Interleukin-10 inhibits pulmonary NF-κB activation and lung injury induced by hepatic ischemia-reperfusion

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Interleukin-10 inhibits pulmonary NF-κB activation and lung injury induced by hepatic ischemia-reperfusion. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L919–L923, 1999.—Hepatic ischemia and reperfusion cause local and remote organ injury. This injury culminates from an integrated cascade of proinflammatory cytokines, chemokines, and adhesion molecules, many of which are regulated by the transcription factor nuclear factor-κB (NF-κB). The anti-inflammatory cytokine interleukin-10 (IL-10) has been shown to have inhibitory effects on NF-κB. The objective of the current study was to determine whether IL-10 could suppress pulmonary NF-κB activation and ensuing lung injury induced by hepatic ischemia-reperfusion. C57BL/6 mice underwent partial hepatic ischemia with or without intravenous administration of IL-10. Hepatic ischemia-reperfusion resulted in pulmonary NF-κB activation, increased mRNA expression of tumor necrosis factor-α (TNF-α), and macrophage inflammatory protein-2 (MIP-2), as well as increased pulmonary neutrophil accumulation and lung edema. Administration of IL-10 suppressed lung NF-κB activation, reduced TNF-α and MIP-2 mRNA expression, and decreased pulmonary neutrophil recruitment and lung injury. The data suggest that IL-10 protects against hepatic ischemia and reperfusion-induced lung injury by inhibiting lung NF-κB activation and the resulting pulmonary production of proinflammatory mediators.

inflammation; tumor necrosis factor-α; neutrophils; mice; nuclear factor-κB

HEPATIC ISCHEMIA-REPERFUSION injury is a complication of liver resectional surgery, liver transplantation, and hemorrhagic shock with fluid resuscitation and may lead to local and remote organ damage (17). There is a preponderance of literature suggesting that the pathogenesis of the resulting tissue injury in remote organs, such as the lung, is related to the hepatic generation of the proinflammatory cytokine tumor necrosis factor-α (TNF-α). After hepatic ischemia and reperfusion, TNF-α initiates a mediator cascade in the lung, including upregulation of the C-X-C chemokines, epithelial neutrophil-activating protein ENA-78, macrophage inflammatory protein-2 (MIP-2), and KC, as well as increased pulmonary vascular expression of intercellular adhesion molecule-1 (ICAM-1) (3–5, 26). The coordinated effects of C-X-C chemokines and adhesion molecules result in pulmonary neutrophil accumulation and ensuing lung injury. The gene expression of these proinflammatory mediators, including TNF-α, C-X-C chemokines, and ICAM-1, is controlled at least in part by the transcription factor nuclear factor-κB (NF-κB) (2, 7, 24, 25). Although activation of NF-κB occurs in liver during ischemia-reperfusion injury (28), it is unknown whether NF-κB is activated in remote organs or whether remote organ NF-κB activation is important for the development of tissue injury.

Recent reports have demonstrated that the anti-inflammatory cytokine interleukin-10 (IL-10) has hepatoprotective effects during liver transplantation (27) and experimental liver injury induced by galactosamine and lipopolysaccharide (18, 23). IL-10 has also been shown to suppress experimental lung inflammatory injury induced by intrapulmonary deposition of IgG immune complexes through inhibitory effects on NF-κB activation (15). In the current studies, we sought to determine whether hepatic ischemia and reperfusion caused NF-κB activation in the lung. In addition, we evaluated the effects of IL-10 on the development of lung inflammatory injury induced by hepatic ischemia and reperfusion.

MATERIALS AND METHODS

Hepatic ischemia and reperfusion injury model. Male C57BL/6 mice (Charles River Laboratories, Wilmington, MA) weighing 22–28 g were used in all experiments. This project was approved by the University of Louisville Animal Care and Use Committee and was in compliance with the guidelines of the National Institutes of Health. The model of partial hepatic ischemia and reperfusion employed was prepared as described previously (16). Briefly, mice were anesthetized with pentobarbital sodium (60 mg/kg ip). Mice received either sterile saline or recombinant murine IL-10 (1 µg; R&D Systems, Minneapolis, MN) via the lateral tail vein before induction of ischemia. A midline laparotomy was performed, and an atraumatic clip was used to interrupt the arterial and the portal venous blood supply to the cephalad lobes of the liver. After 90 min of partial hepatic ischemia, mice again received either sterile saline or IL-10 (1 µg) via the lateral tail vein and the clip was removed, initiating hepatic reperfusion. Sham control mice underwent the same protocol but without vascular occlusion. Mice were killed after the indicated periods of reperfusion, and lung tissues and blood samples were taken for analysis.

Myeloperoxidase assay. Lung myeloperoxidase (MPO) content was assessed by methods described previously (26). Briefly, lung tissue (50 mg) was homogenized in 2 ml of homogenization buffer (3.4 mM KH2PO4 and 16 mM Na2HPO4, pH 7.4). After centrifugation for 20 min at 10,000 g, 10 volumes of resuspension buffer (43.2 mM KH2PO4, 6.5 mM Na2HPO4, 10 mM EDTA, and 0.5% hexadecyltrimethylammonium, pH 6.0) were added to the pellet and the samples were sonicated for 10 s. After heating for 2 h at 60°C, the supernatant was reacted with 3,3′,5,5′-tetramethylbenzidine (Sigma Chemical, St. Louis, MO) and read at 655 nm.

Lung edema. The extent of lung edema was measured by tissue wet-to-dry weight ratios. After dissection, lung samples were weighed and then placed in a drying oven at 55°C until a...

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constant weight was obtained. In this determination, lung edema is represented by an increase in the wet-to-dry weight ratio.

Reverse transcription-polymerase chain reaction. Fresh lung samples were immediately frozen when the mice were killed. The extraction of total RNA was performed using a commercially available kit (Qiagen, Valencia, CA). A 1-µg aliquot of total lung RNA was reverse transcribed to cDNA using the Geneamp RNA polymerase chain reaction (PCR) protocol (Perkin-Elmer, Norwalk, CT) with random hexamers to prime the reverse transcription (RT) with an excess of deoxyribonucleotides. The cDNA products were amplified with 2.5 units of AmpliTaq DNA polymerase, 3 mM MgCl₂, and 0.5 mM primer. After 2 min of initial melting at 95°C, the mixture was amplified for a total of 30 cycles with a three-step cycle process that began with melting at 95°C for 60 s, annealing at 59°C for 90 s, followed by extension at 72°C for 10 s. The final cycle was followed by 12 min of soaking at 72°C. Ten microliters of each RT-PCR were electrophoresed in a 3.5% agarose (GIBCO) gel and stained with ethidium bromide. RT-PCR amplification of a housekeeping gene (β-actin) was performed to verify equal loading of RNA and cDNA in the RT-PCRs. Products of RT-PCRs were photographed.

PCR primers were as follows: MIP-2 sense, 5'-GAA CAA AGG CAA GCC TAA CTG A-3'; MIP-2 antisense, 5'-AAC ATG AGG GGG GCT TCT AGG CAC CA-3'. The cDNA products were amplified with 2.5 units of AmpliTaq DNA polymerase, 3 mM MgCl₂, and 0.5 mM primer. After 2 min of initial melting at 95°C, the mixture was amplified for a total of 30 cycles with a three-step cycle process that began with melting at 95°C for 60 s, annealing at 59°C for 90 s, followed by extension at 72°C for 10 s. The final cycle was followed by 12 min of soaking at 72°C. Ten microliters of each RT-PCR were electrophoresed in a 3.5% agarose (GIBCO) gel and stained with ethidium bromide. RT-PCR amplification of a housekeeping gene (β-actin) was performed to verify equal loading of RNA and cDNA in the RT-PCRs. Products of RT-PCRs were photographed.

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Protein concentrations were determined by bicinchoninic acid (Pierce, Rockford, IL). Binding reactions containing equal amounts of protein (20 µg) and 35 fmol [3H]-labeled reference standard (Pierce, Rockford, IL) were performed for 30 min in binding buffer [4% glycerol, 1 mM MgCl₂, 0.5 mM EDTA, pH 8.0, 0.5 mM dithiothreitol, 50 mM NaCl, 10 mM Tris, pH 7.6, and 50 µg/ml poly(dI-dC); Pharmacia, Piscataway, NJ]. Reaction volumes were held constant to 15 µl. Reaction products were separated in a 4% polyacrylamide gel and analyzed by autoradiography.

Statistical analysis. All data are expressed as means ± SE. Data were analyzed with a one-way analysis of variance, and individual group means were then compared with a Student-Newman-Keuls test. Differences were considered significant when P < 0.05. For calculations of percent change, negative control values were subtracted from positive control and treatment group values.

RESULTS

Activation of NF-κB in lung during hepatic ischemia-reperfusion injury. The time course of NF-κB activation in the lung during hepatic ischemia-reperfusion injury was determined by electrophoretic mobility shift assays of lung nuclear extracts (Fig. 1A). Autoradiograms were digitized and relative band intensity was determined using image-analysis software (Fig. 1B). In lungs from sham-operated controls, there was little NF-κB in nuclear extracts. Unexpectedly, nuclear translocation (activation) of NF-κB was observed after 90 min of hepatic ischemia but before reperfusion (0-h reperfusion). Lung NF-κB activation increased modestly over the 4-h time course studied, with maximal activation occurring 1 and 4 h after hepatic reperfusion.

Effects of IL-10 on lung NF-κB activation and mRNA expression of TNF-α and MIP-2. Because lung NF-κB activation induced by hepatic ischemia and reperfusion was maximally activated within 1–4 h of hepatic reperfusion, we assessed the effects of exogenously administered IL-10 on lung NF-κB activation after 1 h of reperfusion (Fig. 2). As in previous experiments, lung NF-κB activation was increased by hepatic ischemia-reperfusion compared with sham controls. In mice receiving intravenous administration of IL-10, lung NF-κB activation was almost completely inhibited.

To determine whether IL-10-mediated suppression of lung NF-κB activation was associated with decreased proinflammatory mediator generation, lung expression of mRNAs for TNF-α and MIP-2 was assessed in sham control mice and in mice undergoing hepatic ischemia...
administration of IL-10 (Fig. 3A). Hepatic ischemia and 4 h of reperfusion induced a much larger increase in the expression of TNF-α mRNA, which was greatly reduced with administration of IL-10. Pulmonary expression of MIP-2 mRNA was modestly increased after 1 h of reperfusion (Fig. 3B). After 4 h of reperfusion, lung MIP-2 mRNA was greatly increased. Administration of IL-10 resulted in a slight decrease in MIP-2 mRNA expression after 1 h of reperfusion but more effectively inhibited MIP-2 mRNA expression after 4 h of reperfusion (Fig. 3B).

Effects of IL-10 on lung neutrophil recruitment and injury. We have previously shown in this model that pulmonary neutrophil accumulation and lung injury correlate with lung mRNA and serum levels of MIP-2 and TNF-α (26). The recruitment of neutrophils to lung and the development of lung injury occur after 3–4 h of hepatic reperfusion (26). Therefore, we assessed whether the reduction in TNF-α and MIP-2 mRNA expression by IL-10 was associated with decreased recruitment of neutrophils and lung injury after 4 h of hepatic reperfusion. Pulmonary neutrophil recruitment was determined by lung MPO content. Hepatic ischemia and 4 h of reperfusion caused significant increases in lung MPO content compared with the sham controls (Fig. 4). Intravenous administration of IL-10 reduced lung MPO content by 61% (P < 0.016). Lung edema, as measured by lung wet-to-dry weight ratio, was used as an index of lung injury. Hepatic ischemia and 4 h of reperfusion induced significant lung edema (Fig. 5). Administration of IL-10 suppressed increases in lung wet-to-dry weigh ratio by 72% (P = 0.004).

Fig. 2. Effects of interleukin-10 (IL-10) on hepatic ischemia-reperfusion (I/R)-induced lung NF-κB activation. Nuclear translocation of NF-κB was assessed in nuclear extracts from lung tissue obtained from sham mice and mice undergoing hepatic ischemia and 1 h of reperfusion treated with saline (I/R) or IL-10 (I/R + IL-10).

Fig. 3. Effects of IL-10 on lung expression of tumor necrosis factor-α (TNF-α; A) and macrophage inflammatory protein-2 (MIP-2; B) mRNAs. RT-PCR analysis of lung RNA extracts obtained from sham mice and mice undergoing hepatic ischemia-reperfusion treated with saline (I/R) or IL-10 (I/R + IL-10). MWM, Molecular-weight marker. Right panels show image analysis of RT-PCR results.
TNF-α, which initiates a mediator cascade leading to cytes produce proinflammatory cytokines, including initial oxidant-induced injury, Kupffer cells and hepato-
by releasing reactive oxygen species (12). After this initial phase, activated Kupffer cells cause liver injury is associated with two distinct phases. During the ischemia and reperfusion in the presence or absence of IL-10.

Fig. 5. Effects of IL-10 on lung edema induced by hepatic ischemia and reperfusion. Lung edema was determined by tissue wet-to-dry weight ratios in sham control mice and mice undergoing hepatic ischemia and 4 h of reperfusion in the presence or absence of IL-10. For all groups n = 5.

DISCUSSION

Hepatic injury induced by ischemia and reperfusion is associated with two distinct phases. During the initial phase, activated Kupffer cells cause liver injury by releasing reactive oxygen species (12). After this initial oxidant-induced injury, Kupffer cells and hepatocytes produce proinflammatory cytokines, including TNF-α, which initiates a mediator cascade leading to hepatic neutrophil recruitment (6). The ensuing liver injury is mediated by oxidants and proteases released by sequestered neutrophils (11). It has been shown that activation of NF-κB occurs during the initial phase of hepatic injury (28). It is thought that NF-κB activation may be responsible at least in part for the increased hepatic production of proinflammatory cytokines during this phase. Remote organ injury caused by hepatic ischemia and reperfusion, such as that induced in the lung, is thought to be a result of liver-derived TNF-α. In fact, blockade of TNF-α using antibody neutralization greatly reduced hepatic ischemia and reperfusion-induced lung inflammatory injury in rats (6).

Unexpected were the findings that NF-κB was activated in the lung during the period of hepatic ischemia. The precise mechanism of this effect is unknown, but serum TNF-α is not increased at this time (Yoshidome and Lentsch, unpublished data). Our observations of increased lung NF-κB activation during hepatic ischemia are consistent with those of a recent report demonstrating increased lung production of C-X-C chemokines during hindlimb ischemia before reperfusion (1). In those studies, it was noted that there was systemic activation of complement during the ischemic period. Although complement activation products have been shown to augment pulmonary production of TNF-α and C-X-C chemokines (9, 20), these effects are not mediated by activation of NF-κB (9, 14). Alternatively, mediators could be released by the nonischemic liver in response to increased blood flow to that portion of the organ. However, there is no evidence of tissue injury in nonischemic lobes (16).

On the basis of our findings, increased NF-κB activation in lung after hepatic ischemia-reperfusion appears to lead to pulmonary expression of TNF-α and MIP-2. Activation of NF-κB in alveolar macrophages has been shown to be required for the intrapulmonary production of TNF-α and MIP-2, as well as development of lung injury induced by IgG immune complexes (13). IL-10 has been shown to suppress NF-κB in alveolar macrophages by preventing degradation of the NF-κB inhibitory protein IκB-α (15). In the present studies, intravenous administration of IL-10 suppressed lung NF-κB activation induced by hepatic ischemia and reperfusion. These effects were associated with reduced lung expression of TNF-α and MIP-2 mRNAs, both of which are required for full development of lung injury after hepatic ischemia and reperfusion (3, 26). Reductions in TNF-α and MIP-2 corresponded to decreased lung neutrophil accumulation and reduced lung edema. Additionally, IL-10 may reduce neutrophil recruitment into the lung through effects on the pulmonary endothelium. IL-10 has been shown to prevent upregulation of lung vascular ICAM-1 during lung inflammation (19). Furthermore, recent studies showed that blockade of TNF-α decreases pulmonary vascular expression of the adhesion molecule ICAM-1, resulting in reduced lung recruitment of neutrophils (4). Like TNF-α and MIP-2, ICAM-1 is regulated at the transcriptional level by NF-κB (7). Although not investigated in the current study, it is likely that IL-10 reduces pulmonary vascular expression of ICAM-1 either directly through inhibition of NF-κB in endothelial cells or indirectly by suppression of TNF-α production as a contributing mechanism for reduction of lung neutrophil accumulation.

IL-10 has been shown to be beneficial in the setting of organ transplantation, including liver (8, 27) and heart.
hemorrhagic shock. B, may have therapeutic applications for organ
components of the inflammatory response, such as
the use of agents such as IL-10 to target upstream

Thus the current studies suggest that
panied by almost complete inhibition of NF-κB mRNAs. These effects of IL-10 were accom-

increased the extent of lung neutrophil recruitment in
duced by hepatic ischemia and reperfusion. IL-10 de-
ischemia-reperfusion. IL-10 reduced lung injury in-

that IL-10 may also be protective in surgical or trauma-

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