L-Arginine attenuates lipopolysaccharide-induced lung chemokine production

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Chemokines stimulate the influx of leukocytes into tissues. Their production is regulated by nuclear factor-κB (NF-κB), an inducible transcription factor under the control of inhibitory factor κB-α (IκB-α). We have previously demonstrated that l-arginine (L-Arg) attenuates neutrophil accumulation and pulmonary vascular injury after administration of lipopolysaccharide (LPS). We hypothesized that L-Arg would attenuate the production of lung chemokines by stabilizing IκB-α and preventing NF-κB DNA binding. We examined the effect of L-Arg on chemokine production, IκB-α degradation, and NF-κB DNA binding in the lung after systemic LPS. To block nitric oxide (NO) production, a NO synthase inhibitor was given before L-Arg. LPS induced the production of chemokine protein and mRNA. L-Arg attenuated the production of chemokine protein and mRNA, prevented the decrease in IκB-α levels, and inhibited NF-κB DNA binding. NO synthase inhibition abolished the effects of L-Arg on all measured parameters. Our results suggest that L-Arg abrogates chemokine protein and mRNA production in rat lung after LPS. This effect is dependent on NO and is mediated by stabilization of IκB-α levels and inhibition of NF-κB DNA binding.

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may inhibit CINC-1 and MIP-2 production and attenuate LPS-induced ALI. In the resting state, NF-kB is bound to inhibitory factor IkB-α (IkB-α), which confines the complex to the cytoplasm. Inflammatory stimuli such as LPS or ischemia lead to dissociation of IkB-α from NF-kB; the latter element is able to translocate to the nucleus and induce transcription. We hypothesized that in vivo administration of l-Arg would prevent the LPS-induced decrease in IkB-α levels and prevent NF-kB-mediated expression of CINC-1 and MIP-2. The purpose of this study was to determine the effect of l-Arg on LPS-induced CINC-1 and MIP-2 protein and mRNA expression, NF-kB DNA binding, and IkB-α protein in the lung. We found that treatment with l-Arg before LPS attenuated both CINC-1 and MIP-2 protein and mRNA production and NF-kB DNA binding. This mechanism wasmediated through preservation of IkB-α protein levels after LPS.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (body weight 300–350 g) were quarantined and maintained on a standard pelleted diet for 2 wk before the initiation of experimental protocols. All animal experiments were approved by the University of Colorado Health Sciences Center Institutional Animal Care and Use Committee. Animals received humane care in accordance with the revised Guide for the Care and Use of Laboratory Animals (7th ed.; Washington, DC: Natl. Acad. Press, 1996).

Experimental protocol. Awake rats were administered saline or LPS (0.5 mg/kg LPS; Salmonella typhimurium, lot no. 47H4093, Sigma, St. Louis, MO) freshly prepared in 0.9% saline via intraperitoneal injection. The experimental design included the following groups: 1) control treated with normal saline; 2) LPS alone; 3) 300 mg/kg ip of l-Arg given 30 min before LPS (l-Arg-LPS); 4) 300 mg/kg ip of d-Arg given 30 min before LPS (d-Arg-LPS); 5) 0.2 mg/kg ip of a NOS inhibitor, 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine (AMT), 30 min before l-Arg and LPS (AMT-l-Arg-LPS); and 6) AMT alone (AMT). Animals were anesthetized with pentobarbital sodium (0.5 mg/kg ip) at 0.5, 1, 2, and 4 h after LPS or saline administration. Lungs were then harvested, rinsed with normal saline, blotted dry, snap-frozen in liquid nitrogen, and stored at −70°C until analysis. Plasma was obtained by centrifugation (750 g for 15 min at 4°C) of heparinized whole blood. Plasma was determined with a Beckman 6300 amino acid analyzer that employed a 3-lithium-citrate buffer system and a previously published technique (47). Lungs were homogenized in 5 volumes of homogenate buffer (10 mM HEPES, 10 mM KCl, 0.5 M sucrose, 0.1 mM EGTA, and 1 mM dithiothreitol). Homogenates were then centrifuged at 750 g for 10 min at 4°C to isolate crude nuclei. The supernatants, which contained the cytosolic fraction, were stored at −70°C and used for CINC-1 and MIP-2 protein determination. The crude nuclear pellet was then resuspended in 100 μl of ice-cold nuclear extraction buffer (20 mM HEPES, 0.4 M NaCl, 1 mM EGTA, and 1 mM dithiothreitol). Samples were incubated on ice for 30 min with brief, gentle vortexing every 5–10 min. The nuclear extract was then centrifuged at 12,000 g for 5 min at 4°C, and the supernatant was collected and stored at −70°C. Protein was quantified in both cytosolic and nuclear extracts with the Coomassie Plus protein assay kit (Pierce, Rockford, IL).

Electrophoretic mobility shift assay. NF-kB (5'-AGTGA-GGGGACTTTCCAGGC-3'), binding site underlined) consensous oligonucleotide was 5'-end labeled with [α-32P]dATP with T4 polynucleotide kinase. Unincorporated nucleotide was removed with a NucTrap probe purification column (Stratagene, La Jolla, CA). Five micrograms of nuclear protein were incubated with labeled oligonucleotide [100,000–200,000 counts/min (cpm)] in binding buffer [10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5 mM EDTA, 1 mM MgCl2, 0.5 μg ofpoly(dI-dC)-poly(dI-dC), 1% Nonidet P-40, and 4% glycerol] for 25 min at room temperature in a final volume of 25 μl. Free oligonucleotide and oligonucleotide-bound proteins were then separated by electrophoresis on a native 4% polyacrylamide gel. The gel was subsequently dried and exposed to X-ray film between two intensifying screens overnight at −70°C. For supershift studies, antibody (5 μg) to the p65 subunit of NF-kB (Santa Cruz Biotechnology, Santa Cruz, CA) was added before the addition of the labeled oligonucleotide. Binding of the antibody to the DNA-protein complex was indicated by a supershift in the electrophoretic mobility shift assay (EMSA). To further demonstrate specificity, excess unlabeled (cold probe) oligonucleotide was used as a specific competitor.

Immunoblotting. Lung tissue was homogenized with a tissue homogenizer (Virtishearm homogenizer; Virtis, Gard- ner, NY) in 5 volumes of homogenization buffer (25 mM Tris-HCl, 2 mM EGTA, 1 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride, pH 7.4). After centrifugation (3,000 g at 4°C for 20 min), the supernatant was collected. Total protein concentration was determined with the Coomassie Plus protein assay. Samples (20 μg of crude protein) were mixed with an equal volume of sample buffer (100 mM Tris-HCl, 2% SDS, 0.02% bromphenol blue, and 10% glycerol) and boiled for 5 min. Electrophoresis was performed on a 4–20% linear gradient SDS-polyacrylamide gel. Proteins were then electrophoretically transferred onto a nitrocellulose membrane (Bio-Rad). Membranes were blocked for 1 h at room temperature with antibody buffer (PBS containing 0.1% Tween 20 and 5% nonfat dried milk) and then incubated with rabbit polyclonal anti-IkB-α (1:500 dilution with antibody buffer) for 1 h at room temperature. Membranes were washed three times in PBS containing 0.1% Tween 20 and then incubated in peroxidase-labeled goat anti-rabbit IgG (1:10,000 dilution with antibody buffer) for 1 h at room temperature. Membranes were then washed three times, and antigen-antibody complexes were revealed by the enhanced chemiluminescence method (Amersham, Arlington Heights, IL). Quantification of the immunoblot was performed by

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computer-assisted densitometry (NIH application 1.599b4). Density values are expressed as a percentage of the saline control level of each experiment. All densities are means ± SE of three separate experiments.

Northern blotting. Northern blotting was performed as described by Henderson et al. (22). Lung tissue was homogenized with a tissue homogenizer (Virtishear, Virtis) in 10 volumes of TRI Reagent (Molecular Research Center, Cincinnati, OH). Total RNA was extracted with the guanidinium isothiocyanate method (11). Quantification of RNA was performed spectrophotometrically by reading the absorbance at 260 nm. Twelve micrograms of total RNA from each sample were denatured and electrophoresed on a 1.2% agarose-6% formaldehyde gel containing 10 mg/ml of ethidium bromide. Gels were soaked in 10× SSPE for 1 h before overnight capillary transfer (GIBCO BRL, Life Technologies, Gaithersburg, MD) in 10× SSPE onto a nylon membrane (Stratagene). After transfer, the membrane was ultraviolet (UV) cross-linked with a UV Stratalinker (Stratagene), and rRNA loading was visualized and photographed under UV light to assess hybridization efficiency. Membranes were then wet with 2× SSPE and placed in a Mini-6 hybridization oven (Hybaid, Franklin, MA). All prehybridization, hybridization, and high-stringency wash temperatures were calculated based on a previously published method (22) that employed the equation

\[ T_{m50} = 16.6 + 415.5 \times 0.41 \times (\%GC\ content) - 675 \times (\text{no. of bases in probe}), \]

where \( T_{m50} \) is the temperature at which 50% of the DNA probe-RNA hybrids melt, \([\text{Na}]^+ \), and was complementary to rat MIP-2 cDNA nucleotides 170–304–333. Oligonucleotide probes were labeled by terminal deoxynucleotidyltransferase (TdT). The reaction was incubated for 37°C for 1 h in 1× TdT buffer (GIBCO BRL), 10 μl (33 pmol) of [α-32P]dATP (DuPont NEN, Boston, MA), and 2 μl of 15 U/μl of recombinant TdT (GIBCO BRL). The reaction was incubated for 1 h at 37°C. Unincorporated nucleotide was removed by a NucTrap probe purification column (Stratagene). The activity of the probe was determined by scintillation counting.

After hybridization, blots were washed in 1× SSPE buffer containing 0.5% SDS and 0.1% nonfat dry milk at room temperature. A second set of washes was carried out in 0.2× SSPE with 1% SDS at room temperature. A final high-stringency wash was done in 0.1× SSPE with 0.5% SDS at the calculated temperature. The membrane was then dried, covered in plastic wrap, and exposed to X-ray film (BioMax-1 MR-1, Kodak) in the presence of two intensifying screens at −70°C. Quantification of specific mRNA species was accomplished by densitometry (NIH application 1.599b4) and normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA. Membranes were stripped with 2 mM EDTA (pH 8.0), 2 mM Tris (pH 8.0), and 10× Denhardt’s solution for between 2 and 3 h at 65°C and then reprobed for the alternate chemokine or GAPDH.

Rat chemokine protein ELISA. CINC-1 and MIP-2 were measured in lung homogenates (cytoplasmic fraction) as described in Nuclear and cytoplasmic protein isolation with a rat-specific ELISA system. Kits were obtained from Immuno-Biological Laboratories (CINC-1; Fujioka City, Japan) and BioSource International (MIP-2; Camarillo, CA). The absorbency of standards and samples was determined spectrophotometrically with a microplate reader (Bio-Rad). Results were recorded as optical densities and plotted against the linear portion of the standard curve.

Reagents. All antibodies for immunoblotting and EMSA were obtained from Santa Cruz Biotechnology. The IκB-α antibody was a rabbit polyclonal IgG against the carboxy terminus of human IκB-α that cross-reacts with rat IκB-α. The NF-κB antibodies were both goat polyclonal IgGs raised against the carboxy terminus of the human p65 subunit of NF-κB, and both cross-reacted with the rat NF-κB subunits. All other chemicals, unless stated, were obtained from Sigma.

Statistical analysis. Statistical analyses were performed with StatView software (Brain Power, Calabasas, CA). Data are presented as means ± SE. Statistical evaluation used standard one-way ANOVA with post hoc Bonferroni-Dunn test. Significance was accepted at a confidence limit of 95%.

RESULTS

L-Arg plasma determinations. To determine the effect of L-Arg supplementation and LPS on plasma L-Arg concentrations, plasma was harvested from rats 1 h after saline, LPS, or L-Arg treatment and analyzed for L-Arg (n = 3 rats/group). Intrapерitoneal L-Arg supplementation resulted in an increase in plasma L-Arg compared with saline control (L-Arg, 660.23 ± 129.29 nmol/ml vs. saline, 166.97 ± 9.05 nmol/ml; P < 0.05). LPS led to a decrease in circulating plasma L-Arg levels compared with saline control (saline, 166.97 ± 9.05 nmol/ml vs. LPS: 83.80 ± 14.43 nmol/ml; P < 0.05).

Nitrate/nitrite production. To determine the effect of LPS, supplemental L-Arg administration, and NO inhibition on plasma nitrate and nitrite production, rat plasma was assayed for total NOx 2 h after saline or LPS (Fig. 1). Compared with saline, LPS caused an increase in NOx (saline, 13.72 ± 1.00 μmol vs. LPS, 22.14 ± 0.21 μmol; P < 0.05) 2 h after LPS administration. However, L-Arg administration before LPS induced an even greater increase in NOx compared with both saline and LPS (L-Arg-LPS, 35.80 ± 1.34 μmol; P < 0.01). A NOS inhibitor (AMT) given before LPS abolished the LPS-induced increase in NOx (AMT-LPS, 7.47 ± 0.23 μmol; P < 0.05 compared with LPS) and had an inhibitory effect on baseline NOx levels (AMT alone, 9.11 ± 0.25 μmol; P < 0.05 compared with saline).

Effect of L-Arg on lung CINC-1 and MIP-2 protein. To determine the effect of L-Arg on chemokine protein production after LPS, rat lung tissue was examined 2 h after LPS for the presence of CINC-1 (Fig. 2A) and MIP-2 (Fig.
treated with saline, *Salmonella typhimurium*, vs. saline. † P administration before LPS induced an even greater increase in NOx 2 h after LPS administration. However, L-Arg and AMT alone (0.2 mg/kg). Compared with saline, LPS caused an increase in NOx and had an inhibitory effect on baseline NOx levels. *P < 0.05 vs. saline. †P < 0.05 vs. LPS.

2B). Compared with saline treatment, LPS caused a significant increase in both CINC-1 (saline, 27.1 ± 10.1 vs. LPS, 1,275.5 ± 239.3; *P < 0.05) and MIP-2 (saline, 10.3 ± 2.7 vs. LPS, 109.3 ± 19.7; *P < 0.05) in lung homogenate. L-Arg given before LPS attenuated the LPS-induced increase in both CINC-1 (L-Arg-LPS, 257.7 ± 75.0; *P < 0.05 vs. LPS) and MIP-2 (L-Arg-LPS, 17.7 ± 4.3; *P < 0.05 vs. LPS). D-Arg before LPS had no effect on the increase in NOx. As shown). Therefore, to determine the effect of L-Arg on IkB-α after LPS, rat lung tissue was examined for IkB-α 1 h after LPS. L-Arg before LPS attenuated the LPS-induced decrease in IkB-α at 1 h (Fig. 4, C and D). D-Arg had no effect on the level of IkB-α protein after LPS. NOS inhibition prevented the effects of L-Arg on IkB-α levels after LPS. AMT alone had a similar effect to saline-treated animals (data not shown).

**Effect of L-Arg on lung NF-κB DNA binding.** To determine the effect of L-Arg on NF-κB DNA binding after LPS, rat lung tissue was examined 1 h after LPS for NF-κB DNA binding by EMSA (Fig. 5). Compared with saline treatment, LPS challenge caused a significant increase in NF-κB DNA binding within 1 h. L-Arg given before LPS attenuated the LPS-induced NF-κB DNA binding. D-Arg had no effect on LPS-induced NF-κB DNA binding. Treatment with AMT before L-Arg and LPS abolished the effect of L-Arg.

**Effect of L-Arg on lung CINC-1 and MIP-2 mRNA.** To examine the effect of L-Arg on chemokine mRNA transcript expression after LPS, rat lung tissue was examined 2 h after LPS for the presence of CINC-1 (Fig. 3, A and B) and MIP-2 (Fig. 3, C and D) mRNAs with Northern blotting. Compared with saline control, LPS caused a significant increase in both CINC-1 and MIP-2 mRNAs in lung homogenate. L-Arg attenuated the LPS-induced increase in both CINC-1 and MIP-2 messages. D-Arg had no effect on the LPS-induced increase in either CINC-1 or MIP-2 message. Similar to the results obtained for chemokine protein, NOS inhibition (AMT) before L-Arg and LPS abolished the L-Arg-associated attenuation of CINC-1 and MIP-2 mRNA expression.

**Effect of L-Arg on lung IkB-α.** Compared with saline, LPS caused a significant decrease in IkB-α within 1 h, which returned to saline control levels by 4 h (Fig. 4, A and B). Densitometry of Western blots from three separate experiments revealed no significant difference between the 0.5- and 4-h time points in the LPS-treated animals and no difference between saline control and the LPS-treated 4-h time point (data not shown). Therefore, to determine the effect of L-Arg on IkB-α after LPS, rat lung tissue was examined for IkB-α 1 h after LPS. L-Arg before LPS attenuated the LPS-induced decrease in IkB-α at 1 h (Fig. 4, C and D). D-Arg had no effect on the level of IkB-α protein after LPS. NOS inhibition prevented the effects of L-Arg on IkB-α levels after LPS. AMT alone had a similar effect to saline-treated animals (data not shown).

**Effect of L-Arg on lung NF-κB DNA binding.** To determine the effect of L-Arg on NF-κB DNA binding after LPS, rat lung tissue was examined 1 h after LPS for NF-κB DNA binding by EMSA (Fig. 5). Compared with saline treatment, LPS challenge caused a significant increase in NF-κB DNA binding within 1 h. L-Arg given before LPS attenuated the LPS-induced NF-κB DNA binding. D-Arg had no effect on LPS-induced NF-κB DNA binding. Treatment with AMT before L-Arg and LPS abolished the effect of L-Arg. Administration of AMT alone had a similar effect to that in saline-treated animals.
DISCUSSION

Sheridan et al. (51) previously found that L-Arg decreases lung PMN accumulation and pulmonary vascular injury after LPS (51). Our present studies tested the effect of L-Arg treatment before LPS on chemokine production in the lung. Northern blot and ELISA analysis of lung homogenates confirmed that a low dose of LPS caused an increase in lung CINC-1 and MIP-2 proteins and mRNAs as early as 2 h after LPS. L-Arg attenuated both chemokine mRNA expression and protein production in the lung after LPS. Furthermore, we attempted to determine the mechanism whereby L-Arg decreases chemokine production in this model. The attenuation of chemokine production by L-Arg was associated with maintenance of IκB-α levels and a decrease in NF-κB DNA binding. Finally, inhibition of NOS abolished the effects of L-Arg on LPS-induced chemokine mRNA and protein production, IκB-α levels, and NF-κB DNA binding.

L-Arg is the sole substrate for NO synthesis in mammalian systems. The protective anti-inflammatory role of L-Arg or NO in the lung has been evaluated in a number of studies. In animals, previous investigations have demonstrated that L-Arg or NO alleviates the pulmonary hypertension seen after mesenteric ischemia-reperfusion (16). Furthermore, L-Arg or NO decreases alveolar macrophage proinflammatory cytokine (tumor necrosis factor-α, IL-1β) production (34), prevents alveolar edema (25), and attenuates PMN...
acutely respiratory distress syndrome (ARDS), inhaled NO decreases PMN β2-integrin (CD11b/CD18) expression and bronchoalveolar lavage fluid IL-8 levels (10). The in vivo physiological implications of these data are not completely understood. Thus data from the current study, previous work by this laboratory (16, 51), and that of other investigators (20, 45) support the hypothesis that the L-Arg/NO pathway regulates PMN tissue sequestration by downregulation of both chemokine production and adhesion molecule expression.

Despite the beneficial role of NO in ALI, NO may contribute to lung injury by generation of oxidant by-products. Generation of peroxynitrite from the reaction of NO and superoxide can contribute to tissue damage after sepsis (18, 44, 58). Specifically, there is evidence to suggest that the liberation of NO by inducible NOS (iNOS) leads to tissue damage and contributes to lung injury (24). In fact, iNOS inhibition attenuates LPS-induced ALI in rabbits (36) and dogs (41) and hemorrhagic shock-induced ALI in mice and rats (24). However, experiments utilizing iNOS knockout (iNOS−/−) mice to substantiate the pathological role of iNOS in systemic inflammation give conflicting results. In support of iNOS as an effector of sustained inflammation and tissue damage, iNOS(−/−) mice demonstrate reduced levels of NF-κB and signal transducer and activation of transcription-3 (STAT3) activation in the lung after hemorrhagic shock (24). Furthermore, Kristof et al. (28) found that LPS injection in iNOS(−/−) mice elicited no significant change in lactate dehydrogenase activity, lung wet-to-dry weight ratio, or pulmonary nitrotyrosine staining compared with wild-type mice (28).

On the other hand, iNOS inhibition or gene deletion may increase tissue PMN accumulation. Hickey et al. (23) found that leukocyte accumulation in the lung was elevated in iNOS(−/−) mice given systemic LPS, suggesting that induction of iNOS is a homeostatic regulator for leukocyte recruitment. Thus, early in the course of LPS-induced pulmonary inflammation, NO may act by a negative feedback mechanism to inhibit the production of chemokines and thereby attenuate lung PMN accumulation. Indeed, one group of investigators (1) has evaluated the role of iNOS in PMN accumulation in a murine model of peritonitis induced by zymosan. In iNOS(−/−) mice, increased peritoneal levels of MIP-2 and KC (a murine C-X-C chemokine) (1). Furthermore, in a murine model of endotoxin-induced ALI, NOS inhibition increased proinflammatory cytokine protein and mRNA expression and NF-κB DNA binding (57).

The data in this study suggest that the anti-inflammatory effects of L-Arg are mediated by NOS because NOS inhibition in the presence of supplemental L-Arg prevented the attenuation of chemokine production after LPS. We chose AMT given its reported 20-fold selectivity for iNOS inhibition over endothelial NOS (eNOS) (55). However, the constitutive NOS enzymes may regulate PMN accumulation after a proinflammatory stimulus. Indeed, Lefer et al. (31) found that

Fig. 5. Electrophoretic mobility shift assay (A) and representative band density (B) for nuclear factor (NF)-κB DNA binding in rats treated with saline, LPS (0.5 mg/kg ip), D-Arg-LPS (300 mg/kg of D-Arg ip 30 min before LPS), L-Arg-LPS (300 mg/kg of L-Arg ip 30 min before LPS), and AMT-L-Arg-LPS (0.2 mg/kg of AMT 30 min before L-Arg and 1 h before LPS) 1 h after injection of saline or LPS. SS, p65 supershift; CP, cold probe. To further demonstrate specificity, excess unlabeled (cold probe) oligonucleotide was used as a specific competitor. A representative gel and the associated densitometry of 3 separate experiments are shown. Compared with saline, LPS challenge caused a significant increase in NF-κB DNA binding within 1 h. L-Arg but not D-Arg attenuated the LPS-induced NF-κB DNA binding. NOS inhibition before L-Arg and LPS abolished the effect of L-Arg. AMT alone had no effect on NF-κB DNA binding.
transmigration of PMNs into the peritoneum of neuronal NOS(−/−) and eNOS(−/−) mice was increased after thioglycolate injection. Additionally, NO derived from eNOS may play a protective role in LPS-induced ALI (19). Despite the reported high degree of selectivity of AMT for iNOS, it is likely that AMT also inhibited the constitutive NOS isoform. This is further substantiated by the fact that AMT inhibited the production of NO in the saline control animals.

Transcription factor activation is a pivotal step in the pathophysiology of ARDS. Indeed, consensus sequences for NF-κB have been identified in the promoter regions of several genes (TNF-α, IL-1, and ICAM-1) that have been implicated in the pathogenesis of ARDS (37). NF-κB is activated and bound to DNA in alveolar macrophages from patients with ARDS (37, 48). Furthermore, inhibition of NF-κB DNA binding has been shown to be protective against lung injury in animal studies (40). IkB-α regulates the activation of NF-κB by preventing the latter from translocating into the nucleus and affecting gene transcription. Loss of IkB-α has been associated with NF-κB activation and proinflammatory gene transcription in models of ALI (38). Previous studies have demonstrated that transcription of both CINC-1 (5) and MIP-2 (42) are NF-κB dependent.

Multiple in vitro studies have investigated the inhibitory effect of NO on the IkB-α-NF-κB complex. First, endogenously produced NO may act to inhibit the degradation of IkB-α. In vitro, NO donors inhibit NF-κB activation by blocking IkB-α phosphorylation (and subsequent degradation) in rat vascular smooth muscle cells (26, 43). Second, NO may act to induce an increase in IkB-α mRNA transcription independent of NF-κB activation. Despite attenuation of NF-κB DNA binding in human vascular smooth muscle cells after TNF-α in the presence of NO donors, IkB-α mRNA expression was increased (43). Third, NO may enhance IkB-α promoter activity. NO, but not antioxidants, induced human endothelial cell IkB-α promoter activity in vitro (52). And finally, NO may act to inhibit nuclear translocation of NF-κB independent of IkB-α degradation. In murine macrophages, NO leads to S-nitrosylation of the NF-κB p50 subunit and inhibition of NF-κB-dependent DNA binding, promoter activity, and gene transcription (12). This regulatory action of NO is revealed by in vivo NOS inhibition after systemic LPS, which increases proinflammatory cytokine protein and mRNA expression by a NF-κB-dependent mechanism (57).

Our results suggest that, in vivo, supplemental l-Arg acts to inhibit chemokine gene expression by prevention of NF-κB DNA binding and preservation of normal IkB-α levels early in the course of sepsis.

In summary, this work demonstrates that in vivo provision of the NO substrate l-Arg in a rat model of lung injury prevents chemokine production by the maintenance of IkB-α levels and the attenuation of NF-κB DNA binding. Our findings corroborate previously reported in vitro data (34) regarding the effects of NO on the production of proinflammatory mediators and elucidate a mechanism for the attenuation of PMN accumulation in this model of LPS-induced lung injury. Provision of l-Arg early in the course of systemic inflammation may act to downregulate the inflammatory response via a NO-dependent mechanism before the tissue damage that occurs as the result of PMN accumulation and free radical production.

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