Journal of Cellular Biochemistry 89:1206–1214 (2003)

LPS Induces Pulmonary Intravascular Macrophages Producing Inflammatory Mediators via Activating NF-κB

Zheng-Tang Chen, Sheng-Liang Li, En-Qi Cai, Wei-Ling Wu, Jing-Sheng Jin, and Bo Zhu*

Cancer Center of People's Liberation Army, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, People's Republic of China

Abstract Pulmonary intravascular macrophages (PIMs) are often responsible for the clearance of blood-borne pathogens, including endotoxin, lipopolysaccharide of Gram-negative bacteria. It is well accepted that PIMs play a pivotal role in the pathogenesis of endotoxin-induced acute lung injury. However, the mechanisms by which PIMs are involved in the lipopolysaccharide-induced inflammatory responses remain unclear. Through the present study the following results were found: (1) When challenged with lipopolysaccharide (10 μg/ml), PIMs underwent marked cellular enlargement, intercellular adhesion plaques became longer, and some particulates were enwrapped in the pseudopods. (2) Lipopolysaccharide could up-regulate the expression of some inflammatory mediators in PIMs, including TNF-α, IL-1β, IL-6, IL-8, and COX-2, and these up-regulated expression of inflammatory mediators correlated with NF-κB activation. (3) Dexamethasone as well as acetylsalicylic acid reduced the expression of TNF-α in lipopolysaccharide-challenged PIMs, and the decreased expression of TNF-α was also consistent with decreased NF-κB activation. Our results suggest that NF-κB activation in PIMs followed by phagocytizing lipopolysaccharide resulted in the up-regulation of TNF-α, IL-1β, IL-6, IL-8, and COX-2, which could be alleviated by dexamethasone. J. Cell. Biochem. 89: 1206–1214, 2003.

Key words: acute lung injury; cytokines; cyclooxygenase-2

Sepsis with multi-organ failure is the leading cause of death in modern care medicine, with Gram-negative bacteria being involved in the majority of cases. Endotoxin, lipopolysaccharide (LPS) of Gram-negative bacteria, is regarded to mainly contribute to the microcirculatory abnormalities resulting in septic organ failure, among which acute respiratory distress syndrome represents the prototype [Brigham and Meyrick, 1986; Ermert et al., 2000]. Pulmonary intravascular macrophages (PIMs) are lung macrophages that are found apposed to the endothelium of pulmonary capillaries, and are responsible for the clearance of blood-borne particulates and pathogens in many species, including pigs. Their potential role in host defense and acute lung injury has attracted interest, since a number of studies have demonstrated pulmonary localization of circulating particles, microbes, and endotoxin by PIMs [Warner and Brain, 1990].

During endotoxemia, pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-8, are markedly up-regulated. These mediators are known to contribute to the vascular abnormalities and changes in bronchomotor tone occurring in LPS-induced lung injury [Johnston et al., 1998; Hirani et al., 2001]. Moreover, eicosanoid generation is also regarded as the mechanism by which LPS leads to acute lung injury. It is well established that the initial step in the generation of prostaglandins and thromboxane is the conversion of arachidonic acid to prostaglandin H2 via cyclooxygenase [Chang et al., 1989; Uhlig et al., 1996; Steudel et al.,

Grant sponsor: National Nature Science Fund of People's Republic of China; Grant number: 39670311.

*Correspondence to: Bo Zhu, Cancer Center of PLA, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, People's Republic of China.
E-mail: bozhu@mail.tmmu.com.cn

Received 7 April 2003; Accepted 9 May 2003
DOI 10.1002/jcb.10590

1997]. Until now, two isoforms of this key enzyme have been described, cyclooxygenase-1 (COX-1), which was known to be expressed constitutively in various cell types; and cyclooxygenase-2 (COX-2), which was commonly expressed under inflammatory conditions [Seeger et al., 1987; Hempel et al., 1994; Goppelt-Struebe, 1995]. In the present study, we have explored whether TNF-α, IL-1β, IL-6, IL-8, and COX-2 were up-regulated in PIMs stimulated with LPS.

Previous results using other types of macrophage have demonstrated that LPS up-regulated the expression of pro-inflammatory cytokines and COX-2 via activating NF-κB [Ermert et al., 2000; Giroux and Descoteaux, 2000]. On the other hand, dexamethasone (DEX) and acetylsalicylic acid (ASA), as the steroidal and non-steroidal anti-inflammatory drugs in clinic, respectively, could inhibit the activation of NF-κB [Matsumura et al., 2001; Amann and Peskar, 2002]. Therefore, we speculated that DEX and ASA might contribute to the alleviation of acute lung injury via inhibition of NF-κB activation, which down-regulates the expression of inflammatory mediators. In this study we have implicated NF-κB as the mechanism underlying the up-regulation of TNF-α, IL-1β, IL-6, IL-8, and COX-2 induced by LPS in PIMs.

MATERIALS AND METHODS

Animals

Healthy piglets were obtained from The Institute of Animals for Medical Scientific Research located in Third Military Medical University. All experimental procedures were performed in accordance with the guidelines of the National Institute of Health set forth in "Guide for the care and use of laboratory animal."

Observation of PIMs With Transmission Electron Microscopy (TEM)

For observation of normal PIMs, lungs from piglets were removed from the chest cavity and cut to 1 mm^3 pieces, rinsed in cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated in graded ethanols, and transferred through propylene oxide to Embed 812 resin. Thin sections were then examined with a JME-2000EX transmission electron microscope. LPS-treated PIMs were collected and also examined with TEM.

Isolation of PIMs

PIMs were isolated from piglets according to the published methods [Morton and Bertram, 1988] with some modifications. The piglets (male, body weight 20–25 kg) were deeply anesthetized with pentobarbital-Na given i.p. (30 mg/kg body weight), and then the piglets were anti-coagulated with 5,000 U of heparin through i.v. After midsternal thoracotomy, the right and left ventricles were incised, and a cannula was fixed in the right and left ventricle, respectively. The aorta, cava inferior, and cava superior, were then ligated, respectively. Pulsatile perfusions with buffer solution were carried out as following procedures. (I) 250 ml of 0.75% $NaNO_3$ diluted in the 0.15 mol/l PBS; (II) 1,000 ml of 0.04% Na_2 EDTA diluted in the Hank's without Ca^{2+} or Mg^{2+}; (III) 500 ml of Hank's with 0.055% $CaCl_2$; (IV) 250 ml of 0.1% collagenase diluted in Hank's with 0.055% $CaCl_2$; (V) 1,750 ml of Hank's without Ca^{2+} or $Mg.^{2+}$ Finally, the perfusions from the left ventricle were collected.

The above perfusions were centrifuged at 1,000 rpm for 10 min at 4°C and the pelleted cells were collected. Each pellet was suspended in 9 ml of sterile distilled water for 20 s to lyse the red blood cells and then isotonicity was restored by addition of 1 ml of 1.5 mol/l PBS. PIMs were isolated from the remaining cells by adherence to plates (2 h at 37°C, 5% CO_2).

PIMs Stimulation

Freshly isolated PIMs were incubated with RPMI 1640 medium (containing 10 mmol/l HEPES, 2 mmol/l L-glutamine, 100 U/ml penicillin, and 100 μg/ml gentamicin), supplemented with 10% heat-inactivated FCS. For stimulation with LPS, cells were incubated in the appropriate medium with 10 μg/ml LPS. DEX (1 μmol/l) and aspirin (3 mmol/l) were added 2 h prior to stimulation, according to the specified experiment. Following the appropriate treatment, cell supernatants were collected and stored at −20°C until analysis.

ELISA and RIA for the Measurement of Cytokine Concentration

PIMs were stimulated with LPS (Sigma, Missouri, USA, 10 μg/ml) in 24-well flat-bottom plates (2×10^5 cells per well) for various times at 37°C. The production of TNF-α, IL-6, and IL-8

was assessed by ELISA according to manufacturer's protocol. IL-1β was measured by RIA using commercially available reagents.

RNA Extraction and Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

RT-PCR were performed to analyze the expression of COX-2 mRNA in PIMs. Total cellular RNA was extracted from PIMs with Tripure Isolation Regent kit (Promega, Wisconsin, USA). Total cellular RNA from each sample (1 μg) was reverse transcribed at 42°C for 1 h. The same amount of the resulting cDNA was used for amplification by a specific primer for rabbit COX-2. The following oligonucleotides were used as primers for COX-2: sense 5′-ACT CAC TCA GTT TGT TGA GTC ATT C-3′; antisense 5′-TTT GAT TAG TAC TGT AGG GTT AAT G-3′. Primers were also synthesized to amplify the cDNA encoding HPRT, a constitutively expressed gene, as a control. HPRT primers were 5′-GCT GGT GAA AAG GAC CTC TCG -3′ and 5′-GCA GAT GGC CAC AGG ACT AGA-3′. Both primers were synthesized commercially in Sangon company (Shanghai, China). Thirty amplification cycles were carried out with the following parameters: 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. Cycling was ceased with a final extension of 10 min at 72°C. RT-PCR products were visualized with ethidium bromide and semi-quantitatively analyzed by densitometric scanning of the gel images.

Immunohistochemistry

Immunohistochemical staining was used to detect the expression of COX-2 in PIMs with the SP method. The sections were placed into 1% H_2O_2 methanol at room temperature for 20 min and then incubated with goat anti-COX-2 antibody (1:100) (Santa Cruz Laboratories, Santa Cruz, California, USA) at 37°C for 1 h. The slides were then incubated at 4°C overnight. Subsequently, the slides were incubated with biotin-anti-goat-IgG (1:200) (Zhongshan Company, Beijing, China) and SP complex at 37°C for 30 min, respectively. After each procedure, the sections were washed thoroughly with PBS for 5 min, total three times. The images were analyzed by previous published methods [El Samalouti et al., 1999]. However, a 12-bit cooled CCD camera instead of the previously used 8-bit system was used, which significantly enhance the sensitivity for the detection of changes in staining intensity.

Nuclear Extracts and Electrophoretic Mobility Shift Assay

Nuclear extracts were prepared from PIMs according to published methods [Schreiber et al., 1989]. In brief, the cells were washed in a 0.5 ml of cold PBS and then in 400 μl of cold buffer A (10 mmol/l HEPES, pH7.9, 15 mmol/l $MgCl_2$ and 0.5 mmol/l dithiothreitol). The washed cell pellets were then suspended in 50 μl of buffer A plus 0.1% Nonidet P-40 supplemented with 0.1 μg/ml leupeptin and aprotinin, and incubated on ice for 30 min. After incubation, the pellets were mixed briefly by vortex and centrifuged at 10,000 rpm at 4°C for 5 min in a microcentrifuge. The supernatant was carefully removed, and the nuclear pellet was suspended in 20 μl of cold buffer C (20 mmol/l HEPES, 25% glycerol, 0.42 mol/l NaCl, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l EDTA, 0.5 mmol/l dithiothreitol and 0.5 mmol/l phenylnethlsulfory fluoride) containing 1 μg/ml leupeptin and aprotinin, and incubated on ice for 15 min with intermittent vortexing. The extracts were then centrifuged at 10,000 rpm at 4°C for 10 min, and the supernatant was divided into aliquots and frozen at −70°C. Protein concentration was determined with the method of Bradford.

A double-stranded oligonucleotide containing the sequence corresponding to classical NF-κB consensus site (5′-TGC TAA CAA TCA GAT AGA GG-3′) (Gibco, California, USA) was end-labeled with [γ-^{32}P] ATP (Yahui Company, Beijing, China) with the aid of T4 ligase (Biolab, North Carolina, USA). Binding reactions were carried out at room temperature for 20 min in a final volume of 25 μl (10 mmol/l HEPES, pH 7.9, 4 mmol/l Tris-HCl, 60 mmol/l KCl, 1 mmol/l EDTA, 1 mmol/l dithiothreitol, 10% glycerol, 1.5 mg/ml bovine serum albumin, 2 μg of poly (dI-dc), 4 μg of nuclear extracts and 0.5 ng of ^{32}P-labeled oligonucleotide probe). In the experiments of specific competitive inhibition and non-specific competitive inhibition, 0.5 ng unlabeled NF-κB probe or unlabeled GATA3 probe (5′-TGC TAA CAA TCA GAT AGA GG-3′) was added before incubation with ^{32}P-labeled oligonucleotide probe. Binding reactions were subjected to non-denaturing polyacylamide electrophoresis through 60 g/l gels in a 1× Tris borate-EDTA buffer system. Gels were dried and subjected to autoradiography.

Statistical Analysis

Analysis of variance was used to evaluate differences among different groups. Correlation analysis was made between the related parameters. A value of $P < 0.05$ was considered significant. Data are given as mean ± SEM.

RESULTS

Morphological Characteristics of PIMs After Challenged With LPS

Our results showed that PIMs in the lungs of piglets have morphological features of differentiated macrophages, such as irregular shape, an indented nucleus, lysosomal granules, pseudopods, phagosomes and phagolysosomes, tubular micropinocytosis vermiformis structures (TMVS), and a fuzzy glycocalyx, which are in accordance with other reports [Morton and Bertram, 1988]. PIMs were also adhered to pulmonary capillary endothelial cells through intercellular adhesion plaques (ICAP) (Fig. 1B). Enlarged and increased pseudopods, and increased amount of lysosomes and phagosomes were found in the in vitro isolated PIMs after stimulated with LPS. Moreover, some particulates were enwrapped in the pseudopods exruded from PIMs, which seems like in the period of phagocytosis (Fig. 1C).

Increased Pro-Inflammatory Cytokine Production in PIMs Stimulated With LPS

Freshly prepared PIMs from piglets were stimulated in vitro with LPS (10 μg/ml), and the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 or IL-8) in the culture supernatant was determined by ELISA or RIA. The LPS-induced TNF-α and IL-1β production was significantly increased at 1 h after stimulation, and the production of IL-1β kept to increase and reached to peak level at 2 h after stimulation. In detecting of IL-6 level, significant increase was only found at 4 h, whereas, sustained increases of IL-8 level were detected from 4 to 8 h after stimulation with LPS. Interestingly, the time for reaching the peak level for TNF-α production was earlier (at 1 h), whereas they were later for IL-6 and IL-8 (at 4 and 6 h after LPS-stimulation, respectively). More importantly, NF-κB activation was prior to the production of inflammatory mediators mentioned above in PIMs stimulated with LPS (10 μg/ml), and a positive correlation was found between the activated NF-κB and the concentration of TNF-α at 1 h after LPS stimulation ($r = 0.991$, $P < 0.01$). These results suggest that the production of pro-inflammatory cytokines is increased in LPS- stimulated PIMs in comparison with wild-type PIMs (Fig. 2), and NF-κB activation is an early event in LPS-stimulated PIMs, which results in an up-regulation of inflammatory mediators.

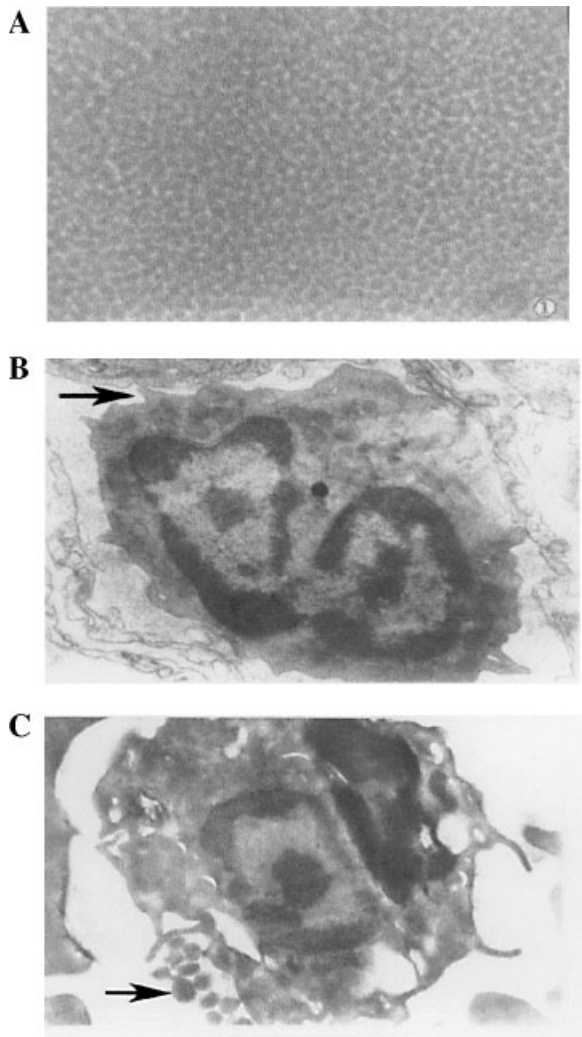

Fig. 1. Morphological characteristics of naïve PIMs and that of LPS-induced PIMs. Experimental details are described in Materials and Methods. **A**: Morphological characteristic of naïve PIMs with phase-difference microscope (×100). **B**: Naïve PIMs have irregular shape, an indented nucleus, lysosomal granules, pseudopods, phagosomes, and phagolysosomes, TMVS, and a fuzzy glycocalyx. Naïve PIMs adhered to pulmonary capillary endothelial cells through ICAP (→) (×15,000). **C**: Enlarged and increased pseudopods, and increased amount of lysosomes and phagosomes were found in the PIMs after stimulated with lipopolysaccharide (10 μg/ml). Some particulates were enwrapped in the pseudopods extruded from PIMs, which seems like in the period of phagocytosis (→) (×15,000).

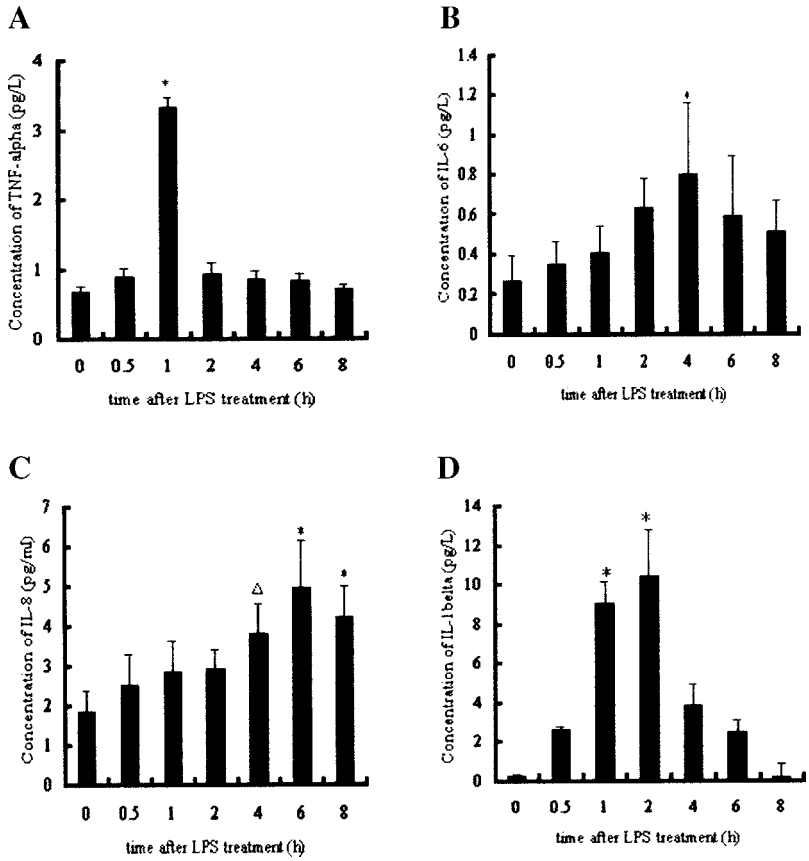

Fig. 2. Expression of TNF-α, IL-1β, IL-6, and IL-8 in LPS-induced PIMs. TNF-α, IL-6, and IL-8 were determined with ELISA, IL-1β were determined with RIA, described in Materials and Methods. **A**: TNF-α. **B**: IL-6. **C**: IL-8. **D**: IL-1β. *: $P < 0.01$, △: $P < 0.05$ versus control group.

Expression of COX-2 in PIMs Stimulated With LPS

COX-2, one of the most important cyclooxygenases converting arachidonic acid to prostaglandin H_2, plays a key role in the initiation and enlargement of inflammatory response. Thus, we next explored whether up-regulation of COX-2 occurred in LPS-stimulated PIMs. Our results with RT-PCR and immunohistochemistry showed that COX-2 was not detectable in unstimulated PIMs (data not shown). Treatment of PIMs with LPS (10 μg/ml) for increasing times resulted in expression of COX-2, which was maximal by 4 h on the level of mRNA and by 8 h on the level of protein (Figs. 3 and 4B).

DEX Inhibited the Expression of TNF-α and COX-2 in LPS-Stimulated PIMs, But Aspirin Only Inhibited TNF-α Expression

Due to the importance of DEX and aspirin in the treatment of acute lung injury and acute respiratory distress syndrome, we next explored the effects of these two drugs on the production of pro-inflammatory cytokine (TNF-α) in the LPS-induced PIMs. Our results showed that DEX and aspirin could inhibit the increased production of TNF-α in PIMs challenged with LPS (Fig. 4A). These results implicated the mechanism by which DEX and aspirin might be used to treat acute lung injury and ARDS.

DEX (2 μmol/l) incubated for 2 h prior to LPS challenge diminished the amount of COX-2 significantly. Reduction of COX-2 mRNA resulted in attenuation of COX-2 protein levels, as assessed by immunohistochemistry. However, no inhibition was observed in the PIMs pretreated with aspirin before LPS stimulation. (Fig. 4B).

DEX and Aspirin Inhibited LPS-Induced NF-κB Activation in PIMs

It is well known that NF-κB plays a pivotal role in the expression of several pro-inflammatory

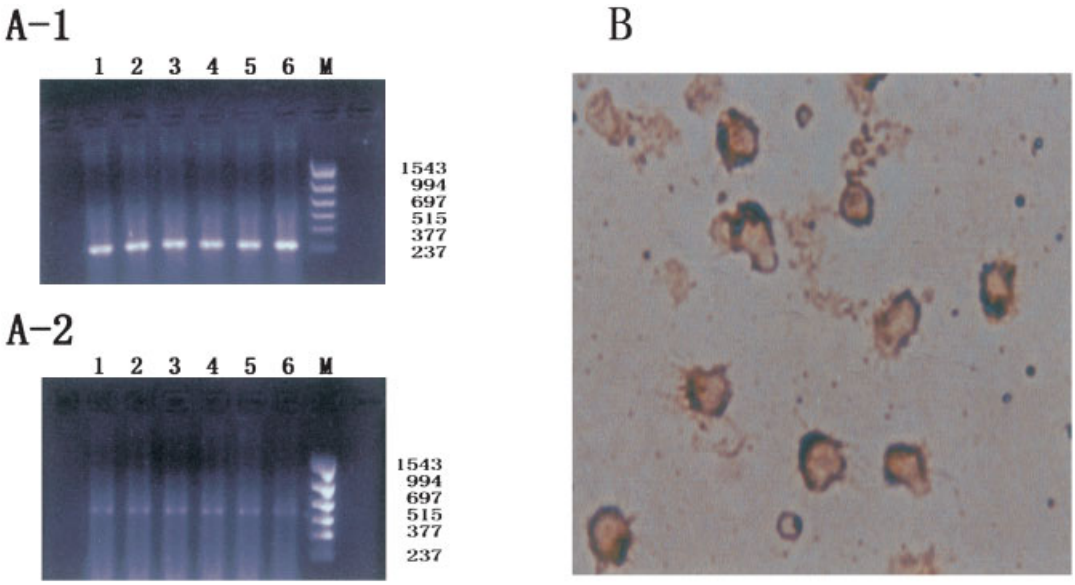

Fig. 3. COX-2 expression at the level of mRNA and protein in LPS-induced PIMs. **A**: PCR amplification was performed with specific primers, and PCR products were demonstrated through electrophoresis on a 1.2% agarose gel with ethidium bromide staining and densitometric scanning of the gel images were used to analyze semi-quantitatively. **1**: HPRT product, **lanes 1–6**: 0.5 h, 1 h, 2 h, 4 h, 6 h, and 8 h after stimulation with LPS, respectively. **2**: COX-2 product, **lanes 1–6**: 0.5 h, 1 h, 2 h, 4 h, 6 h and 8 h after stimulation with LPS, respectively. **B**: Representative for immunohistochemistry analysis of COX-2 expression in LPS-induced PIMs, which is on 8 h after stimulation with LPS. The first antibody to COX-2 is a polyclonal, a goat antibody against mosue COX-2 protein (×200). Image analysis was used to determine staining intensity of COX-2 protein semi-quantitatively.

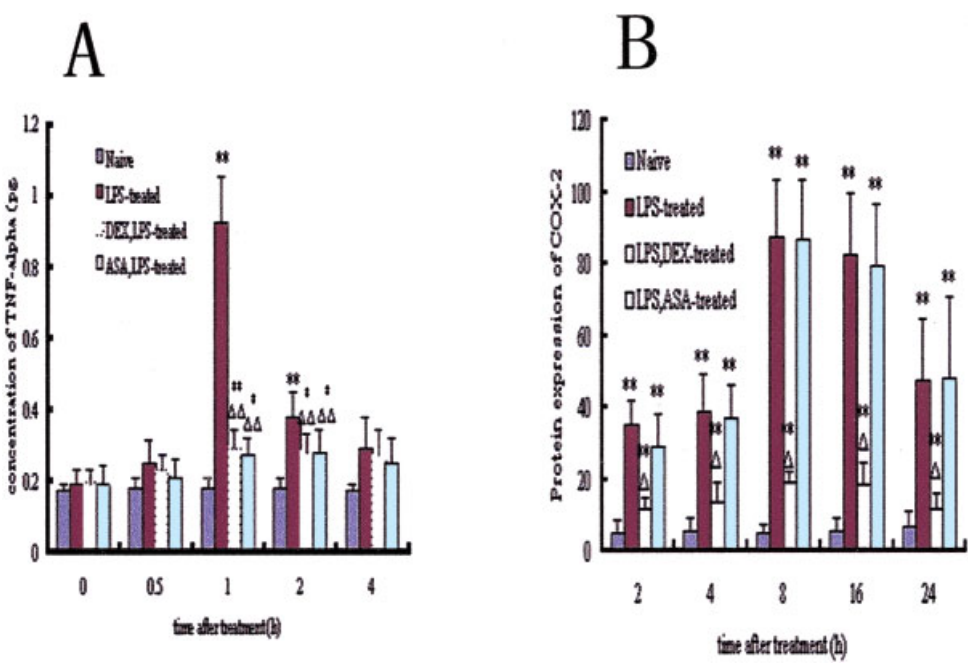

Fig. 4. The effects of DEX and aspirin on the expression of TNF-α, IL-1β, IL-6 IL-8, and COX-2 in LPS-induced PIMs. **A**: DEX and aspirin inhibited the expression of TNF-α, IL-1β, IL-6, and IL-8 in LPS-induced PIMs. **B**: DEX inhibited the expression of COX-2 mRNA and protein in LPS-induced PIMs, but aspirin did not. *: $P < 0.05$, **: $P < 0.01$ versus control group; △: $P < 0.05$, △△: $P < 0.01$ versus LPS-stimulated group.

cytokines and COX-2. Therefore, we next explored the effect of LPS on the activation of NF-κB in PIMs, on the basis of finding that LPS up-regulate the expressions of TNF-α, IL-1β, IL-6, IL-8, and COX-2. Nuclear extracts of PIMs challenged with LPS (10 μg/ml) for 30 min formed a DNA-protein complex with the specific NF-κB-binding sequence (Fig. 5A) which was prevented if a 100-fold excess of unlabeled NF-κB probe has been added (data not shown). This is in agreement with results published in the same cell type.

To further explore the mechanisms involved in the effect of DEX and aspirin on the expression of TNF-α, IL-1β, IL-6, IL-8, and COX-2, the pattern of NF-κB activation in LPS-stimulated PIMs pretreated with DEX or aspirin was evaluated. Our results showed that DEX or aspirin could partly inhibit the NF-κB activation in LPS-stimulated PIMs, and the inhibitory effects by aspirin were stronger than that by DEX. (Fig. 5B).

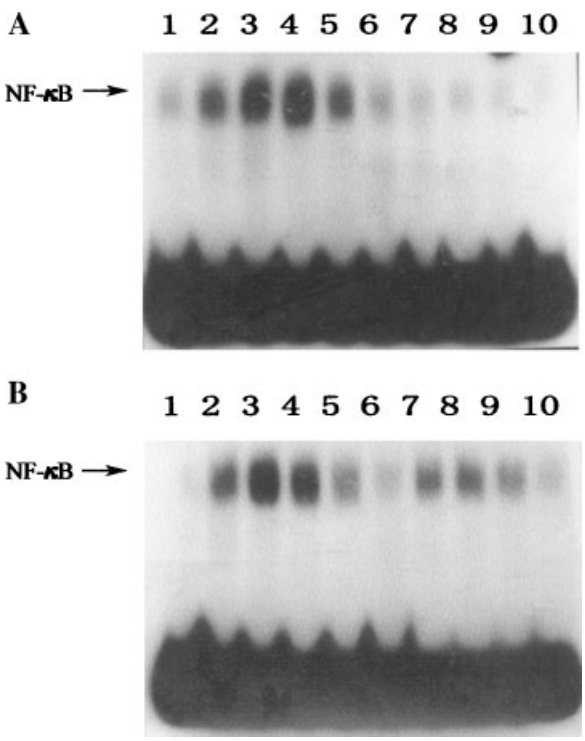

Fig. 5. LPS induced NF-κB activation in PIMs, whereas DEX and aspirin partially inhibited this activation. **A**: EMSAs for the distal NF-κB probe were performed on nuclear extracts of PIMs treated with LPS (10 μg/ml). **Lanes 1–5**: 0, 0.5 h, 1 h, 2 h, 4 h after stimulation with LPS, respectively; **lanes 6–10**: 0, 0.5 h, 1 h, 2 h, 4 h after stimulation with PBS, respectively. **B**: PIMs treated with LPS (10 μg/ml) and DEX (1 μmol/l) or aspirin (3 mmol/l), simultaneously. **Lanes 1–5**: 0, 0.5 h, 1 h, 2 h, 4 h after stimulation with LPS and DEX, respectively; **lanes 6–10**: 0, 0.5 h, 1 h, 2 h, 4 h after stimulation with LPS and aspirin, respectively.

DISCUSSION

PIMs are a resident cell population, junctionally adherent to the capillary endothelium of lungs and morphologically similar to hepatic Kupffer cells, which are a pulmonary constituent of the mononuclear phagocyte system with respect to secretory, endocytic, and functional properties [Winkler, 1988]. Removal of blood-borne particulates in calves, sheep, goats, cats, and pigs occurs predominantly in these macrophages [Winkler, 1988]. In this study we isolated PIMs from piglets and found that PIMs have morphological features of differentiated macrophages, such as irregular shape, an indented nucleus, lysosomal granules, pseudopods, phagosomes and phagolysosomes, TMVS, and a fuzzy glycocalyx, which are in accordance with other reports. When challenged with LPS, PIMs underwent marked cellular enlargement, the ICAP became longer and some particles were enwrapped in the pseudopods, which suggested that PIMs are highly activated after phagocytizing LPS.

Previous reports have shown that inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-8 play pivotal roles in the pathogenesis of acute lung injury [Walley et al., 1999; Kolb et al., 2001; Kabir et al., 2002; Liaudet et al., 2002]. The substances mediating acute lung injury followed by PIM activation, however, remain unknown. In the present study we found up-expression of these cytokines in LPS-challenged PIMs. Interestingly, after stimulation with LPS, the production of IL-6 and IL-8 was peaked at 4 h or 6 h, while TNF-α was peaked at 1 h. These results suggest that TNF-α itself could up-regulate the expression of cytokines, including IL-6 and IL-8. Therefore, our results may implicated that TNF-α played a key role in initiation and enlargement of inflammatory responses through inducing and up-regulating other pro-inflammatory cytokines. TNF-α, IL-1β, IL-6, and IL-8 could do damage to lung tissues through chemotaxis, recruitment of polymorphonuclear neutrophils, and accelerate them adherent to vascular endothelia. The results from our and other groups suggested that the up-regulation of TNF-α, IL-1β, IL-6, and IL-8 might be one of the mechanisms by which PIMs are involved in the pathogenesis of

acute lung injury, even acute respiratory distress syndrome.

On the other hand, prostanoids with various physiological effects in the lung have been shown to be one of the important factors in acute lung injury [Goggel et al., 2002]. COX-2, one of the most important cyclooxygenases converting arachidonic acid to prostaglandin H2 [McAdam et al., 1999], has been proven to play a key role in the initiation and enlargement of the inflammatory response [Gust et al., 1999]. Accordingly, we explored whether up-regulation of COX-2 occurred in LPS-stimulated PIMs. Results from RT-PCR and immunohistochemistry showed that LPS could up-regulate the expression of COX-2 in the PIMs. These results clearly confirmed that COX-2 in PIMs plays a critical role in the development of the inflammatory response by altering key components of the inflammatory cascade.

NF-κB is required for the transcription of many inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-8 [Blackwell and Christman, 1997; Christman et al., 1998; Sharma et al., 1998; Fan et al., 2001], which are thought to be important in the pathogenesis of acute lung injury. Furthermore, mitogen-activated protein kinase and protein kinase C are involved in COX-2 expression by LPS. These pathways act, in a coordinate way, with several transcription factors, among which NF-κB is the most important [Hwang et al., 1997; Inoue and Tanabe, 1998; Giroux and Descoteaux, 2000]. Therefore, we hypothesized that LPS-induced NF-κB activation in PIMs might also contribute to the up-regulation of inflammatory mediators, including TNF-α, IL-1β, IL-6, IL-8, and COX-2. Our present data indicated that NF-κB activation was prior to the production of inflammatory mediators mentioned above in PIMs stimulated with LPS (10 μg/ml), and a positive correlation was found between the activated NF-κB and the concentration of TNF-α at 1 h after LPS stimulation ($r = 0.991$, $P < 0.01$). These apparently correlated results allowed the conclusion that NF-κB activation is an early event in LPS-stimulated PIMs, which results in the up-regulation of inflammatory mediators.

It is generally accepted that DEX and aspirin, as the most important anti-inflammatory agents, have potent effectiveness in the treatment of pulmonary inflammatory disorders, including acute lung injury and acute respiratory distress syndrome [Chelucci et al., 1993; Corbanese, 2001]. On the other hand, via secreting inflammatory mediators, PIMs play a pivotal role in the pathogenesis of acute lung injury. The mechanisms by which these two anti-inflammatory agents alleviate PIMs-mediated damage to lung tissues, however, remain to be established. Our present results showed that DEX might reduce the expression of TNF-α, IL-1β, IL-6, IL-8, and COX-2 in LPS-treated PIMs by inhibiting NF-κB activation. Interestingly, aspirin could not reduce the COX-2 expression although it could partially inhibit NF-κB activation. Our results (data not shown), however, showed that COX-2 activity was significantly reduced by aspirin in LPS-stimulated PIMs, which implicated other mechanisms might be involved in clinical use of aspirin for treatment of acute inflammatory diseases including acute lung injury.

In conclusion, our present data indicates that NF-κB activation in PIMs followed by phagocytizing endotoxin results in the up-regulation of TNF-α, IL-1β, IL-6, IL-8, and COX-2, which might be involved in the pathogenesis of endotoxin-induced acute lung injury. Moreover, the alleviative effects of DEX on acute lung injury might involve the inhibition of expression of TNF-α, IL-1β, IL-6, IL-8, and COX-2 via decreasing NF-κB activation. Aspirin was only found to inhibit the expression of TNF-α, IL-1β, IL-6, and IL-8 in LPS-stimulated PIMs.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Dr. Jianxin Zhou, Department of Biochemistry, Third Military Medical University.

REFERENCES

Amann R, Peskar BA. 2002. Anti-inflammatory effects of aspirin and sodium salicylate. Eur J Pharmacol 447:1–9.

Blackwell TS, Christman JW. 1997. The role of nuclear factor-kappa B in cytokine gene regulation. Am J Respir Cell Mol Biol 17:3–9.

Brigham KL, Meyrick B. 1986. Endotoxin and lung injury. Am Rev Respir Dis 133:913–927.

Chang S-W, Westcott JY, Pickett WC, Murphy RC, Voelkel NF. 1989. Endotoxin-induced lung injury in rats: Role of eicosanoids. J Appl Physiol 66:2407–2418.

Chelucci GL, Boncinelli S, Marsili M, Lorenzi P, Allegra A, Linden M, Chelucci A, Merciai V, Cresci F, Rostagno C. 1993. Aspirin effect on early and late changes in acute lung injury in sheep. Intensive Care Med 19(1):13–21.

Christman JW, Lancaster LH, Blackwell TS. 1998. Nuclear factor kappa B: A pivotal role in the systemic

inflammatory response syndrome and new target for therapy. Intensive Care Med 24:1131–1138.
Corbanese U. 2001. Late steroid therapy in primary acute lung injury. Intensive Care Med 27(2):453–454.
El Samalouti VT, Schletter J, Chyla I, Lentschat A, Mamat U, Brade L, Flat HD, Ulmer AJ, Hamann L. 1999. Identification of the 80-kDa LPS-binding protein (LMP80) as decay-accelerating factor (DAF, CD55). FEMS Immunol Med Microbiol 23:259–269.
Ermert L, Ermert M, Merkle M, Goppelt-Struebe M, Duncker HR, Grimminger F, Seeger W. 2000. Rat pulmonary cyclooxygenase-2 expression in response to endotoxin challenge: Differential regulation in the various types of cells in the lung. Am J Pathol 156: 1275–1287.
Fan J, Ye RD, Malik AB. 2001. Transcriptional mechanisms of acute lung injury. Am J Physiol Lung Cell Mol Physiol 281:L1037–L1050.
Giroux M, Descoteaux A. 2000. Cycooxygenase-2 expression in macrophages: Modulation by protein kinase C-alpha. J Immunol 165:3985–3991.
Goggel R, Hoffman S, Nusing R, Narumiya S, Uhlig S. 2002. Platelet-activating factor-induced pulmonary edema is partly mediated by prostaglandin E(2), E-prostanoid 3-receptors, and potassium channels. Am J Respir Crit Care Med 166:657–662.
Goppelt-Struebe M. 1995. Regulation of prostaglandin endoperoxide synthase (cyclooxygenase) isozyme expression. Prostaglandins Leukot Essent Fatty Acids 52:213–222.
Gust R, Kozlowski JK, Stephenson AH, Schuster DP. 1999. Role of cyclooxygenase-2 in oleic acid-induced acute lung injury. Am J Respir Crit Care Med 160:1165–1170.
Hempel SL, Monick MM, Hunninghake GW. 1994. Lipopolysaccharide induces prostaglandin H synthase-2 protein and mRNA in human alveolar macrophages and blood monocytes. J Clin Invest 93:391–396.
Hirani N, Antonicelli F, Strieter RM, Wiesener MS, Ratcliffe PJ, Haslett C, Donnelly SC. 2001. The regulation of interleukin-8 by hypoxia in human macrophages—a potential role in the pathogenesis of the acute respiratory distress syndrome (ARDS). Mol Med 7:685–697.
Hwang D, Jang BC, Yu G, Boudreau M. 1997. Expression of mitogen-inducible cyclooxygenase induced by lipopolysaccharide: Mediation through both mitogen-activated protein kinase and NF-κB signaling pathways in macrophages. Biochem Pharmacol 54:87–96.
Inoue H, Tanabe T. 1998. Transcriptional role of the nuclear factor κB site in the induction by lipopolysaccharide and suppression by dexamethasone of cyclooxygenase-2 in U937 cells. Biochem Biophys Res Commun 244:143–148.
Johnston CJ, Finkelstein JN, Gelein R, Oberdorster G. 1998. Pulmonary cytokine and chemokine mRNA levels after inhalation of lipopolysaccharide in C57BL/6 mice. Toxicol Sci 46:300–307.
Kabir K, Gelinas JP, Chen M, Chen D, Zhang D, Luo X, Yang JH, Carter D, Rabinovici R. 2002. Characterization of a murine model of endotoxin-induced acute lung injury. Shock 17:300–303.
Kolb M, Margetts PJ, Anthony DC, Pitossi F, Gauldie J. 2001. Transient expression of IL-1beta induces acute lung injury and chronic repair leading to pulmonary fibrosis. J Clin Invest 107:1529–1536.
Liaudet L, Mabley JG, Pacher P, Virag L, Soriano FG, Marton A, Hasko G, Deitch EA, Szabo C. 2002. Inosine exerts a broad range of antiinflammatory effects in a murine model of acute lung injury. Ann Surg 235:568–578.
Matsumura M, Kakishita H, Suzuki M, Banba N, Hattori Y. 2001. Dexamethasone suppresses iNOS gene expression by inhibiting NF-kappaB in vascular smooth muscle cells. Life Sci 69:1067–1077.
McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, FitzGerald GA. 1999. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: The human pharmacology of a selective inhibitor of COX-2. Proc Natl Acad Sci USA 96:272–277.
Morton D, Bertram TA. 1988. Isolation and preliminary in vitro characterization of the porcine pulmonary intravascular macrophage. J Leukoc Biol 43:403–410.
Schreiber E, Matthias P, Muller MM, Schaffner W. 1989. Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. Nucleic Acids Res 17:6419.
Seeger W, Menger M, Walmrath D, Becker G, Grimminger F, Neuhof H. 1987. Arachidonic acid lipoxygenase pathways and increased vascular permeability in isolated rabbit lungs. Am Rev Respir Dis 136:964–972.
Sharma SA, Tummuru MK, Blaser MJ, Kerr LD. 1998. Activation of IL-8 gene expression by Helicobacter pylori is regulated by transcription factor nuclear factor-kappa B in gastric epithelial cells. J Immunol 160:2401–2407.
Steudel W, Krämer H-J, Degner D, Rosseau S, Schütte H, Walmrath D, Seeger W. 1997. Endotoxin priming of thromboxane-related vasoconstrictor responses in perfused rabbit lungs. J Appl Physiol 83:18–24.
Uhlig S, Nüsing R, Bethmann AV, Featherstone RL, Klein T, Brasch F, Müller KM, Ullrich V, Wendel A. 1996. Cyclooxygenase-2-dependent bronchoconstriction in perfused rat lungs exposed to endotoxin. Mol Med 2:373–383.
Walley KR, McDonald TE, Higashimoto Y, Hayashi S. 1999. Modulation of proinflammatory cytokines by nitric oxide in murine acute lung injury. Am J Respir Crit Care Med 160:698–704.
Warner AE, Brain JD. 1990. The cell biology and pathogenic role of pulmonary intravascular macrophages. Am J Physiol 258:L1–L12.
Winkler G-C. 1988. Pulmonary intravascular macrophages in domestic animal species: Review of structural and functional properties. Am J Anat 181:217–234.