue as shown in other studies (30–32).

Total antioxidative capacity was significantly decreased in Prdx 6 (−/−) mice. Thus, Prdx 6 functions as an antioxidant enzyme to protect lung cells against LPS-induced inflammation.

Previous reports have shown that LPS and ROS production could lead to intensified proinflammatory cytokines release, such as TNF-α and IL-1β (25, 26, 33), and activation of transcription factors such as NF-κB (9, 27), each of them then could act as a stimulator to amplify a subsequent cascade signal. Of note, NF-κB activation and cytokines release from Prdx 6 (−/−) mice were significantly increased after LPS challenge, likely mediated by higher levels of ROS. Therefore, lung injury in Prdx 6 (−/−) mice was more severe than that in WT mice. Similar to our *in vitro* study, several other reports (17, 34) showed that Prdx’s could regulate cytokine production in response to LPS stimulation. Thus, Prdx 6 might modulate neutrophil inflammation through reducing cytokines release, suggesting that Prdx 6 can be considered as an anti-inflammatory enzyme as well as a bulk antioxidant.

Alveolar epithelial/capillary endothelial permeability is increased in ALI, usually accompanied with elevated protein concentration and polymorphonuclear neutrophils in BALF. In our study, LPS-treated Prdx 6 (−/−) mice presented higher protein concentrations in BALF and increased MPO activity in the lungs, which indicated that lung vascular endothelial/epithelial permeability might be increased in Prdx 6 (−/−) mice compared with WT mice. Degradation of extracellular matrix components is an important mechanism for increased vascular endothelial permeability mediated by MMPs. MMPs are mainly secreted by macrophages, PMNs, and structural cells in response to inflammatory stimuli such as LPS’ proinflammatory cytokines or ROS (35–37) through the NF-κB (38, 39) pathway. In the present study, MMP-9 mRNA expression and protein activation were more markedly increased in LPS-induced lung injury in Prdx 6 (−/−) mice, which were probably due to more ROS production and TNF-α secretion through more activated NF-κB pathway in Prdx 6 (−/−) mice. Consistent with our results, negative relationships between Prdx 3 and Prdx 4 with MMPs have been reported (40, 41) in other studies. Therefore, MMP-9 rather than MMP-2 played pivotal role in extracellular matrix
H2O2 thus leads to excessive lipid and
ular macrophages in response to LPS
Prdx
intraperitoneal macrophages contain
may not represent a full profile of live
ROS dependant-manner (46). In our
tribute to MMP-9 production (43–45) in a
nase, ERK, and c-JNK pathways may con-
ings (22, 42).

deletion evidenced by our and other find-
illustration of working hypothesis. 

Figure 7. 

ACKNOWLEDGMENTS

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sously gifting Prdx 6 knockout mice.

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ischemia/reperfusion produces lung injury

CONCLUSIONS

There are some limitations in this
study, such as LPS-induced lung injury
may not represent a full profile of live
bacteria-induced lung injury. Although
intraperitoneal macrophages contain
Prdx 6 and have similar function as alve-
olar macrophages in response to LPS
stimulation, direct evidence from alve-
olar macrophages is needed.

In conclusion, the present study dem-
onstrates that Prdx 6 plays key role in
regulating LPS-induced ALI and inflam-
mation in mice, which suggested Prdx 6
might be a new target for ALIARDS

CONCLUSIONS

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study, such as LPS-induced lung injury
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intraperitoneal macrophages contain
Prdx 6 and have similar function as alve-
olar macrophages in response to LPS
degradation in the lungs after Prdx 6
deletion evidenced by our and other find-
ings (22, 42).

Several studies reported that p38 ki-

nase, ERK, and c-JNK pathways may con-
tribute to MMP-9 production (43–45) in a
ROS dependant-manner (46). In our
study, macrophages from Prdx 6 (−/−)
mice presented a higher ROS level, which
might mediate more LPS-induced MMP-9
mRNA and protein expression. Our find-
ings also showed that treatment with ei-
ther PD98059 (inhibitor of ERK) or
SP600125 (inhibitor of c-JNK) down-
regulated LPS-induced MMP-9 expres-
sion. Thus, JNK and ERK1/2 but not p38
MAPK pathways are involved in the reg-
ulation of MMP-9 production in LPS-
duced inflammation and deletion of
Prdx 6 increased MMP-9 expression
through up-regulation of ROS.

The working hypothesis is that (Fig. 7)
deletion of Prdx 6 results in impaired
degradation/scavenge of H2O2 since H2O2
is the substrate of Prdx 6. The increased
H2O2 thus leads to excessive lipid and
protein oxidation and cytokine produc-
tion. H2O2 could also activate NF-κB,
ERK, and c-JNK pathways to activate
MMP-9 and thus induce matrix degrada-
tion and increased alveolar-capillary per-
meability.

CONCLUSIONS

There are some limitations in this
study, such as LPS-induced lung injury
may not represent a full profile of live
bacteria-induced lung injury. Although
intraperitoneal macrophages contain
Prdx 6 and have similar function as alve-
olar macrophages in response to LPS

Figure 7. Illustration of working hypothesis. 

NF-κB, nuclear factor-κB; TNF-α, tumor necrosis
factor-α; c-Jun N-terminal kinase; MMP, matrix metalloproteinase; mRNA, messenger ribonu-
cleic acid; SOD, superoxide dismutase; LPS, lipopolysaccharide; IL-1β, interleukin-1β; H2O2, hydrogen
peroxide; A-C, alveolar capillary.
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