Endotoxin-induced acute lung injury requires interaction with the liver

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Siore, Amsel M., Richard E. Parker, Arlene A. Stecenko, Chris Cuppels, Martha McKeon, Brian W. Christman, Roberto Cruz-Gervis, and Kenneth L. Brigham. Endotoxin-induced acute lung injury requires interaction with the liver. Am J Physiol Lung Cell Mol Physiol 289: L769–L776, 2005. First published July 8, 2005; doi:10.1152/ajplung.00137.2005.—Clinical and laboratory data indicate that the liver plays an important role in the incidence, pathogenesis, and outcome of acute lung injury/acute respiratory distress syndrome. To distinguish direct effects of endotoxin on the lungs from liver-dependent effects during the early phase of the response to endotoxemia, we used an in situ perfused piglet preparation in which only the ventilated lung or both the lung and liver could be included in a blood perfused circuit. We monitored pulmonary vascular resistance, oxygenation, neutrophil count, lung edema as reflected by wet-dry weights of lung tissue, perfusate concentrations of TNF-α, IL-6, and 8-isoprostane (a marker of oxidative stress), and activation of the transcription factor (NF-κB) in lung tissue before and for 2 h after endotoxin. When only the lung was perfused, endotoxin caused pulmonary hypertension and neutropenia; but oxygenation was maintained; TNF-α, IL-6, and 8-isoprostane levels were minimally elevated; and there was no lung edema. When both the liver and lung were perfused, endotoxin caused marked hypoxemia, large increases in perfusate TNF-α, IL-6, and 8-isoprostane concentrations, and severe lung edema. NF-κB activation in the lung was greatest when the liver was in the perfusion circuit. We conclude that the direct effects of endotoxemia on the lungs include vasoconstriction and leukocyte sequestration as a direct effect, but not lung injury. Maximal activation of lung NF-κB, a proinflammatory transcription factor, cytokine release, oxidant stress, and lung edema required the presence of the liver. Although our studies do not permit an unambiguous definition of specific roles for circulating mediators, we can conclude that interactions between the liver and the lung early in the response to endotoxemia are essential to the severe acute inflammatory response and lung injury characteristic of clinical ALI and ARDS.

METHODS

In Situ Perfused Piglet Preparation

We developed an in situ perfused piglet preparation that permits perfusion of either the lung alone or the lung and the liver in series (Fig. 1). We used pathogen-free young pigs (Palmetto Farm, Reevesville, SC) weighing 8–12 kg. The study adhered to National Institute of Health guidelines on use of experimental animals and was approved by the institutional animal care and use committee of Emory University. Animals were maintained in the Emory University Division of Animal Resources, an American Association for Accreditation of Laboratory Animal Care-approved facility.

Piglets were anesthetized, a tracheostomy was performed, and cannulas were placed in a carotid artery and a jugular vein. After heparinization, we exsanguinated the animals by drawing blood vascular permeability, and leukocyte activation in the lungs (29). Several functions of the liver, including endotoxin and bacterial clearance, generation of proinflammatory cytokines and eicosanoids, and synthesis of acute phase proteins, could modulate lung injury in the setting of sepsis (27). However, whether interactions between the lungs and the liver are essential to the pathogenesis of sepsis-induced ALI is not clear.

We hypothesized that endotoxemia affects the lungs by both direct and liver-dependent mechanisms and that both effects are necessary to trigger the severe inflammatory response that leads to ALI. To test that hypothesis and to elucidate the mechanisms and consequences of liver-lung interactions, we determined effects of endotoxemia in an in situ perfused swine preparation that permitted perfusion of only the lungs or the liver and lungs in series. We chose swine as an experimental animal because, like humans, they are especially sensitive to endotoxin and the pathophysiology of the response is similar to that in humans (10).

We determined effects of endotoxemia on physiological, biochemical, and molecular responses of the lungs with and without the liver in the perfusion circuit. Endotoxemia caused pulmonary vasoconstriction and neutrophil sequestration as a direct effect, but not lung injury. Maximal activation of lung NF-κB, a proinflammatory transcription factor, cytokine release, oxidant stress, and lung edema required the presence of the liver. Although our studies do not permit an unambiguous definition of specific roles for circulating mediators, we can conclude that interactions between the liver and the lung early in the response to endotoxemia are essential to the severe acute inflammatory response and lung injury characteristic of clinical ALI and ARDS.

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Experimental Protocols and Data Collection

Four groups of experiments were done with five animals in each group: in situ perfusion of only the lungs (with and without endotoxin) or lungs and liver perfused in series (with and without endotoxin).

The groups were designated as lung + liver + endotoxin (LL+E), lung only + endotoxin (L+E), lung + liver − endotoxin (LL−E), and lung only − endotoxin (L−E).

For groups receiving endotoxin, 5 μg/kg body weight of *Escherichia coli* endotoxin (serotype O55:B5; Difco, Detroit, MI) dissolved in normal saline was introduced into the blood reservoir after a stable baseline period.

Perfusate blood samples were collected twice before endotoxin administration (30 min before and immediately before) and at three time points thereafter (30 min, 1 h, and 2 h after endotoxin). Automated cell count and differential counts were performed every 15 min with a device that is specifically designed for use in laboratory animals (Hemevet; CDC Technologies, Oxford, CT). Blood gas analysis was performed every 15 min for the duration of the experiment by a portable blood gas analyzer (Radiplanb 248; Diamond Diagnostics, East Walpole, MA). For pH values outside the range of 7.35–7.45, we made adjustment either by changing the ventilation rate or by administering sodium bicarbonate. Serum samples were separated and stored at −80°C for measurement of TNF-α, interleukin-6 (IL-6), and 8-isoprostanone.

At each time point, we recorded pressures and flows in and out of the lungs and flow to the liver. Pulmonary vascular resistance (PVR) was calculated as in-flow pressure (pulmonary artery pressure) minus out-flow pressures (left atrial pressure) divided by total blood flow. We measured perfusate temperature continuously and kept it between 38 and 40°C by adjusting the temperature of the water in the water-jacket surrounding the reservoir.

Lung and liver tissue specimens were obtained at four time points (30 min before endotoxin and 30 min, 1 h, and 2 h after endotoxin) for the determination of NF-κB activity. The study was terminated 2 h after endotoxin, and both lungs were taken for determination of wet-to-dry weight ratio.

Cytokine Measurements

Plasma concentrations of TNF-α and IL-6 were measured separately using Quantikine ELISA kits (R&D Systems, Minneapolis, MN), according to the manufacturer’s instructions. In brief, samples were dispensed into 96-well microtiter plates that are precoated with porcine monoclonal or polyclonal antibodies specific to the above cytokines. After washing away any unbound substances, we added to the wells an enzyme (horseradish peroxidase)-linked monoclonal (TNF-α) or polyclonal (IL-6) antibody specific to the above cytokines. After washing away any unbound antibody-enzyme reagent, a substrate solution (hydrogen peroxide/tetramethylbenzidine) was added to the wells. The reaction was terminated with a stop buffer and absorbance read at 450 nm using an MRX Revelation (Dynex Technologies, Chantilly, VA) multwell plate reader. We determined values of IL-6 and TNF-α by reference to a standard curve constructed using the porcine proteins and computer software capable of generating a four-parameter logistic curve fit.

Measurement of NF-κB Activity in the Lung Tissue

ELISA. Nonradioactive NF-κB assays were performed by Chemicon NF-κB p65 transcriptional factor binding assay according to the manufacturer’s suggested protocol (Chemicon International, Temecula, CA). This immunoassay measures NF-κB p65 antigen that remains tethered to streptavidin plates via its binding to biotinylated NF-κB oligonucleotide after washing. Whole cell extracts (WCE) of lung tissue were prepared from frozen lung tissue by a protocol adapted from that of Boothby et al. (8). In brief, 100 mg of frozen

through the carotid artery with simultaneous infusion of equal volume of normal saline through the jugular vein. The blood was used to fill the warm water-jacketed blood reservoir and the perfusion circuit. The chest and the abdomen were then opened. For lung and liver preparation, the hepatic artery was isolated and ligated. The in-flow and the out-flow cannulas to the liver were placed in portal vein and inferior vena cava, respectively. The cannulas were then connected to the extracorporeal perfusion circuit containing blood at 40°C. The lungs were perfused at a constant flow rate of 40 ml·kg⁻¹·min⁻¹ (up to 400 ml/min), whereas the liver was perfused at 200 ml/min. Lung in-flow pressure was not allowed to rise >40 cmH2O by including an overflow circuit that reduced flow to the lung when in-flow pressure exceeded that level (see the circuit diagram in Fig. 1). The blood to both organs was circulated by two separate minipumps (Renal Systems, Minneapolis, MN). The lungs were ventilated with a piston ventilator (Harvard Apparatus, Dover, MA) with room air and 5% CO2. The minute ventilation was adjusted to maintain perfusate pH between 7.35 and 7.45. Throughout the experiments, pressures in the pulmonary artery, left atrium, and portal vein and flow in and out of both organs were monitored continuously, and the data were stored in a computer for later analysis. The lung-only preparation was identical to the lung and liver preparation described above, except that the liver was excluded from the circuit by ligation of both the aorta and the inferior vena cava at the level of the diaphragm.
tissue were dissected from tortilla-pressed and frozen biopsy samples and placed in 1.5 ml of cold ELB buffer [50 mM HEPES, pH 7.0, 250 mM NaCl, 5 mM EDTA (pH 8.0) and 0.1% Nonidet P-40, 1 mM PMSF, 1 mM DTT, and 1× protease cocktail (Roche complete protease inhibitor cocktail tablets, catalog no. 1 697 4980)]. Samples were homogenized with an IKA tissue homogenizer, and the homogenate was allowed to sit on ice for 5–10 min. Homogenate was transferred to microfuge tubes and spun at 14,000 rpm for 10 min, at 4°C, to obtain a clarified extract. Supernatant was carefully removed, aliquoted, frozen, and stored at −80°C. Protein concentration of the WCE was determined by the method of Bradford (9) using the Bio-Rad protein assay reagent protocol. Thawed aliquots of whole cell extracts were used for each assay and diluted with isolation buffer to obtain concentrations of 1 mg/ml protein. Duplicate assays were made on each sample, and results agreed within 20%. Values obtained were compared with positive HeLa control extracts from human cell line provided with the Chemicon assay kit.

EMSA. WCE, obtained by the same method described under ELISA, was evaluated for NF-kB binding activity using EMSA as described by Venkatakrisnan et al. (39). In summary, NF-kB double-stranded oligonucleotide probe was made by end-labeling single-stranded complementary oligonucleotides with 32P by T4 kinase transfer of gamma-labeled phosphate from ATP to the 5’ end of the oligonucleotide. End-labeled nucleotides were annealed under stringent conditions overnight at 45°C. Unincorporated nucleotide and phosphate were removed by molecular exclusion chromatography (Princeton separations spin column). Incorporation of radioactivity was assessed by liquid scintillation counting (Beckman LS6500), and counts/min (cpm) per microliter was calculated. Typically, 50,000 cpm of labeled NF-kB probe was used in the binding cocktail (5 μg of freshly thawed WCE aliquot, 0.030 pmol of NF-kB probe). Protein-DNA complexes were resolved by 5% PAGE in 0.5× Tris-borate buffer. Gels were vacuum dried and autoradiographed.

**Measurement of 8-Isoprostane**

This lipid peroxidation product was measured by stable isotope dilution and mass spectrometry based on the methods of Morrow and Roberts (30). In brief, to extract and hydrolyze isoprostane-containing phospholipids from perfusate samples, ice-cold Folch solution was added to a perfusate sample, and the mixture was agitated and centrifuged. The organic layer was concentrated and then incubated in methanol containing butylated hydroxytoluene and KOH to effect hydrolysis of the isoprostanes. After acidification and addition of tetra-deuterated standard (Cayman Chemical, Ann Arbor, MI), the mixture was purified with sequential reverse and straight-phase silica cartilages (Waters Associate, Milford, MA) and concentrated. Isoprostanes were then converted to pentfluorobenzyl (PFB) ester and purified by thin-layer chromatography. Isoprostanes were eluted from the silica, then converted to trimethylsilyl ether derivatives, and analyzed by mass spectrometry using a Hewlett-Packard 5982A mass spectrometer interfaced with an IBM Pentium computer system. Using the relative abundance of the peak to that of the heavy isotope internal standard, we calculated the concentration of 8-isoprostanes by interpolation from a standard curve and expressed it in ng/ml.

**Measurements of Lung Water**

At the completion of each experiment, both lungs were harvested and weighed (wet weight). The lungs were then homogenized, and the homogenate was dried to constant weight in an oven (dry weight). The ratio of wet-dry lung weight was calculated as a measure of the amount of lung water (edema) that was present.

**Statistical Analysis**

Statistical significant differences were determined by using analysis of variance and Tukey-Kramer for post hoc test. P < 0.05 was considered significant. In most cases the data are presented as change from baseline, with the baseline calculated as the mean of values at 30 min before and immediately before administration of endotoxin.

**RESULTS**

**Oxygenation and PVR**

As shown in Fig. 2A, endotoxemia caused a significant increase in PVR that reached peak at 30 min after endotoxin. This transient elevation of PVR was similar whether only the lung or both the lungs and the liver were perfused. In groups without endotoxin, PVR was stable throughout the experimental period. In the LL+E group, endotoxemia caused a statistically significant drop in perfusate blood oxygen tension at 30, 60, and 120 min after endotoxin. In the L+E group, oxygenation remained stable throughout the experimental period. The greatest decline in mean P O2 value from baseline in the LL+E group at the end of the experiment was ~70 mmHg compared with a decline of only ~15 mmHg in the L+E group (Fig. 2B).

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Fig. 2. A: change from baseline pulmonary vascular resistance (PVR) (means ± SE) in the 4 experimental groups with and without endotoxin (5 μg/kg) being added to the perfusate. PVR was calculated as follows: pulmonary artery pressure minus left atrial pressure divided by flow out of the lungs, and expressed in cmH2O·ml−1·min−1. Baseline values are the mean of the values at 30 min before and immediately before addition of endotoxin. The PVR was significantly increased in lung + liver + endotoxin (LL+E) and lung only + endotoxin (L+E) groups with peak reached 30 min after endotoxin and gradually returned to near baseline value by the end of the study. PVR was stable throughout the study period in groups without endotoxin. B: change from baseline perfusate oxygen tension expressed in mmHg (means ± SE) in 4 experimental groups over the 2-h experimental period. Perfusate blood P O2 was measured with a blood gas analyzer (Rapidlab 248; Diamond diagnostics, East Walpole, MA). The LL+E group was the only group with a significant drop in P O2 starting within 30 min of endotoxin administration, and with continuous decline for the duration of the experiment. For both A and B, statistical analysis was done by 2-way ANOVA and Tukey-Kramer for post hoc test.
Perfusate White Blood Cell and Neutrophil Counts

The pattern of change in perfusate white blood cell (WBC) and neutrophil counts is shown in Fig. 3. Endotoxemia caused similar degrees of leukopenia (Fig. 3A) whether both liver and lungs or only lungs were perfused. Leukocyte counts were stable throughout the experimental period in the absence of endotoxiaemia. The pattern of neutropenia was similar to that for leukopenia (Fig. 3B). Neutrophil counts began to fall early in the response and continued to fall to very low levels by the end of the experiment. Neutrophil counts did not change significantly in the absence of endotoxin, although there was a tendency for the counts to rise when only the lungs were perfused, presumably a result of release of a normal marginated neutrophil pool.

Lung Water Content

The wet-to-dry weight ratios of the lungs are shown in Fig. 4. The lungs were taken at the end of the experiment (120 min after endotoxin). Endotoxin caused pulmonary edema only when the liver was included in the perfusion circuit. The lung wet-to-dry weight ratios in the LL+E group was significantly higher compared with the other three groups. In L+E experiments, endotoxemia did not cause pulmonary edema, and the lung wet-to-dry weight ratio was not significantly different from the groups without endotoxin.

Cytokine Measurements

Concentrations of TNF-α and IL-6 in perfusate blood were measured at base line and at three time points after endotoxin administration. When both lung and liver were perfused, endotoxin caused a significant increase in the concentrations of both TNF-α and IL-6. Highest level of TNF-α level was seen at 2 h after endotoxin, but the levels at both 60 and 120 min after endotoxin were significantly higher than the values in the LL+E group (Fig. 5A). In the LL+E group, there was a significant increase in lung wet-to-dry weight ratio compared with the other groups, indicating significant pulmonary edema. There were no significant differences between the L+E group and the groups without endotoxin. LL−E, lung + liver − endotoxin; L−E, lung only − endotoxin. Statistical analysis was done by 2-way ANOVA.

NF-κB Activity

The DNA binding activities of NF-κB in whole cell extracts of lung tissues from all four groups were determined by both ELISA and EMSA, and that was specific for the p65 component. Figure 6A summarizes the ELISA, and Fig. 6B shows a representative EMSA. No activation of NF-κB was observed in the experiments without endotoxin, but NF-κB was activated in the lungs after endotoxemia both in the LL+E group and L+E group. At 60 min after endotoxin, NF-κB activation was significantly higher with perfusion of both the liver and lungs compared with perfusion of the lungs alone.
Isoprostane

We evaluated oxidative stress by measuring circulating concentrations of the lipid peroxidation product, 8-isoprostane (Fig. 7). The only group that showed increased perfusate concentrations of 8-isoprostane was the LL/E/H experiments. In this group, significantly higher values were seen at 60 and 120 min after endotoxin compared with the L/E/H group.

DISCUSSION

The most common cause of ALI in humans is sepsis, commonly with gram negative bacteria. Thus, infusion of gram negative bacterial endotoxin has been used as a model of sepsis induced-ALI in several animal species. Although virtually all mammalian species react to endotoxin, the magnitude of the response varies. Rodents require much larger doses of endotoxin for a similar response than do pigs, sheep, or humans (36), perhaps because endotoxin-sensitive species have prominent pulmonary intravascular macrophages that may play an important role in mediating lung injury (16, 31). Investigators have generally assumed that endotoxin responses in animals are relevant to those in humans, and swine have been used because their cardiovascular responses and sensitivity to endotoxin are similar to humans (40, 41).

ALI occurs most often in the setting of multiple organ failure (28), and, on the basis of clinical observations and experiments in intact animals, the liver has been implicated as actively contributing to the lung injury (12, 13, 15). In order to study interactions between the liver and the lungs more precisely, we developed an in situ autologous blood perfused preparation that permits study of endotoxin effects on either the lungs or the lungs and liver isolated from other organs. Comparisons of the effects of endotoxemia on physiological, cellular, biochemical, and molecular events in the lungs in the presence and absence of the liver provide unique information relevant to interactions between the two organs in the pathogenesis of ALI.

Pulmonary vasoconstriction is an acute effect of endotoxemia in intact animals, an effect that appears to depend on...
generation of the vasoconstrictor prostanooid thromboxane (36). In the perfused liver-lung preparation, we found that endotoxemia caused a marked increase in PVR peaking at 30 min after endotoxin and declining toward baseline thereafter, a time course like that reported in intact animals (32). That this response was a direct effect of endotoxin on the lungs is indicated by the fact that the response was identical whether the liver was included or not. In contrast, hypoxemia and pulmonary edema (both measures of lung injury) did not occur when only the lung was exposed to endotoxin but required the presence of the liver in a common perfusion circuit. Although oxygen consumption would be greater with the liver in the perfusion circuit, hypoxemia after endotoxemia must reflect lung dysfunction since the ventilated normal lung would fully oxygenate blood entering at venous $P_O_2$, as shown in the experiments where both liver and lung were perfused but endotoxin was not given. We conclude that endotoxin-induced ALI in this preparation depends on communication from the liver to the lungs through a common circulation. In whole animal preparations, it has been proposed that hepatic release of proximal cytokines, most often TNF-$\alpha$, mediates liver-dependent effects on the lungs (25).

Sequestration of circulating leukocytes, primarily neutrophils, in the lungs as reflected by neutropenia is an early event in the endotoxin response. This appears to be important for the pathogenesis of the injury as the early stages of endotoxin-induced lung injury are inhibited in neutrophil-depleted animals (11, 22). Both leukopenia and bronchoalveolar lavage neutrophilia are poor prognostic signs in humans with ARDS (37). Activation of sequestered neutrophils results in activation of NF-$\kappa$B, generation of TNF-$\alpha$ (and other proinflammatory cytokines), and generation of reactive oxygen species that cause oxidative tissue injury. Thus, neutrophils are thought to be early effector cells in hemorrhagic or endotoxin-induced ALI (3). We found that endotoxemia caused marked neutropenia beginning as early as 30 min after endotoxin administration and that the degree of neutropenia (and presumably lung neutrophil sequestration) was similar whether only the lung or both the lung and liver were exposed to endotoxin. This was true even though lung injury required the presence of the liver. These findings are consistent with the concept that neutrophil sequestration and activation are separate events, sequestration occurring as a direct effect of endotoxin on neutrophils, lung endothelium, or both, and activation of the sequestered leukocytes and consequent lung injury requiring a signal from the liver (14, 35).

Endotoxin induces expression of proinflammatory cytokines including TNF-$\alpha$ and IL-6 (7, 17), excess production of which has been associated with multiple organ dysfunction and mortality in humans (23). In clinical sepsis, circulating concentrations of TNF-$\alpha$ increase early and trigger an inflammatory cascade that includes other cytokines, lipid mediators, cell adhesion molecules, and generation of reactive oxygen species (7). The liver is a major source of circulating TNF-$\alpha$ after endotoxemia in humans (25, 26), and IL-6 production from normal or diseased liver is increased following endotoxin challenge (24, 38). We found modest increases in circulating concentrations of TNF-$\alpha$ and IL-6 following endotoxia when only the lung was perfused and large increases when the liver was included in the perfusion circuit. However, concentrations of IL-6 increased very late, and concentrations of TNF-$\alpha$ were first increased 1 h after endotoxia, whereas hypoxemia and neutropenia (and evidence of oxidant-mediated tissue injury, vide infra) began by 30 min. Circulating levels of cytokines would be a consequence of production from both liver and lung when both organs are perfused, so that the difference from results when only the lung is perfused may represent production by the liver or some more complicated interaction between the two organs. We conclude that TNF-$\alpha$ and IL-6 produced locally in the lungs as well as that released from the liver may play an important role in perpetuating and intensifying the injury, but the time course of the response compared with alterations in other variables suggests that neither of these cytokines is likely to be the mediator from the liver that initiates the lung injury process.

The transcription factor NF-$\kappa$B regulates expression of cytokines (including TNF-$\alpha$ and IL-6) and other mediators that participate in acute inflammatory responses (2). NF-$\kappa$B activation results from its nuclear translocation consequent to phosphorylation of a cytoplasmic binding protein, IxB. In both isolated cells and in whole animals, endotoxin causes activation of NF-$\kappa$B, mediated at least in part by oxidant stress, and its activation in the lungs is generally considered an early event in endotoxin-induced ALI (4, 34). We found that NF-$\kappa$B in the lungs was activated whether or not the liver was present but that the magnitude of activation was much greater when the liver was present. The time course and magnitude of lung NF-$\kappa$B activation are consistent with the conclusion that NF-$\kappa$B was driving expression of TNF-$\alpha$ and IL-6. However, there was minimal lung NF-$\kappa$B activation at 30 min after endotoxin administration and no difference at that time between the values for experiments where only the lungs were perfused and those where both organs were perfused. Because hypoxemia, neutropenia, and evidence of oxidant stress (vide infra) began by 30 min after endotoxin, we cannot implicate activation of NF-$\kappa$B as the initial trigger for the response, although it remains possible that activation of this transcription factor in a subpopulation of lung cells occurred earlier in the
LIVER-LUNG INTERACTION IN ENDOTOXIN-INDUCED ACUTE LUNG INJURY

response but that this was not detected in measurements in whole lung tissue. Activation of NF-κB detected in whole lung tissue after endotoxemia has been attributed to the presence of activated neutrophils sequestered in the lung microcirculation (1). In our studies, we saw similar degrees of neutropenia (and presumed neutrophil sequestration) following endotoxemia whether only the lung or both organs were perfused but considerably less NF-κB activation when only the lung was present. This would be consistent with similar degrees of neutrophil sequestration in both circumstances, but less activation of sequestered neutrophils in the absence of the liver.

ALI is associated with oxidative stress, at least in part due to reactive oxygen species produced by activated neutrophils, which is felt to be the proximate cause of the tissue injury (21). Although direct measurements of reactive oxygen species in biological systems are difficult (12, 20), measurements of the lipid peroxidation products, isoprostanes, appear to accurately reflect the magnitude of oxidative tissue injury (5, 33). We measured circulating concentrations of one of these species, 8-isoprostane, by gas chromatography/mass spectrometry over the course of the endotoxin response as an index of oxidative stress. We found no increase in circulating 8-isoprostane in any of the experimental groups except when both the lung and the liver were exposed to endotoxin. In that group, levels began to increase at 30 min after endotoxin, reached significance compared with controls by 1 h, and continued to increase to 2 h. These results are consistent with oxidative stress as a major cause of liver-dependent endotoxin-induced lung injury. Because circulating isoprostane levels may reflect endotoxin-induced oxidative stress in both the liver and the lungs, we cannot determine the relative contribution of the two organs to this result. However, the fact that there was no increase in isoprostanes when only the lung received endotoxin indicates that endotoxin-induced oxidant stress in the system required the presence of the liver. The time course of this index of oxidant injury may suggest a relationship to the early hypoxemia that is peculiar to the endotoxin response when both liver and lung are perfused.

In summary, we determined physiological, biochemical, cellular, and molecular responses of the lungs to endotoxemia in a newly developed in situ autologous blood perfused swine preparation that permitted perfusion with or without endotoxin of only the lungs or both the liver and the lungs. When only the lungs were perfused, endotoxin caused pulmonary hypertension and neutropenia, but oxygenation was maintained, circulating levels of proximal cytokines and lipid peroxidation products were minimally or not at all elevated, and there was no lung edema. When both the liver and lung were perfused, endotoxin caused marked hypoxemia, large increases in perfusate cytokines and lipid peroxidation products, and severe lung edema. Lung tissue NF-κB was activated when only the lung was perfused, but to a much greater degree when the liver was present. We conclude that the direct effects of endotoxemia on the lungs include vasoconstriction and leukocyte sequestration, but not lung injury. Endotoxemia-induced ALI requires that the lungs and the liver share a common circulation. The time course of the responses that we measured makes it unlikely that TNF-α is the early signal from the liver that initiates lung injury. Our data support the concept that oxidant stress triggered by a circulating signal from the liver to the lungs is a pivotal early event in the pathogenesis of the endotoxin response.

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