

# Decreased Expression of Peroxisome Proliferator-Activated Receptor $\gamma$ in Endotoxin-Induced Acute Lung Injury

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## Summary

Peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ), a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors, possesses anti-inflammatory properties. The purpose of the present study was to investigate the profile of PPAR- $\gamma$  expression in the lung and to explore its functional significance in lipopolysaccharide (LPS)-induced acute lung injury. Thirty male Wistar rats were randomly assigned to one of the following five groups: saline control group and different LPS groups (2 h, 4 h, 6 h and 8 h after LPS 6 mg/kg i.v.). At predefined time points, blood samples were collected to measure plasma level of tumor necrosis factor (TNF)- $\alpha$  and lungs were removed to assay histopathological changes, wet-to-dry weight (W/D) ratio, myeloperoxidase (MPO) activity and TNF- $\alpha$  level. Expression of PPAR- $\gamma$  and activation of nuclear factor (NF)- $\kappa$ B p65 in lung tissues were also examined in each group. LPS injection resulted in marked lung damage and elevated levels of W/D ratio and MPO activity in the lung. Increased levels of TNF- $\alpha$  were also observed in the plasma and lung. These inflammatory events were associated with reduced expression of PPAR- $\gamma$  protein and with activation of NF- $\kappa$ B in the lung. Our data suggest that decreased expression of PPAR- $\gamma$  protein in lungs may contribute to the ongoing pulmonary inflammation and tissue injury in endotoxemia.

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## Key words

Peroxisome proliferator-activated receptor- $\gamma$  • Endotoxin • Acute lung injury • Nuclear factor- $\kappa$ B

## Introduction

Severe sepsis results in multiple organ dysfunctions including acute lung injury (Balibrea and Arias-Díaz 2003). While the exact mechanisms of sepsis-induced acute lung injury remain undefined, it is generally accepted that bacterial endotoxin (lipopolysaccharide, LPS) released into the circulation activates interconnected inflammatory cascades in the lung, ultimately leading to lung damage (Ghosh *et al.*

1993, Kabir *et al.* 2002, Bhatia and Moochhala 2004).

Peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors with pleiotropic effects on lipid and glucose homeostasis, cellular proliferation and inflammation control (Blanquart *et al.* 2003). There are two known isoforms of PPAR- $\gamma$ : PPAR- $\gamma$ 1 and PPAR- $\gamma$ 2. PPAR- $\gamma$ 2 is mostly expressed in adipose tissue while PPAR- $\gamma$ 1 is more widely distributed including lungs (Michael *et al.* 1997). Various PPAR- $\gamma$

agonists have been reported to possess anti-inflammatory properties *in vitro* and *in vivo* (Jiang *et al.* 1998, Guyton *et al.* 2003, Cuzzocrea *et al.* 2004, Genovese *et al.* 2005). More recently, we have also demonstrated that rosiglitazone, a synthetic PPAR- $\gamma$  agonist, exerts protective effect against acute lung injury in endotoxemic rats (Liu *et al.* 2005). In addition, previous studies have suggested that PPAR- $\gamma$  expression may be altered by an inflammatory process (Hill *et al.* 1997, Katayama *et al.* 2003). However, little is known about the kinetics of the expression of intrapulmonary PPAR- $\gamma$  and its association with local proinflammatory responses in the development of endotoxin-induced acute lung injury.

Thus, the present study was undertaken to investigate the time course profile of PPAR- $\gamma$  expression in lungs of rats with endotoxin-induced acute lung injury and to determine its relationship to pulmonary inflammation.

## Methods

### Animals

Male pathogen-free Wistar rats weighing 180–220 g were obtained from the Laboratory Animal Center of Tongji Medical College, China. All rats were fed a standard laboratory chow and were provided water *ad libitum* until the day of the experiment. The Animal Use and Care Committee of our University approved the experimental protocol.

### Experimental protocol

All animals were anesthetized with sodium pentobarbital (50 mg/kg *i.p.*), and a 24-gauge angiocatheter was inserted into the right external jugular vein for the administration of fluid and drugs. Rats were randomly assigned to one of five experimental groups ( $n = 6$  per group): control, LPS 2 h, LPS 4 h, LPS 6 h, or LPS 8 h. Animals in the four LPS groups received 6 mg/kg of LPS (*E. coli* serotype O55:B5, Sigma-Aldrich, USA) intravenously and control animals received only the vehicle (normal saline). Previous studies have shown that LPS at the selected dose induced neutrophilic lung inflammation, edema, and parenchymal injury in rats (Yoshinari *et al.* 2001, Tian *et al.* 2004). During the course of experiment, rats were given warm saline solution (1.5 ml·kg<sup>-1</sup>·h<sup>-1</sup> *i.v.*) to maintain hydration and breathed spontaneously without any mechanical ventilatory support or supplementary oxygen. Rectal temperature was maintained between 37.0 and 38.5 °C by

a heating lamp located above the animal.

### Lung perfusion and lung tissue procurement

At predefined time points (2, 4, 6, and 8 h after LPS injection or immediately after saline application), the rats were killed by exsanguinations. Blood sample were collected *via* the right carotid artery in heparinized syringes, and plasma was prepared by centrifugation and stored at –20 °C. Subsequently, the thorax was opened by a midline thoracotomy and the pulmonary circulation was flushed with 10 ml of cold saline (4 °C) *via* right ventricle injection. Then, the lungs were removed *en bloc*. The right lung lower lobe was fixed in 10 % (w/v) phosphate-buffered saline (PBS) buffered formaldehyde solution (pH 7.0) until processing in paraffin wax, and the left lung was cut off, snap frozen in liquid nitrogen, and stored at –70 °C until subsequent analysis.

### Histopathological examination

Paraffin-embedded lungs were sectioned at 4  $\mu$ m, stained with hematoxylin and eosin, and examined under a light microscope. A blinded pathologist scored lung injury according to the following four aspects: alveolar congestion, hemorrhage, infiltration or aggregation of neutrophils in airspace or vessel wall, and thickness of alveolar wall/hyaline membrane formation. Each item was graded with five point scales based on the report previously (Takao *et al.* 2005): 0 = minimal (little) damage, 1 = mild damage, 2 = moderate damage, 3 = severe damage, and 4 = maximal damage. A total lung injury score was calculated as the sum of the four items. Thus, minimum and maximum possible scores were 0 and 16, respectively.

### Measurement of lung wet-to-dry weight ratio

To quantify the magnitude of pulmonary edema, we evaluate the wet/dry weight (W/D) ratio of the lung. Briefly, portions of the harvested wet lungs were weighed and then placed in an oven for 24 h at 80 °C, and weighed again when they were dry. The W/D ratio was then calculated.

### Measurement of lung myeloperoxidase activity

The activity of myeloperoxidase (MPO), an indicator of neutrophil accumulation, was determined by a modification of the method of Gray *et al.* (2003). Upon thawing, the lung tissues were homogenized in a phosphate buffer (20 mM, pH 7.4) and centrifuged at

30 000 $\times$ g for 30 min. The pellet was resuspended in another phosphate buffer (50 mM, pH 6.0) with 0.5 % hexadecyltrimethyl ammonium bromide. MPO activity in the resuspended pellet was assayed by measuring absorbance changes spectrophotometrically at 460 nm, using 0.167 mg/ml O-dianisidine hydrochloride and 0.0005 % hydrogen peroxide. One unit of MPO activity was defined as the quantity of enzyme degrading 1  $\mu$ mol of peroxide/min, and results were expressed as units per gram of wet tissue.

#### *Measurement of plasma and lung levels of TNF- $\alpha$*

TNF- $\alpha$  levels in plasma and lung homogenate was determined using an enzyme-linked immunosorbent assay kit for rat TNF- $\alpha$  according to the manufacturer's instructions (R & D Systems, Minneapolis, MN, USA).

#### *Reverse transcription-polymerase chain reaction (RT-PCR)*

Total RNA was isolated from lung tissue using the Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol. First-strand complementary DNA (cDNA) was synthesized using random primers and the AMV reverse transcriptase (Promega Corp., USA). RT-generated cDNA encoding PPAR $\gamma$ 1, PPAR $\gamma$ 2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified using PCR. The housekeeping gene GAPDH was used as an internal standard. The primers used were specific for rat PPAR $\gamma$ 1 (forward: 5'-TGCTGGTGATCAGAAGGCTG-3'; reverse: 5'-ACGCAGGCTCTACTTTGATCG-3'), for PPAR $\gamma$ 2 (forward: 5'-CTTCGCTGATGCACTGCCTA-3'; reverse: 5'-ACGCAGGCTCTACTTTGATCG-3'), and for GAPDH (forward: 5'-CTCTACCCACGGCAAGTTCAA-3'; reverse: 5'-GGGATGACCTTGCCACAGC-3'). The expected PCR products of PPAR $\gamma$ 1, PPAR $\gamma$ 2 and GAPDH were 373, 287 and 516 base pairs in length, respectively. PCR reactions were initiated at 94 °C for 3 min, followed by 35 cycles of amplification (denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min) with a final primer extension at 72 °C for 7 min. The PCR products were separated on 2 % agarose gel and stained with ethidium bromide. The intensity of each PPAR $\gamma$ 1 and PPAR $\gamma$ 2 mRNA band was quantified by densitometry using a gel documentation and analysis system (GDS 8000, Ultra-Violet Products, UK) and normalized to values for GAPDH.

#### *Nuclear protein extraction and Western blot analysis*

Nuclear extracts of lung tissues were prepared

by hypotonic lysis followed by high salt extraction (Tian *et al.* 2004). Briefly, 100 mg of frozen lungs were homogenized in 0.5 ml of ice-cold buffer A, composed of 10 mM HEPES (pH 7.9), 10 mM KCl, 2 mM MgCl<sub>2</sub>, 0.1 mM EDTA, 1.0 mM dithiothreitol (DTT), and 0.5 mM phenylmethylsulfonyl fluoride (PMSF) (all from Sigma Co., USA). The homogenate was incubated on ice for 15 min, after which 50  $\mu$ l of 10 % Nonidet P-40 solution was added (Sigma Co.); the mixture was vortexed for 30 sec and centrifuged for 1 min at 6 000 $\times$ g at 4 °C. The crude nuclear pellet was resuspended in 200  $\mu$ l of buffer B, containing 20 mM HEPES (pH 7.9), 420 mM NaCl, 1.5 mM MgCl<sub>2</sub>, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, and 25 % (v/v) glycerol, and was incubated on ice for 30 min with intermittent mixing. The suspension was centrifuged at 12 000 $\times$ g at 4 °C for 15 min. The supernatant containing the nuclear proteins was collected and kept at -70 °C. The protein concentration was assayed by the Bradford procedure.

For Western blot analysis, nuclear protein (80  $\mu$ g) were electrophoresed in a 10 % SDS-PAGE, and transferred to a nitrocellulose membrane. The membranes were incubated with anti-PPAR- $\gamma$  antibody or anti-p65 subunit of NF- $\kappa$ B antibody (Santa Cruz Biotechnology, Santa Cruz, USA; 1:500 dilution) after non-specific binding sites had been blocked. Then, the blots were incubated with a horseradish peroxidase-labeled secondary antibody for 1 h at room temperature. Immunoreactive proteins were visualized using enhanced chemiluminescence detection (Amersham Biosciences, USA) and exposed to X-ray films. The densitometry results for PPAR- $\gamma$  and NF- $\kappa$ B p65 were assessed using gel documentation and analysis system (GDS8000, Ultra-Violet Products, UK) and expressed as the ratios to the mean of the control group.

#### *Immunohistochemistry*

Tissue sections (4  $\mu$ m thick) were prepared from paraffin embedded lung sample. After deparaffinization, endogenous peroxidase was quenched with 3 % hydrogen peroxide for 30 min. The sections were permeabilized with 0.1 % (w/v) Triton X-100 in PBS for 20 min. Non-specific adsorption was minimized by incubating the section in 5 % (v/v) normal goat serum in PBS for 20 min. Immunohistochemical staining was performed using indirect streptavidin/peroxidase technique (SP kit, Zymed Co., USA). The sections were incubated overnight with primary anti-PPAR $\gamma$  polyclonal antibody (Santa Cruz, 1:200 dilution), and then incubated with an

**Table 1.** Changes in wet-to-dry weight (W/D) ratio, myeloperoxidase (MPO) activity, and lung injury score in LPS-treated rats.

Groups	W/D ratio	MPO activity (U/g)	Lung injury score
Control	3.81 ± 0.14	1.77 ± 0.20	1.0 (0-2)
LPS 2 h	4.13 ± 0.22	3.94 ± 0.33**	5.0 (4-7)**
LPS 4 h	5.06 ± 0.24**	7.97 ± 0.41**	8.5 (7-11)**
LPS 6 h	5.33 ± 0.31**	8.18 ± 0.46**	10.5 (7-12)**
LPS 8 h	5.43 ± 0.28**	8.67 ± 0.44**	12.0 (8-14)**

\*\* $P < 0.01$  vs. control group. Values are expressed as mean ± S.D. or median (range) of  $n = 6$  animals per group.

affinity-purified peroxidase-conjugated secondary antibody for 60 min, developed with diaminobenzidine and counterstained with hematoxylin. Negative control staining was performed using normal goat serum instead of primary antibody.

#### Statistical analysis

Data were expressed as mean ± S.D. except that acute lung injury scores were presented as median (range). The statistical analysis was carried out using SPSS 11.0 for Windows (SPSS Inc., USA). The acute lung injury scores were analyzed using the Kruskal-Wallis rank test followed by Mann-Whitney U test, and the others were analyzed by one-way analysis of variance followed by Dunnett *post hoc* tests.  $P < 0.05$  value was considered significant.

## Results

#### Histological evaluation

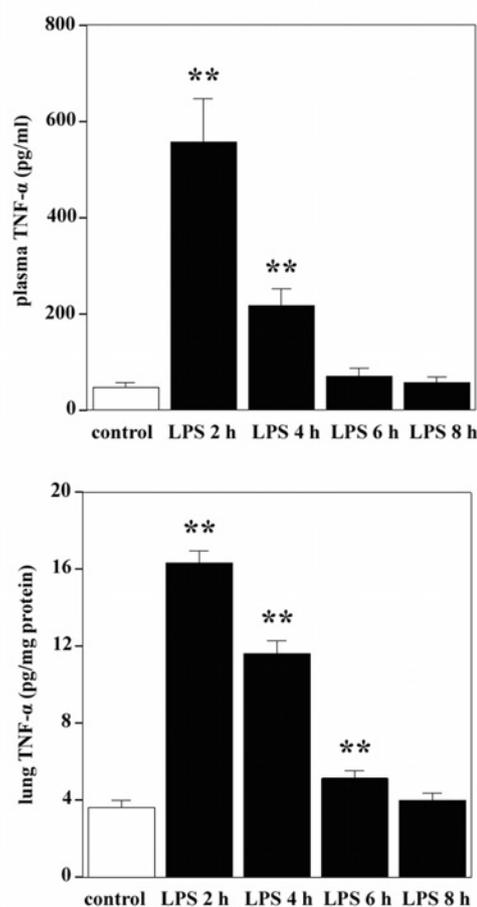
Control animals injected with saline showed normal lung histology, whereas the LPS challenge caused extensive morphological lung damage: edema, thickening of the alveolar wall, and infiltration of inflammatory cells into alveolar spaces and interstitial spaces. The extent of these histological abnormalities as assessed by lung injury score increased markedly at 2 h, and peaked at 8 h after the LPS injection (Table 1).

#### W/D ratio and MPO activity in lung tissue

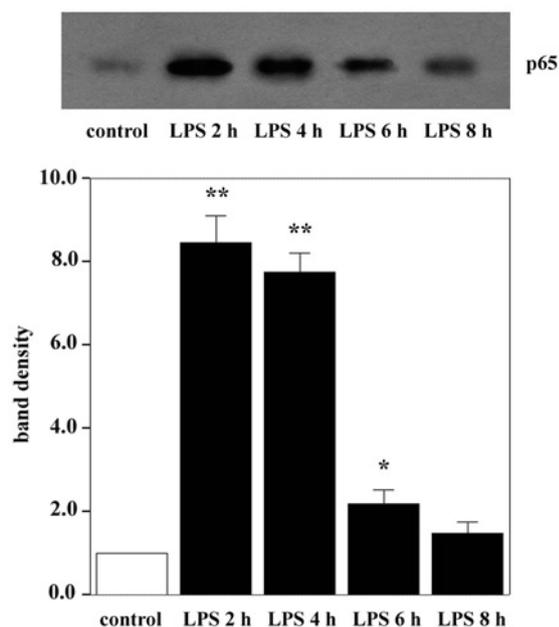
The lung W/D ratio significantly increased after 4 h and peaked at 8 h after the LPS injection (Table 1). The lung MPO activity was significantly elevated at 2 h, and reached the maximum at 8 h after LPS injection (Table 1).

#### TNF- $\alpha$ levels in plasma and lung tissue

The temporal changes in TNF- $\alpha$  levels are shown in Figure 1. After LPS administration, TNF- $\alpha$  in both plasma and lung increased significantly, peaking at 2 h, and subsequently decreased slowly, dropping to near control levels by 6 and 8 h after LPS treatment, respectively.



**Fig. 1.** TNF- $\alpha$  levels in plasma and lung tissue from controls and LPS-treated rats. Values are expressed as mean ± S.D. of  $n = 6$  animals per group. \*\* $P < 0.01$  vs. control group.



**Fig. 2.** Western blot analysis of activated NF- $\kappa$ B in lung tissues from controls and LPS-treated rats. The *top* panel shows Western blot analysis of NF- $\kappa$ B p65 in nuclear extracts of lungs from controls and LPS-treated rats. The *bottom* panel shows densitometric analysis of three separate experiments. Densitometry of p65 bands are expressed in arbitrary units and the relative densitometric signal from control rats was arbitrarily set to 1. \* $P$  < 0.05, \*\* $P$  < 0.01 vs. control group.

#### NF- $\kappa$ B activation in lung tissue

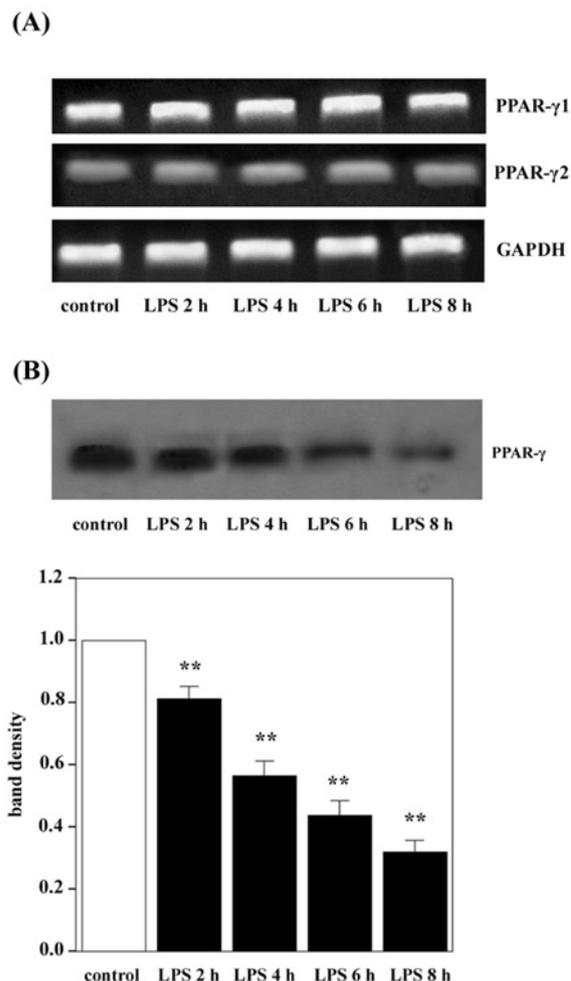
Western blot analysis revealed a low level of p65, the active subunit of NF- $\kappa$ B, in nuclear extracts from lungs of control rats. However, treatment with LPS resulted in a marked increase in the nuclear localization of p65 in lungs from LPS-treated rats, reaching maximum at 2-4 h after LPS treatment ( $P$  < 0.01, Fig. 2). LPS treatment did not affect the cytoplasmic localization of p65 as compared to saline control (data not shown).

#### Expression of PPAR- $\gamma$ mRNA in lung tissue

PCR analysis of lung tissue samples showed that normal animals constitutively expressed high mRNA levels of PPAR- $\gamma$ 1 and low levels of PPAR- $\gamma$ 2 in lung tissue (Fig. 3A). However, LPS treatment had no effect on either PPAR- $\gamma$ 1 or PPAR- $\gamma$ 2 mRNA levels at all time points.

#### Expression of PPAR- $\gamma$ protein in lung tissue

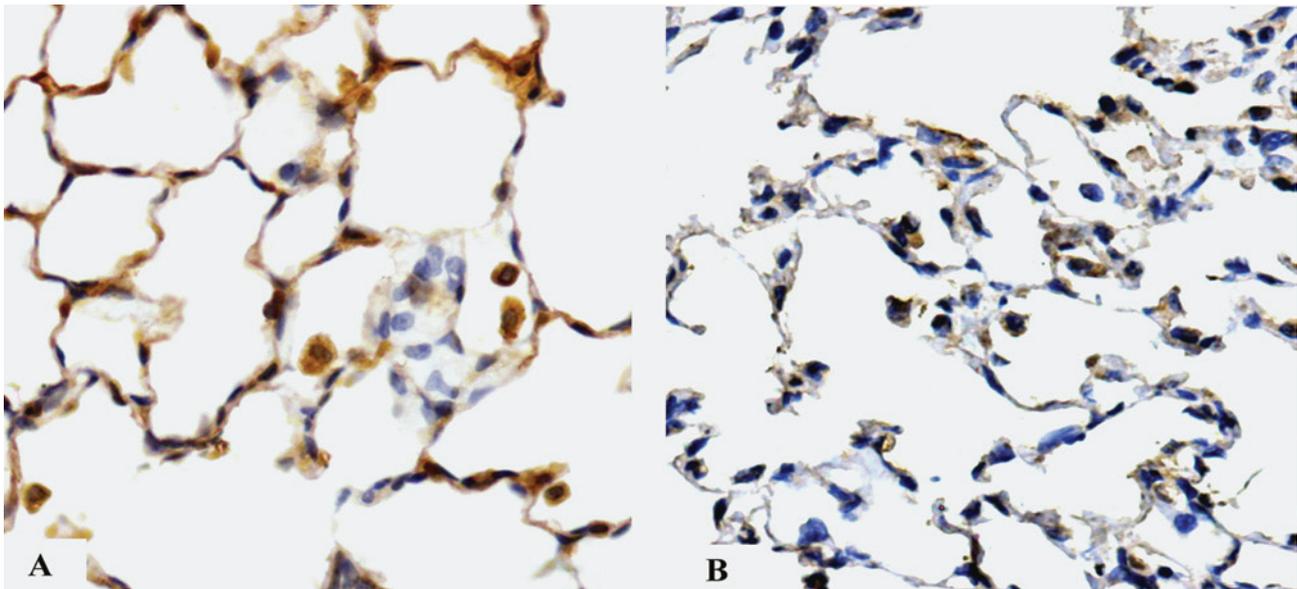
Later, we performed Western blot analysis to determine whether induction of endotoxemia was



**Fig. 3.** Expression of PPAR- $\gamma$  mRNA and protein in rat lung. (A) Ethidium bromide stained gels of PPAR- $\gamma$ 1, PPAR- $\gamma$ 2, and GAPDH RT-PCR products in lung tissues from controls and LPS-treated rats. The expression of GAPDH was served as an internal control. (B) The *top* panel shows Western blot analysis of PPAR- $\gamma$  in nuclear extracts of lungs from controls and LPS-treated rats. The *bottom* panel shows densitometric analysis of three separate experiments. Densitometry of PPAR- $\gamma$  bands are expressed in arbitrary units and the relative densitometric signal from control rats was arbitrarily set to 1. \*\* $P$  < 0.01 vs. control group.

associated with changes in PPAR- $\gamma$  protein expression. As shown in Figure 3B, nuclear PPAR- $\gamma$  protein began to decrease 2 h after LPS treatment, and progressively decrease lasted up to 8 h after LPS treatment.

In normal lungs, immunohistochemistry revealed a ubiquitous expression of PPAR- $\gamma$  in alveolar epithelial cells, alveolar macrophages and in small vessel endothelium. However, the expression of PPAR- $\gamma$  appeared to be reduced in alveolar epithelial cells and macrophages in the lungs of LPS-treated rats (Figs 4A and 4B).



**Fig. 4.** PPAR- $\gamma$  immunohistochemistry of lung tissues. (A) Control group shows ubiquitous staining for PPAR- $\gamma$  in alveolar epithelial cells, alveolar macrophages, and in small vessel. (B) LPS 4 h group shows decreased staining for PPAR- $\gamma$  in alveolar epithelial cells and alveolar macrophages. Original magnification:  $\times 400$ .

## Discussion

In the present study, we clearly demonstrated that LPS administration resulted in acute lung inflammation and injury, as evidenced by marked histological alterations, pulmonary edema, and neutrophil infiltration as well as increased plasma and lung levels of TNF- $\alpha$ . Furthermore, these changes are associated with reduced expression of PPAR- $\gamma$  protein and with activation of NF- $\kappa$ B in the lung. To our knowledge, this is the first study to demonstrate that endotoxemia down-regulates the lung expression of PPAR- $\gamma$  protein. Our findings support the hypothesis that decreased expression of PPAR- $\gamma$  in lungs may be involved in the pathophysiology of LPS-induced acute lung injury.

Neutrophils are key players in the pathogenesis of acute lung injury, releasing lipid and enzyme mediators and oxygen radicals (Abraham 2003, Tian *et al.* 2004). The accumulation of neutrophils within the lung in the setting of LPS-caused insults is probably dependent on the coordinated expression of pro-inflammatory cytokines, adhesion molecules, and the establishment of chemotactic gradients *via* the local generation of chemotactic factors. TNF- $\alpha$ , an early pro-inflammatory cytokine, is believed to trigger activation of other pro-inflammatory cytokines, such as interleukins IL-1 $\beta$  and IL-6. It also has high potency in inducing neutrophil activation where it acts in conjunction with adhesion molecules such as the intercellular adhesion molecule (ICAM)-1 and E-selectin (Condliffe *et al.* 1996,

Drost and MacNee 2001). NF- $\kappa$ B is known to be one of the crucial transcription factors required for maximal transcription of a wide array of pro-inflammatory molecules including TNF- $\alpha$  and other mediators. In endotoxemia, NF- $\kappa$ B released from I $\kappa$ B translocates into the nucleus, where it enhances the transcription of cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and ICAM-1 (Ali and Mann 2004). The pivotal role of the activation of NF- $\kappa$ B in inflammation during acute lung injury has been well elucidated in previous studies (Liu *et al.* 1999, Lim *et al.* 2004, Tian *et al.* 2004). In the present study, administration of LPS induced a time-dependent increase in the magnitude of lung edema and accumulation of neutrophils as well as morphological lung damages. These changes confirm a well-recognized acute lung injury associated with endotoxemia (Yoshinari *et al.* 2001, Tian *et al.* 2004). In addition, administration of LPS also caused a marked increase in circulating and pulmonary TNF- $\alpha$ , which ran parallel with the magnitude of NF- $\kappa$ B activation in overall trends. Our findings are in accordance with other *in vivo* studies (Liu *et al.* 1999, Lim *et al.* 2004, Tian *et al.* 2004), indicating that activation of NF- $\kappa$ B appears to be a key molecular event in the initiation and exacerbation of LPS-induced lung injury.

PPAR- $\gamma$  expression is ubiquitous within the lung and has been shown to be constitutive in epithelial cells, endothelial cells, smooth muscle cells, and alveolar macrophages (Wang *et al.* 2001, Ameshima *et al.* 2003, Patel *et al.* 2003, Reddy *et al.* 2004). Previous studies

have suggested that PPAR- $\gamma$  expression may be changed in an inflammatory process. In adipose tissue, PPAR- $\gamma$  mRNA and protein expression decreased after mice were challenged *in vivo* with endotoxin (Hill *et al.* 1997). In other studies, inflammatory cytokines, including TNF- $\alpha$ , IL-1, and IL-6, also decreased PPAR- $\gamma$  expression in adipose tissue (Tanaka *et al.* 1999). In the present study, we observed that endotoxemia did not alter the expression of PPAR- $\gamma$ 1 and PPAR- $\gamma$ 2 mRNA in rat lung. Our data are in agreement with a previous *in vivo* report demonstrating that PPAR- $\gamma$  mRNA was not altered in mouse heart during endotoxemia (Feingold *et al.* 2004). Interestingly, we found that the expression of PPAR- $\gamma$  nuclear protein decreased in a time-dependent fashion during endotoxemia, suggesting post-transcriptional modifications of PPAR- $\gamma$  may be occurring. In addition, the expression of PPAR- $\gamma$  protein was down-regulated in the alveolar epithelium and macrophages. Several mechanisms which induced a reduction in the PPAR- $\gamma$  levels during endotoxemia may be hypothesized. Mitogen-activated kinases, such as extracellular signal-regulated kinases 1 and 2 (ERK1/2), have been proposed to induce the suppression of PPAR- $\gamma$  transcriptional activation through phosphorylation of the receptor (Adams *et al.* 1997). Additional mechanisms may also be operative, such as nitration of PPAR- $\gamma$  protein which inhibits the receptor ability to translocate into the nucleus. This effect has been demonstrated in macrophage-like cells stimulated with endotoxin or TNF- $\alpha$  (Shibuya *et al.* 2002). However, it is difficult to establish from our *in vivo* data whether down-regulation of PPAR- $\gamma$  correlates with decreased PPAR- $\gamma$  function.

Accumulating evidence has suggested that PPAR- $\gamma$  plays an important role in the regulation of multiple inflammatory processes. For example, PPAR- $\gamma$  activation *in vitro* inhibits the expression of TNF- $\alpha$ , IL-1 $\beta$ , and inducible nitric oxide synthase (iNOS) from LPS-activated macrophages and monocytes (Jiang *et al.* 1998). It has also been reported that PPAR- $\gamma$  activation significantly suppressed the expression of adhesion molecules in cultured human endothelial cells and the ensuing leukocyte recruitment *in vitro* (Pasceri *et al.* 2000). All of these molecules are thought to be important

in the pathophysiology of acute lung injury, and a decrease in PPAR- $\gamma$  level could add to their excessive production. In line with these *in vitro* findings, we and others have recently reported that rosiglitazone, a potent PPAR $\gamma$  agonist, can attenuate the expression of iNOS, TNF- $\alpha$  and ICAM-1 as well as tissue neutrophil infiltration associated with endotoxemia, zymosan-induced nonseptic shock, or bleomycin-induced lung injury (Cuzzocrea *et al.* 2004, Genovese *et al.* 2005, Liu *et al.* 2005). It has been proposed, in fact, that PPAR- $\gamma$  may inhibit the activation of NF- $\kappa$ B and activator protein (AP)-1, leading to transrepression of inflammatory genes (Fahmi *et al.* 2001). We demonstrated here that the decrease in nuclear PPAR- $\gamma$  protein correlated well with the occurrence of and severity of pulmonary inflammation and injury. Similarly, Zingarelli *et al.* (2003) have also reported that the cardiovascular hypodynamic phase of septic shock is associated with down-regulation of PPAR- $\gamma$  expression on the endothelium of thoracic aorta and in the bronchial epithelium in rats. The results from this and our current study suggest that the reduction of PPAR- $\gamma$  protein level may contribute to the ongoing pulmonary inflammation and NF- $\kappa$ B activation, ultimately leading to acute lung injury. However, it is unclear why the duration and degree of the reduction of PPAR- $\gamma$  protein expression were not paralleled with that of NF- $\kappa$ B activation in the endotoxemic lungs, which should be elucidated in the future.

In conclusion, our data demonstrate that acute endotoxemia can induce acute pulmonary inflammation and lung injury. Furthermore, it appears that the decreased expression of PPAR- $\gamma$  in lung tissue may be implicated in the pathogenesis of endotoxin-induced acute lung injury. This novel pathophysiological insight may provide new basis for the development of tools for the treatment of acute lung injury related to endotoxemia.

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