

# Phosphorylation of Extracellular Signal-Regulated Kinase (ERK)-1/2 Is Associated with the Downregulation of Peroxisome Proliferator-Activated Receptor (PPAR)- $\gamma$ during Polymicrobial Sepsis

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Peroxisome proliferator-activated receptor (PPAR)- $\gamma$  is a ligand-activated transcription factor and regulates inflammation. Post-translational modifications regulate the function of PPAR $\gamma$ , potentially affecting inflammation. PPAR $\gamma$  contains a mitogen-activated protein kinase (MAPK) site, and phosphorylation by extracellular signal-regulated kinase (ERK)-1/2 leads to inhibition of PPAR $\gamma$ . This study investigated the kinetics of PPAR $\gamma$  expression and activation in parenchymal and immune cells in sepsis using the MAPK/ERK kinase (MEK)-1 inhibitor, an upstream kinase of ERK1/2. Adult male Sprague Dawley rats were subjected to polymicrobial sepsis by cecal ligation and puncture. Rats received intraperitoneal injection of vehicle or the MEK1 inhibitor PD98059 (5 mg/kg) 30 min before cecal ligation and puncture. Rats were euthanized at 0, 1, 3, 6 and 18 h after cecal ligation and puncture. Control animals used were animals at time 0 h. Lung, plasma and peripheral blood mononuclear cells (PBMCs) were collected for biochemical assays. In vehicle-treated rats, polymicrobial sepsis resulted in significant lung injury. In the lung and PBMCs, nuclear levels of PPAR $\gamma$  were decreased and associated with an increase in phosphorylated PPAR $\gamma$  and phosphorylated ERK1/2 levels. Treatment with the MEK1 inhibitor increased the antiinflammatory plasma adipokine adiponectin, restored PPAR $\gamma$  expression in PBMCs and lung, and decreased lung injury. The inflammatory effects of sepsis cause changes in PPAR $\gamma$  expression and activation, in part, because of phosphorylation of PPAR $\gamma$  by ERK1/2. This phosphorylation can be reversed by ERK1/2 inhibition, thereby improving lung injury.

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## INTRODUCTION

Peroxisome proliferator-activated receptor (PPAR)- $\gamma$  is a ligand-activated transcription factor. Activation of PPAR $\gamma$  plays a role in controlling the inflammatory response. Several studies have demonstrated that activation of PPAR $\gamma$  by specific ligands significantly improves survival in clinically relevant models of septic shock (1–3). The beneficial effect of PPAR $\gamma$  activation is likely to be secondary to inhibition of the production of several inflammatory mediators, as shown *in vivo* in septic rodents (1–3) and

*in vitro* in activated macrophages and monocytes (4).

Sepsis and other inflammatory states affect PPAR $\gamma$  expression and correlate with the inflammatory response. We have previously demonstrated that PPAR $\gamma$  expression is downregulated in the lung and vascular endothelium in rodent models of septic shock and that treatment with PPAR $\gamma$  ligands reverses the sepsis-induced reduction (1). In adipose tissue, PPAR $\gamma$  expression decreased after mice were challenged *in vivo* with endotoxin, and cytokine-induced sup-

pression of PPAR $\gamma$  was reversed with synthetic agonists (5,6). However, it remains unclear what mechanisms lead to a decrease in PPAR $\gamma$  activity in sepsis.

Posttranslational modifications are mechanisms that regulate the function of PPAR $\gamma$  and may contribute to the downregulation of PPAR $\gamma$  in sepsis (7). The activation function (AF)-1 domain of PPAR $\gamma$  contains a consensus mitogen-activated protein kinase (MAPK) site, and phosphorylation by extracellular signal-regulated kinase (ERK)-1/2 at serine residue 82 (or 112 for PPAR $\gamma$ 2) leads to inhibition of PPAR $\gamma$  transactivation (8,9). This phosphorylation-induced repression is due to conformational changes that can lead to altered affinity for ligands and cofactors (8,9). In addition, phosphorylation promotes degradation of PPAR $\gamma$  by the ubiquitin-proteasome system (10).

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In cultured adipocytes, using a specific ERK inhibitor reverses the reduction in PPAR $\gamma$  (11).

Therefore, in this study, we investigated the kinetics of altered PPAR $\gamma$  expression and activation in immunologic and parenchymal cells from rats subjected to polymicrobial sepsis. To gain a better understanding of the molecular mechanism by which PPAR $\gamma$  expression is affected, we investigated the effects of polymicrobial sepsis on the phosphorylation of PPAR $\gamma$  by ERK1/2. Furthermore, we investigated whether *in vivo* inhibition of MAPK/ERK kinase (MEK)-1 by PD98059 may restore PPAR $\gamma$  expression and afford protective effects in sepsis.

## MATERIALS AND METHODS

The primary antibodies for PPAR $\gamma$  and  $\alpha$ -tubulin were obtained from Thermo Fisher Scientific (Rockford, IL, USA). The primary antibodies for p-PPAR $\gamma$ , p-ERK1/2 and ERK1/2 and the oligonucleotide for PPARs were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

### Rat Model of Cecal Ligation and Puncture

The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health and was reviewed and approved by our Institutional Animal Care and Use Committee. Polymicrobial sepsis was induced in male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA, USA), weighing 175–250 g, by cecal ligation and puncture (CLP) as previously described (1). Rats were anesthetized with thiopentone sodium (70 mg/kg) injected intraperitoneally. After opening the abdomen, the cecum was exteriorized and ligated with a 3.0 silk suture at its base without obstructing the intestinal continuity. The cecum was punctured twice with an 18-gauge needle and returned to the peritoneal cavity. The abdominal

incision was closed with 3.0 silk running sutures.

Animals underwent intraperitoneal injection of vehicle (dimethyl sulfoxide [DMSO]) or the MEK1 inhibitor PD98059 (5 mg/kg) 30 min before CLP. Rats were sacrificed at 0, 1, 3, 6 and 18 h after CLP ( $n = 3-6$  for each group). In the control group (CLP 0 h), surgery was performed, but the cecum was neither ligated nor punctured. Saline solution (0.9%, 5 mL) was given subcutaneously to replace the fluid and blood loss during the operation. Whole blood, plasma and lungs were collected for the biochemical studies described below.

### Histopathological Analysis

Lungs were fixed in 4% paraformaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin and evaluated by three independent observers unaware of the experimental protocol. Specifically, lung injury was analyzed by a semiquantitative score as previously reported (12) based on the following histologic features: (a) alveolar congestion, (b) hemorrhage, (c) infiltration or aggregation of neutrophils in the airspace or vessel wall and (d) thickness of alveolar wall/hyaline membrane formation. Each feature was graded from 0 to 4 (i.e., no injury, minimal, mild, significant or severe). The four variables were summed to represent the lung injury score (total score, 0–16).

### Peripheral Blood Mononuclear Cell Isolation

Peripheral blood mononuclear cells (PBMCs) were isolated by Percoll density gradient centrifugation (Amersham Biosciences, Piscataway, NJ, USA). Whole blood was layered onto a Percoll gradient, and samples were centrifuged at 1,500g for 30 min at 4°C. After centrifugation, the buffy coat layer was removed and washed in Hanks solution with bovine serum albumin. Samples were centrifuged again, and the pellet was resuspended in Hanks solution and stored at –80°C.

### Subcellular Fractionation and Protein Extraction

PBMCs were rinsed and lung tissue was homogenized with a cold buffer containing 0.32 mmol/L sucrose, 10 mmol/L Tris-HCl, 1 mmol/L EGTA, 2 mmol/L EDTA, 0.2  $\mu$ mol/L NaN<sub>3</sub>, 50 mmol/L NaF, 20  $\mu$ mol/L leupeptin, 0.15  $\mu$ mol/L pepstatin A and 0.2 mmol/L phenylmethylsulfonyl fluoride (PMSF). The cell suspension or tissue homogenate was centrifuged (1,000g at 4°C for 10 min). The supernatant (cytosol + membrane extract) was collected and stored at –80°C. The pellet was solubilized in Triton buffer (1% Triton X-100, 150 mmol/L NaCl, 10 mmol/L Tris-HCl [pH 7.4], 1 mmol/L EGTA, 1 mmol/L EDTA, 0.2 mmol/L sodium orthovanadate, 20  $\mu$ mol/L leupeptin A and 0.2 mmol/L PMSF). The lysates were centrifuged (15,000g at 4°C for 30 min), and the supernatant (nuclear extract) was collected and stored at –80°C. The amount of protein was quantified by the Bradford assay.

### Western Blot Analysis

The nuclear or cytosol content of PPAR $\gamma$ , phosphorylated PPAR $\gamma$  (p-PPAR $\gamma$ ), ERK1/2, phosphorylated ERK1/2 (p-ERK1/2) and  $\alpha$ -tubulin in PBMCs and lung was determined by immunoblot analyses. Extracts were boiled in equal volumes of loading buffer (125 mmol/L Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol), and 50  $\mu$ g protein was loaded per lane on an 8–16% Tris-glycine gradient gel. Proteins were separated electrophoretically and transferred to nitrocellulose membranes. For immunoblotting, membranes were blocked with 5% nonfat dried milk in Tris-buffered saline for 1 h and then incubated with primary antibodies against PPAR $\gamma$ , ERK 1/2, p-ERK 1/2 and p-PPAR $\gamma$ . The membranes were washed in Tris-buffered saline with 0.1% Tween 20 and incubated with secondary peroxidase-conjugated antibody. Membranes were reprobed with primary antibody against  $\alpha$ -tubulin to ensure equal loading. Detection was enhanced by

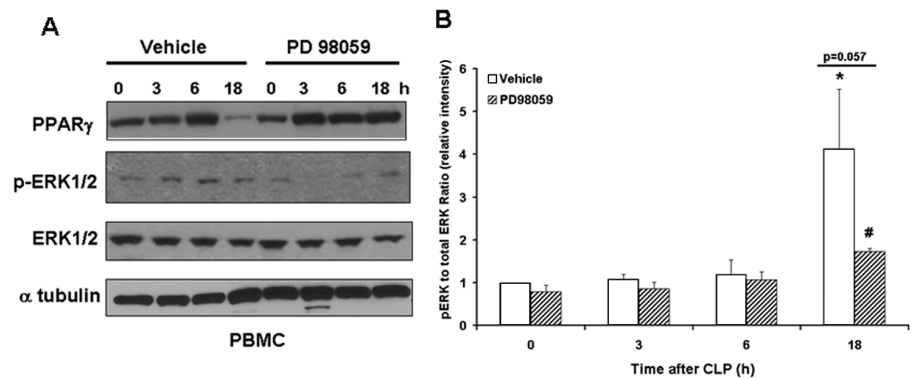
chemiluminescence and exposed to photographic film. Densitometric analysis of blots was performed using ImageQuant (Molecular Dynamics, Sunnyvale, CA, USA).

### Electrophoretic mobility shift assay

Electrophoretic mobility shift assay (EMSA) was performed as described previously (1). An oligonucleotide probe corresponding to PPARs consensus sequence (5'-GAA AAC TAG GTC AAA GGT CA-3') was labeled with [ $\gamma$ - $^{32}$ P]ATP using T4 polynucleotide kinase and purified in Bio-Spin chromatography columns (Bio-Rad, Hercules, CA, USA). A total of 10  $\mu$ g nuclear protein was preincubated with EMSA buffer (12 mmol/L HEPES, pH 7.9, 4 mmol/L Tris-HCl, pH 7.9, 25 mmol/L KCl, 5 mmol/L MgCl<sub>2</sub>, 1 mmol/L EDTA, 1 mmol/L dithiothreitol, 50 ng/mL poly[d(I-C)], 12% glycerol vol/vol and 0.2 mmol/L PMSF) on ice for 10 min before adding radio-labeled oligonucleotide for an additional 10 min. The specificity of the binding reactions was determined by co-incubating duplicate nuclear extract samples with a 10-fold molar excess of unlabeled oligonucleotides (competitor assays), anti-PPAR $\alpha$ , anti-PPAR $\beta$  or anti-PPAR $\gamma$  antibody (supershift assay). Protein-nucleic acid complexes were resolved using a nondenaturing polyacrylamide gel consisting of 5% acrylamide (29:1 ratio of acrylamide:bisacrylamide) and run in 0.5  $\times$  Tris borate-EDTA (45 mmol/L Tris-HCl, 45 mmol/L boric acid and 1 mmol/L EDTA) for 1 h at a constant current (30 mA). Gels were transferred to 3M paper (Whatman, Clifton, NJ, USA), dried under a vacuum at 80°C for 1 h and exposed to photographic film at -70°C with an intensifying screen. Densitometric analysis was performed using ImageQuant (Molecular Dynamics).

### Plasma Levels of Adiponectin

Plasma levels of adiponectin were measured with a multiplex assay kit (Millipore, Billerica, MA, USA) using the protocol recommended by the manufacturer.



**Figure 1.** Effect of polymicrobial sepsis on nuclear PPAR $\gamma$  and p-ERK1/2 in PBMCs. Rats were subjected to CLP and sacrificed at various time points. Animals received either vehicle (DMSO) treatment or the MEK1 inhibitor PD98059. (A) Representative radiograph of Western blot analysis for PPAR $\gamma$ , p-ERK1/2 and total ERK1/2. Equal loading was confirmed by  $\alpha$ -tubulin immunoblotting. (B) Relative densitometric analysis of p-ERK to total ERK content ( $n = 4$ /group). \* $P \leq 0.05$  versus sham (time 0) by analysis of variance, Tukey test. # $P \leq 0.05$  versus sham (time 0), ANOVA, Holm-Sidak test.

### Statistical Analysis

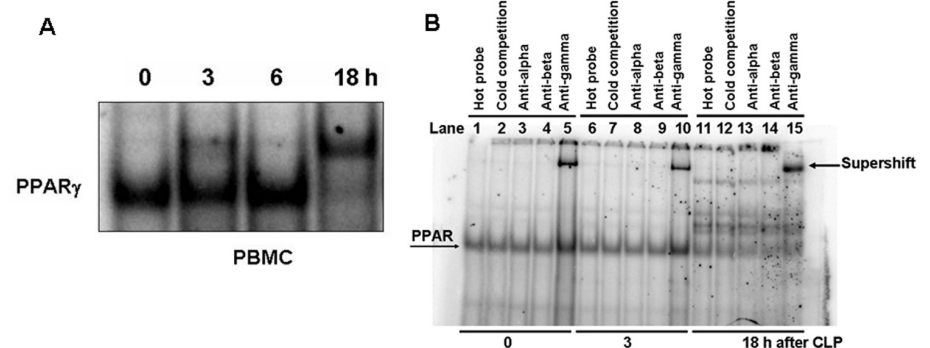
All values in the figures and text are expressed as mean  $\pm$  SEM of  $n$  observations ( $n = 3-6$  animals for each group). Statistical analysis of damage scores and adiponectin levels were performed using the  $t$  test. Densitometric statistical analysis of p-ERK to total ERK was compared with the Kruskal-Wallis analysis of variance with the Tukey test for the vehicle group and Holm-Sidak method for the PD98059 group. The  $t$  test was used to

compare vehicle-treated versus PD98059-treated groups at 18 h after CLP. A value of  $P \leq 0.05$  was considered significant.

### RESULTS

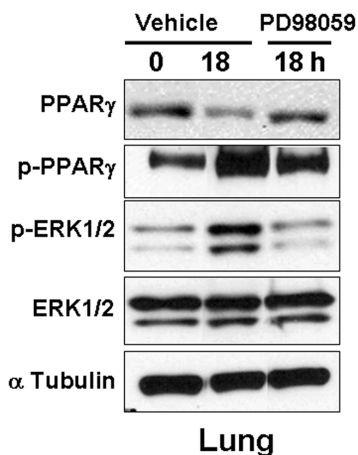
#### Polymicrobial Sepsis Is Associated with a Decrease in Nuclear PPAR $\gamma$ and an Increase in p-ERK1/2 in PBMCs

To determine the effect of sepsis on changes in activation of PPAR $\gamma$  in PBMCs, rats were subjected to CLP and



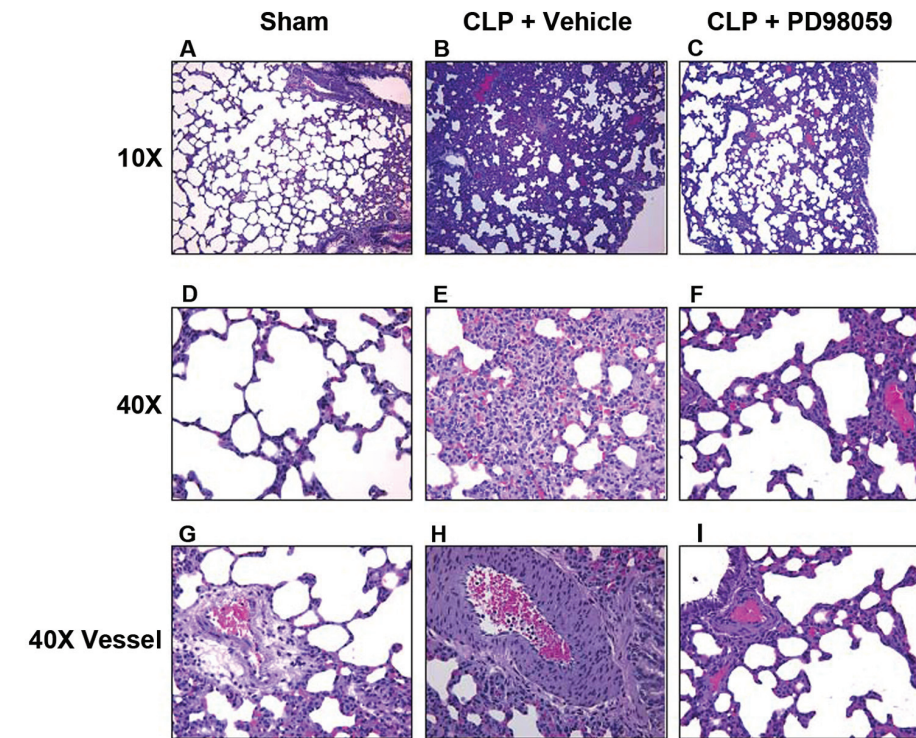
**Figure 2.** Effect of polymicrobial sepsis on PPAR-DNA binding in PBMCs. Nuclear extracts were obtained from vehicle-treated rats at 0, 3, 6 and 18 h after CLP (A) Representative autoradiograph of EMSA for PPAR. (B) Representative autoradiograph of EMSA with cold competition and supershift at 0, 3 and 18 h after CLP. Lanes 1, 6, and 11 represents hot probe; lanes 2, 7, and 12 represent cold competition; lanes 3, 8, and 13 represent anti-PPAR $\alpha$ ; lanes 4, 9, and 14 represents anti-PPAR $\beta$ ; and lanes 5, 10, and 15 represent supershift with anti-PPAR $\gamma$ .





**Figure 3.** Effect of polymicrobial sepsis on PPAR $\gamma$ , phosphorylated PPAR $\gamma$ , p-ERK1/2 and ERK1/2 in the lung. Rats were subjected to CLP and euthanized at various time points. Significant findings were demonstrated at the 18-h time point; therefore, this is the only time point depicted. The Western blot was modified to demonstrate the 0-h and 18-h time points only. The results for each protein have not been otherwise modified. Animals received either vehicle treatment or the MEK1/2 inhibitor PD98059. A representative radiograph of Western blot analysis for PPAR $\gamma$ , p-PPAR $\gamma$ , p-ERK1/2 and total ERK1/2 is shown. Equal loading was confirmed by  $\alpha$ -tubulin immunoblotting.

euthanized at various time points. Constitutive nuclear expression of PPAR $\gamma$  ( $1.05 \pm 0.08$  relative intensity) was observed in PBMCs in control animals (time 0, before CLP), as evaluated by Western blot analysis (Figure 1A). A marked decrease of nuclear PPAR $\gamma$  was observed at 18 h after CLP ( $0.35 \pm 0.13$  relative intensity) when compared with control PPAR $\gamma$  content ( $P < 0.05$ ) (see Figure 1A). A reduction of PPAR $\gamma$  nuclear expression correlated with a significant reduction of PPAR DNA binding, as evaluated by EMSA (Figure 2A). Specific PPAR $\gamma$  DNA binding was confirmed by supershift analysis (Figure 2B). To gain further insights into the molecular mechanisms involved in the decreased nuclear PPAR $\gamma$  content, we evaluated the effect of CLP on p-ERK1/2. In a similar time course analysis, nuclear expres-



**Figure 4.** Effect of polymicrobial sepsis on lung injury at 18 h after CLP. Representative histology of lung sections stained with hematoxylin and eosin is shown. A, D, and G represent normal lung of a sham rat at 10x and 40x and blood vessel at 40x (respectively). B, E, and H represent lung after CLP with vehicle treatment only. Interstitial hemorrhage and accumulation of inflammatory cells were observed in lung. C, F, and I represent lung after CLP with PD98059 treatment and demonstrate an ameliorated CLP-induced lung injury and reduced infiltrate. Animals received either vehicle or PD98059. A similar pattern was seen in  $n = 3$  different tissue sections in each experimental group.

sion of p-ERK1/2 increased as early as 3 h after CLP and was further enhanced thereafter (Figure 1A, B).

To confirm the physiological role of ERK1/2 activation on the regulation of PPAR $\gamma$ , we treated rats subjected to CLP with a selective inhibitor of the upstream MEK1 kinase, PD98059. Treatment with PD98059 decreased nuclear p-ERK1/2 and restored PPAR $\gamma$  expression in PBMCs from rats subjected to CLP (see Figure 1A, B).

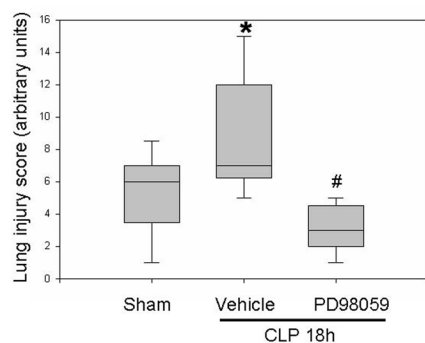
**Polymicrobial Sepsis Is Associated with a Decrease in PPAR $\gamma$  and an Increase in p-PPAR $\gamma$  and p-ERK1/2 in the Lung**

We have previously demonstrated that in polymicrobial sepsis, PPAR $\gamma$  expression exhibits maximal degradation in the

lung at 18 h after CLP (1). Therefore, we chose to highlight the changes that occur at this critical time point. Similar to results demonstrated in PBMCs, PPAR $\gamma$  was decreased in lung tissue at 18 h after CLP (Figure 3). This decrease correlated with an increase in the phosphorylated form of PPAR $\gamma$  and an increase in p-ERK1/2 expression in the lung at 18 h after CLP (see Figure 3). In animals that received the MEK1 inhibitor PD98059, PPAR $\gamma$  expression was restored and p-PPAR $\gamma$  was decreased in the lung during polymicrobial sepsis (see Figure 3).

**Effect of ERK1/2 Inhibition on Lung Injury**

In the lungs of vehicle-treated rats, histologic examination revealed extravasation of red cells and accumulation of in-

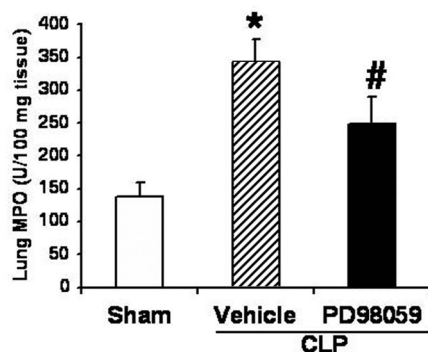


**Figure 5.** Effect of polymicrobial sepsis on lung injury score at 18 h after CLP. Histopathologic scores of lung sections are shown ( $n = 3$  for each group). Lung injury was scored from 0 (no damage) to 16 (maximum damage). Box plots represent 25th percentile, median and 75th percentile; error bars define 10th and 90th percentiles. \* $P < 0.05$  versus sham; # $P < 0.05$  versus vehicle-treated rats subjected to CLP.

flammatory cells into the air spaces. Margination and adhesion of neutrophils were seen in blood vessels at 18 h after CLP (Figure 4). Vehicle-treated rats at 18 h after CLP had a significantly higher lung injury score ( $9 \pm 1.15$ ) when compared with sham animals ( $5.3 \pm 0.8$ ) ( $P < 0.05$ ) (Figure 5). However, animals that received PD98059 had a significantly lower lung injury score ( $3.2 \pm 0.46$ ;  $P < 0.05$ ) and amelioration of inflammatory cells when compared with values of vehicle-treated rats.

### Effect of ERK1/2 Inhibition on Lung Neutrophil Infiltration

A serious consequence of sepsis is the occurrence of multiple organ failure, which is preceded by accumulation of neutrophils in major vital organs (13). We next quantified neutrophil infiltration in the lung by measuring the activity of myeloperoxidase, an enzyme specific to granulocyte lysosomes. Myeloperoxidase activity was significantly elevated at 18 h after CLP in the lung from vehicle-treated rats indicating marked neutrophil infiltration ( $330.2 \pm 46.5$  U/100 mg tissue). In contrast, animals that received PD98059 had a significant reduction in



**Figure 6.** Effect of *in vivo* treatment with PD98059 or vehicle on myeloperoxidase (MPO) activity in the lung after CLP. Each data point represents the mean  $\pm$  SEM of three animals for each group. \* $P < 0.05$  versus sham rat; # $P < 0.05$  versus vehicle-treated rat. Animals received either vehicle or PD98059 (5 mg/kg).

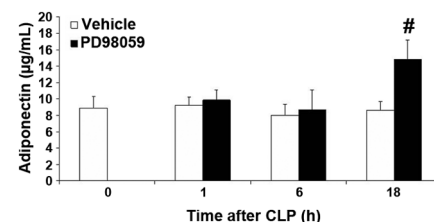
myeloperoxidase activity ( $268.1 \pm 25.5$  U/100 mg tissue) at 18 h after CLP ( $P < 0.05$ ) (Figure 6).

### Effect of ERK1/2 Inhibition on Plasma Levels of Adiponectin

Adiponectin has a peroxisome proliferator response element in its promoter region (14). Consequently, changes in PPAR $\gamma$  activity may be reflected as changes in adiponectin (15). To determine the downstream effects of PPAR $\gamma$  activation, we measured plasma adiponectin expression as a biomarker for PPAR $\gamma$  activity. In vehicle-treated rats, there was no significant change in plasma adiponectin levels after CLP. However, in PD98059-treated rats, plasma adiponectin levels were significantly higher at 18 h after CLP when compared with vehicle-treated animals (Figure 7).

### DISCUSSION

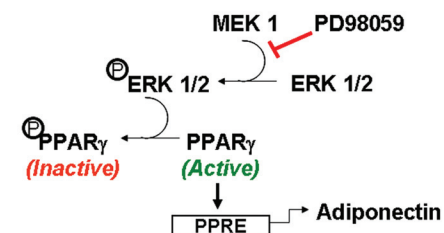
The present study demonstrates that PPAR $\gamma$  is reduced in immunomodulatory and parenchymal cells during polymicrobial sepsis. Furthermore, the downregulation of PPAR $\gamma$  involves its phosphorylation by ERK1/2, and inhibition of ERK1/2 causes a decrease in phosphorylated PPAR $\gamma$  and restores PPAR $\gamma$ . This



**Figure 7.** Effect of polymicrobial sepsis on plasma levels of adiponectin in rats after CLP. Each data point represents the mean  $\pm$  SEM of  $n = 3-6$  animals for each group. # $P < 0.05$  versus vehicle-treated rats at 18 h.

restoration of PPAR $\gamma$  correlates with an increase in plasma levels of the anti-inflammatory adipokine adiponectin (Figure 8).

In the present study, we observed that PPAR $\gamma$  is altered in PBMCs and lung parenchymal cells during polymicrobial sepsis. Our findings are in agreement with previous studies that demonstrate that the expression, production and activity of PPAR $\gamma$  are affected in other inflammatory conditions. In adipose tissue, PPAR $\gamma$  mRNA and protein expression decreased after mice were challenged *in vivo* with endotoxin (5). Previous results in our laboratory support the idea that PPAR $\gamma$  is important in controlling inflammation and correlates



**Figure 8.** Mechanisms of activation of PPAR $\gamma$  through inhibition of MEK1. MEK1 phosphorylates ERK1/2, leading to phosphorylation and inactivation of PPAR $\gamma$ . Inhibition of MEK1 by PD98059 retains PPAR $\gamma$  in the non-phosphorylated active form. Therefore, PPAR $\gamma$  can subsequently bind to the peroxisome proliferator response element in the promoter region of target genes and affect transcription. One known target of PPAR $\gamma$  is the adipokine adiponectin.

with clinical outcomes. In the cardiovascular hypodynamic phase of septic shock, PPAR $\gamma$  expression was downregulated in the lung and in thoracic aortas in rats (1). Furthermore, the sepsis-induced reduction in PPAR $\gamma$  expression was reversed by *in vivo* treatment with PPAR $\gamma$  ligands. In an experimental model of polymicrobial sepsis, Zhou *et al.* (16) demonstrate that hepatic PPAR $\gamma$  protein and gene expression is downregulated in the late stages of sepsis. Although many studies confirm the effects of sepsis on decreasing PPAR $\gamma$  expression in tissue, contradictory results exist in immune cells. In porcine white blood cells, PPAR $\gamma$  expression increased in the first 6 hours after *in vivo* lipopolysaccharide (LPS) challenge (17). However, PPAR $\gamma$  expression normalized to control levels by 8 hours post-LPS. Similarly, the expression of PPAR $\gamma$  was increased in peripheral blood mononuclear cells and T-cells from patients with septic shock and sepsis (15,18).

Our results provide a mechanism through which a decrease in PPAR $\gamma$  in sepsis may be partially explained and are consistent with previous results demonstrated in endotoxic shock (19). We hypothesized that posttranslational modifications, including phosphorylation of PPAR $\gamma$  by ERK1/2, may alter PPAR $\gamma$  in a model of polymicrobial sepsis. Our current data demonstrate that nuclear content of p-ERK1/2 increases in PBMCs after CLP and correlates with a decrease in PPAR $\gamma$ . In the lung, an increase of p-ERK1/2 also correlated with phosphorylation of PPAR $\gamma$ . Posttranslational modifications are mechanisms that regulate the function of PPAR $\gamma$  (7). The AF-1 domain of PPAR $\gamma$  contains a consensus MAPK site, and phosphorylation at serine residue 82 (or 112 for PPAR $\gamma$ 2) leads to inhibition of PPAR $\gamma$  transactivation (8,9,20). This phosphorylation-induced repression is due to conformational changes that can lead to altered affinity for ligands and cofactors (8,9). Additionally, phosphorylation of PPAR $\gamma$  promotes its degradation through the ubiquitin-proteasome system (10). In

MCF-7 breast cancer cells, inhibition of p-ERK1/2 with  $\alpha$ -eleostearic acid correlated with decreased PPAR $\gamma$  in a time-dependent manner (21). An alternative mechanism resulting in decreased nuclear PPAR $\gamma$  expression and activity could occur through direct interaction of nuclear PPAR $\gamma$  with MEKs, resulting in the nuclear export of PPAR $\gamma$ , thereby preventing its nuclear activation (22). Thus, it is possible that during the inflammatory process, alteration of protein conformation by posttranslational mechanisms may affect the expression of the receptor (19).

Adiponectin is an adipocyte-derived protein that is secreted into plasma. Adiponectin has beneficial effects including an antiatherosclerotic action, it improves insulin sensitivity and it activates glucose uptake in skeletal muscle cells (23). Additionally, adiponectin is an antiinflammatory cytokine that inhibits nuclear factor (NF)- $\kappa$ B activation in endothelial cells and macrophages (24,25). Furthermore, adiponectin is induced by PPAR $\gamma$  agonists via direct binding to the peroxisome proliferator response element in the adiponectin promoter (14,23). Tsuchihashi *et al.* (26) previously demonstrated that plasma adiponectin levels were decreased in rats subjected to polymicrobial sepsis. We demonstrate that adiponectin levels were significantly increased in animals who received the ERK1/2 inhibitor PD98059 during polymicrobial sepsis. This increase in adiponectin reflects the changes in the kinetics of PPAR $\gamma$  after CLP. This finding is in agreement with previous studies that demonstrate that alteration of PPAR $\gamma$  through treatment with PPAR $\gamma$  ligands alters adiponectin expression (27–29). Combs *et al.* (28) demonstrate that adiponectin levels were significantly increased in healthy male subjects treated with the PPAR $\gamma$  ligand rosiglitazone. Moreover, in patients with the dominant-negative PPAR $\gamma$  mutation, adiponectin levels were lower compared with patients with severe insulin resistance with no mutation.

The inflammatory effects of polymicrobial sepsis cause changes in PPAR $\gamma$  expression and activation in PBMCs and lung tissue in rats. These changes in PPAR $\gamma$  are, in part, due to the phosphorylation of PPAR $\gamma$  by ERK1/2 and can be reversed by ERK1/2 inhibition. Furthermore, adipokines are altered during polymicrobial sepsis and adiponectin plasma levels correlate with PPAR $\gamma$  expression and can be augmented with ERK1/2 inhibition. More studies are necessary to investigate the molecular link between adipokines and the inflammatory response in sepsis.

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## DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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