Roles of iNOS and nNOS in sepsis-induced pulmonary apoptosis

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**Rudkowski, Jill C., Esther Barreiro, Rania Harfouche, Peter Goldberg, Osama Kishta, Pedro D’Orleans-Juste, Julie Labonte, Olivier Lesur, and Sabah N. A. Hussain.** Roles of iNOS and nNOS in sepsis-induced pulmonary apoptosis. Am J Physiol Lung Cell Mol Physiol 286: L793–L800, 2004. First published December 5, 2003; 10.1152/ajplung.00266.2003.—Apoptosis (programmed cell death) is induced in pulmonary cells and contributes to the pathogenesis of acute lung injury in septic humans. Previous studies have shown that nitric oxide (NO) is an important modulator of apoptosis; however, the functional role of NO derived from inducible NO synthase (iNOS) in sepsis-induced pulmonary apoptosis remains unknown. We measured pulmonary apoptosis in a rat model of Escherichia coli lipopolysaccharide (LPS)-induced sepsis in the absence and presence of the selective iNOS inhibitor 1400W. Four groups were studied 24 h after saline (control) or LPS injection in the absence and presence of 1400W pretreatment. Apoptosis was evaluated using DNA fragmentation, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling staining, and caspase activation. LPS administration significantly augmented pulmonary cell apoptosis and caspase-3 activity in airway and alveolar epithelial cells. Pretreatment with 1400W significantly enhanced LPS-induced pulmonary apoptosis and increased caspase-3 and -7 activation. The antipapoptotic effect of iNOS inhibition was confirmed in iNOS−/− mice, which developed a greater degree of pulmonary apoptosis than the corresponding wild-type controls both under control conditions and in response to LPS compared with wild-type mice. By comparison, genetic deletion of the neuronal NO synthase 1 had no effect on LPS-induced pulmonary apoptosis. We conclude that NO derived from iNOS plays an important protective role against sepsis-induced pulmonary apoptosis.

Nitric oxide synthase; inducible; neuronal; lungs; caspase; acute lung injury

**ACUTE RESPIRATORY DISTRESS syndrome (ARDS),** which is a significant cause of morbidity and mortality in critically ill patients, is characterized by bilateral pulmonary infiltrates, hypoxemia, neutrophil pulmonary sequestration, shunt fraction increase, pulmonary edema secondary to disruption of pulmonary capillary integrity, intravascular coagulation, and endothelial and epithelial cell apoptosis (9, 27, 28). The importance of pulmonary cell apoptosis in the pathogenesis of acute lung injury has recently been underscored in studies of mice receiving bacterial lipopolysaccharide (LPS). The degree of acute lung injury and animal mortality was significantly attenuated when pulmonary apoptosis was inhibited in these animals by using a pan-caspase inhibitor (19).

Numerous mediators have been found to be associated with sepsis-induced acute lung injury such as proinflammatory cytokines, prostaglandins, thromboxanes, reactive oxygen species, and nitric oxide (NO). Despite many attempts to modulate these mediators in therapeutic trials in humans, none have proven effective thus far in either decreasing the severity of lung injury or preventing death. NO is a secondary messenger molecule synthesized by a group of hemoproteins known as NO synthases (NOS). There are two recognized constitutive forms in the lung: the neuronal (nNOS, type I) and endothelial (eNOS, type III) isoforms (11). Both isoforms are expressed in airway epithelium, pulmonary vascular endothelial cells, and noncholinergic and nonadrenergic nerve fibers of normal lungs. The third isoform is the inducible isofrom (iNOS, type II). Expression of iNOS is induced in a variety of cells in response to exposure to proinflammatory cytokines and LPSs and in animals and humans with sepsis or septic shock. Several reports have identified constitutive iNOS expression in large airway epithelial cells of humans and animals, in vascular smooth muscle cells, endothelial cells, and macrophages (2, 13).

Many reports have documented an increase in pulmonary NO production in response to severe sepsis or LPS administration (for review, see Ref. 37). However, there is considerable debate about the cellular origin and functional significance of enhanced pulmonary NO production to the pathogenesis of acute lung injury. Fujii et al. (8) reported that macrophages contribute significantly to pulmonary iNOS expression and enhanced NO production in the lungs of septic rats. However, these authors did not evaluate whether macrophage NO production participates in the progression and induction of acute lung injury. We (22) and others (36) have reported that mice deficient in iNOS expression (iNOS-knockout mice) develop smaller increases in pulmonary wet/dry ratios and significantly less pulmonary albumin vascular leakage in response to LPS administration compared with wild-type mice. These findings strongly support the notion that iNOS plays a detrimental role in the development of sepsis-induced acute lung injury. Contrasting with this conclusion are observations that inhaled NO inhibits neutrophil migration and cytokine production, and that NO inhibition can worsen lung injury (31). These results
suggest that augmentation of NO production in septic lungs may serve a beneficial role in maintaining lung function during the course of sepsis. One possible mechanism through which this role may be mediated is modulation of pulmonary apoptosis. NO is a well-known regulator of apoptosis and has been shown to inhibit the activity of many caspases under both in vitro and in vivo conditions (6, 24). Based on these findings, we hypothesized, on the basis of the rate of NO production by the three NOS isoforms in pulmonary cells and the time course of endogenous NO production in septic lungs, that the iNOS isoform rather than nNOS or eNOS plays an important role in attenuating pulmonary apoptosis in septic animals, and that iNOS-derived NO inhibits pulmonary apoptosis by attenuating caspase activation in pulmonary cells. In this study, our hypothesis was tested in LPS-induced acute lung injury in rats by using the highly selective iNOS inhibitor N-[3-(aminomethyl)benzyl]acetamidine (1400W). In addition, we used mice deficient in iNOS to evaluate the role of this isoform in pulmonary apoptosis both under normal conditions and in response to LPS injection.

METHODS

Rat Experiments

Four groups of male Sprague-Dawley rats (body wt, 225–250 g; n = 6 per group) were studied. Two groups were given an intraperitoneal injection of either normal saline or 20 mg/kg of Escherichia coli LPS (serotype 055:B5). Groups 3 and 4 were injected with 1400W (20 mg/kg ip) every 8 h for a total of 3 injections, and 30 min after the first 1400W injection, animals were given either saline or E. coli LPS (20 mg/kg). All animals were anesthetized with pentobarbital sodium and killed 24 h after the first injection.

Mice Experiments

Adult (8–12-wk old) wild-type C57/B6 (iNOS+/+; n = 12) and C57/B6 background (iNOS−/−; n = 12; Ref. 23) mice were studied at Sherbrooke University and were injected intraperitoneally with either saline (n = 6 per group) or E. coli LPS (20 mg/kg; n = 6 per group) and were killed 24 h later. Similarly, wild-type (nNOS+/+; n = 12) and nNOS-knockout (nNOS−/−; n = 12; Ref. 18) mice were studied at the McGill University Animal Facility and were administered either normal saline (n = 6 per group) or E. coli LPS (20 mg/kg; n = 6 per group) by intraperitoneal injection and killed 24 h later.

Lung Tissue Sampling

Lungs were removed, blotted dry, frozen in liquid nitrogen, and stored at −80°C. Frozen lung samples were homogenized with a metal homogenizer in 6 vol/wt ice-cooled homogenization buffer that contained (pH 7.5) 50 mM HEPES, 5 mM EDTA, 10% glycerol, 0.50% Triton X-100, 1 mg/ml PMSF, 1 mM sodium orthovanadate, 5 μg/ml aprotinin, 2 μg/ml leupeptin, and 10 μg/ml pepstatin A. The crude homogenates were centrifuged at 4°C for 30 min at 5,000 rpm, and the supernatants were used for all experiments. Protein concentrations of homogenates were determined using the Bradford technique. For immunohistochemistry, the animals were anesthetized with...
pentobarbital sodium, and the lungs were removed en bloc. After removal, lungs were placed in Tissue-Tek OCT (Sakura Finetek, Torrance, CA) and normal saline via a tracheal catheter for 30 min at 25 cmH2O. After removal, lungs were placed in Tissue-Tek OCT (Sakura Finetek, Torrance, CA) and perfused with a 1:1 mixture of Tissue-Tek OCT and normal saline via a tracheal catheter for 30 min at 25 cmH2O. After fixation, the lungs were covered in Tissue-Tek OCT and snap-frozen in isopentane immersed in liquid nitrogen before being stored at −80°C. The lungs were then immersed for 20 s in cold isopentane, frozen in liquid nitrogen, and stored at −80°C.

Detection of Apoptosis

DNA apoptotic ladder. DNA was isolated from frozen lungs (DNeasy kit, Qiagen, Mississauga, Ontario) and electrophoretically separated (10 μg per sample) on a 2% agarose gel. The gel was stained with SYBRgreen I (Molecular Probes, Eugene, OR) and visualized and photographed under UV epillumination.

Cell death detection ELISA. Cytoplasmic histone-associated DNA fragments were detected in 5-μg protein samples of lung homogenate using a commercial kit (Roche Diagnostics, Laval, Quebec). Homogenate samples were placed into strepavidin-coated wells and incubated with antihistone biotin and anti-DNA peroxidase conjugate for 2 h at room temperature. Wells were washed, reaction substrate was added, and absorbance was read at 405 nm.

Caspase-3 activity assay. Caspase-3 activity was determined by measuring the proteolytic cleavage of the fluorogenic substrate N-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin (Ac-DEVD-AMC, BioMol Research Laboratories). Briefly, 40–100 μg of crude lung lysate protein was incubated at 37°C for 30 min with 20 nM of substrate in 500 μl of lysis buffer (25 mM HEPES, pH 7.4, 5 mM EDTA, 2 mM DTT, and 0.2% Triton X-100) supplemented with protease inhibitors. The fluorescence of the cleaved reporter group was measured at excitation (380 nm) and emission (469 nm) in 30-min intervals to determine the rate of substrate hydrolysis. Values obtained were adjusted to a standard curve of AMC alone. Results were expressed as nanomoles per minute per milligram of protein.

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay. Frozen lungs were sectioned (5-μm thickness), and sections were fixed with 4% paraformaldehyde and prepared according to manufacturer’s instructions (In Situ Death Detection kit, Roche, Mannheim, Germany). Slides were visualized with a fluorescence microscope (Olympus) equipped with a digital camera (CoolSNAP cf, Roper Scientific, Trenton, NJ).

Immunoblotting

Specific proteins in lung homogenates (20–80 μg of total protein per sample) were detected via 1-D immunoblotting using Tris-glycine polyacrylamide gels and electrophoretic transfer to PVDF membranes. Cleaved caspase-3 and -7, cleaved poly(ADP-ribose) polymerase (PARP; New England Biolabs, Beverly, MA), and survivin (Novus Biologicals, Littleton, CO) were detected with polyclonal antibodies and cleaved x-linked inhibitor of apoptosis (XIAP), Bcl-2, and Bcl-xL (BD Biosciences, Franklin Lakes, NJ) were detected with monoclonal antibodies. Blots were scanned, and optical densities of specific protein bands were quantified using Image Pro Plus software (Media Cybernetics, Carlsbad, CA). To validate equal protein loading among various lanes, PVDF membranes were stripped (0.1 N NaOH solution for 30 min) and reprobed with monoclonal anti-α-tubulin antibody (Sigma).

Localization of Cleaved Caspase-3

Lung sections (5 μm) were prepared for immunohistochemistry as described previously (22) and incubated overnight with a primary
polyclonal anticleaved-caspase-3 antibody (New England Biolabs). Slides were then washed and probed with biotinylated secondary antibody and Cy3-labeled streptavidin. For identification of nuclei, sections were also stained with 0.1% 4,6-diamino-2-phenylindol dihydrochloride (DAPI; Boehringer, Mannheim, Germany) for 15 min at room temperature. All sections were mounted with antifading mounting medium for fluorescence and were stored in darkness at −20°C until examination with a fluorescence microscope (Olympus).

NOS Assay

NOS activity in lung homogenates (10-μl volume) was measured using an NOSdetect assay kit according to the supplier’s specifications (Stratagene, La Jolla, CA). Rat cerebellum was used as a positive control, and the addition of Nω-nitro-L-arginine methyl ester served as a negative control. Statistical analysis was carried out using SigmaStat software (SPSS, Chicago, IL). Data shown are means ± SE. Groups were compared with χ²-analysis or the Mann-Whitney rank sum test for nonnormal data. Differences were considered significant with a P < 0.05.

RESULTS

Rat Experiments

Apoptosis and caspase activation. Within 24 h, LPS injection elicited a significant increase in lung NOS activity from a mean value of 2.6 ± 1.4 in control lungs to 9.2 ± 3.3 pmol-min⁻¹·mg⁻¹ (P < 0.01). In addition, LPS injection evoked DNA fragmentation in lung samples, which indicates that the level of apoptosis increased significantly (Fig. 1A). This was confirmed by a prominent increase in lung caspase-3 activity after 24 h of LPS injection (Fig. 1B). In addition to measuring caspase-3 activity using the rate of substrate cleavage, we used a polyclonal antibody that detects cleaved (active) caspase-3 components (17 and 19 kDa; Ref. 15). Prominent 17- and 19-kDa-protein bands were detected in the lungs after 24 h of LPS injection (P < 0.01 compared with control; Fig. 1, C and D).

Caspase-7 is an effector caspase that is activated by many enzymes including caspase-3, -6, -8, and -9 and is cleaved into a main active unit of 20 kDa, which in turn cleaves several proteins including PARP. No detectable cleaved caspase-7 was evident in control rats, whereas prominent caspase-7 cleavage was detected in LPS-injected animals (P < 0.01 compared with control; Fig. 2, A and B). PARP is a nuclear protein that is involved in DNA repair particularly in response to oxidative stress and cytokine exposure. Its activity is critical for cell viability (33). PARP is one of the main cleavage targets for caspase-3 (34); when cleaved, it yields a DNA-binding domain (24 kDa) and a catalytic domain (89 kDa). We used a polyclonal rat-specific antibody to detect the active (89-kDa) subunit of cleaved PARP. Although no cleaved PARP protein band was detectable in lungs of control rats, LPS injection was associated with the appearance of cleaved PARP protein bands in lung homogenates, which confirms that apoptosis developed in these animals (Fig. 2, C and D).

To localize pulmonary apoptosis, we performed terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay staining on lungs obtained from control, LPS-injected, and LPS- with 1400W-injected rats. Positive TUNEL staining was evident in few pulmonary cells localized in the alveolar septa in normal rats (Fig. 3A). Injection of LPS resulted in the detection of a larger number of TUNEL-positive cells in alveolar septa (Fig. 3B) and endothelial cells of large pulmonary vessels. Very few large airway epithelial cells stained positive for TUNEL in control and LPS-injected rats. Figure 4 illustrates the localization of cleaved (active) caspase-3 in the lungs of control and LPS-injected animals. Few alveolar epithelial cells stained positive for cleaved caspase-3 in control rats (Fig. 4A). A clear increase in the number of alveolar epithelial cells that stained positive for cleaved caspase-3 was detected in response to LPS injection (Fig. 4B) compared with control lungs, whereas very few airway cells showed positive staining for cleaved caspase-3 in LPS-injected animals (Fig. 4C).
Effects of 1400W on pulmonary apoptosis. Administration of 1400W to saline-treated rats reduced lung NOS activity from a mean value of 2.6–1.4 pmol/min/mg of protein (P < 0.05) but had no significant effects on caspase-3, -7, and PARP activation (see Figs. 1 and 2). In septic rats, pretreatment with 1400W reduced lung NOS activity from 9.2 to 2.5 pmol/min/mg (P < 0.01) and elicited a significant augmentation of caspase-3 activity and cleavage (see Fig. 1). Injection of 1400W also elicited substantial activation of caspase-7 and PARP cleavage to levels significantly higher than those observed with LPS alone (Fig. 5A). Pulmonary apoptosis in control iNOS+/− mice was significantly greater than in control iNOS+/+ mice (Fig. 5A). Injection of LPS in iNOS−/− mice resulted in additional augmentation of apoptosis to >300% of that observed in iNOS+/+ mice (Fig. 5A). Pulmonary apoptosis in control iNOS−/− mice was significantly greater than in control iNOS+/− mice (Fig. 5A). Injection of LPS in iNOS−/− mice resulted in additional augmentation of apoptosis to >300% of that observed in iNOS+/+ mice (Fig. 5A).

Mice Experiments

We evaluated the role of iNOS in LPS-induced pulmonary apoptosis by comparing the degree of apoptosis elicited by LPS injection in iNOS+/+ and iNOS−/− mice. Cell death detection ELISA revealed that LPS injection in iNOS+/+ mice more than doubled the degree of pulmonary apoptosis compared with control iNOS+/+ mice (P < 0.05; Fig. 5A). Pulmonary apoptosis in control iNOS−/− mice was significantly greater than in control iNOS+/− mice (Fig. 5A). Injection of LPS in iNOS−/− mice resulted in additional augmentation of apoptosis to >300% of that observed in iNOS+/+ mice (Fig. 5A). Similarly, the intensities of cleaved caspase-3 and caspase-3 activities were significantly greater in control iNOS−/− compared with control iNOS+/+ mice (P < 0.05; Fig. 5, B and C), and injection of LPS resulted in further elevation of pulmonary caspase-3 cleavage and activity in iNOS−/− compared with iNOS+/+ mice injected with LPS (P < 0.05; Fig. 5, B and C).

No significant differences in the degree of pulmonary apoptosis (measured with cell death detection ELISA) could be detected among control nNOS+/+ and control nNOS−/− mice.
and among LPS-treated nNOS\textsuperscript{+/-} and nNOS\textsuperscript{-/-} mice. Moreover, LPS injection evoked a similar degree of pulmonary caspase-3 cleavage among nNOS\textsuperscript{+/-} and nNOS\textsuperscript{-/-} mice (mean values of cleaved caspase-3 optical densities: 180 and 172 arbitrary units for nNOS\textsuperscript{+/-}-LPS and nNOS\textsuperscript{-/-}-LPS, respectively).

DISCUSSION

The main findings of this study are as follows: 1) E. coli LPS injection produced significant augmentation of apoptosis and caspase-3 activation in the lungs of rats and mice; 2) pharmacological inhibition of iNOS activity in rats before and after LPS injection augmented LPS-induced pulmonary apoptosis and caspase-3 activation; 3) augmentation of pulmonary apoptosis in septic rats in response to iNOS inhibition was not associated with alterations in the expression of prosurvival proteins (Bcl2, Bcl-xL, XIAP, and survivin); and 4) absence of the iNOS gene but not the nNOS gene resulted in augmentation of pulmonary apoptosis both under control conditions and in response to LPS injection.

Pulmonary Apoptosis and Acute Lung Injury

It has been established that significant apoptosis develops in lymphoid organs of septic patients (16) and that inhibition of apoptosis in these organs is associated with improved survival in experimental animals (17). In addition to inducing apoptosis in lymphoid organs, sepsis and endotoxemia also induce apoptosis in pulmonary cells including endothelial and respiratory epithelial cells (9). Apoptosis has also been described in type II alveolar (3), airway epithelial (25), and pulmonary endothelial (9) cells of humans and animals with acute lung injury. Our findings of a significant induction of apoptosis in alveolar and airway epithelial cells after 24 h of LPS injection in rats are therefore in agreement with previous studies. The functional significance of parenchymal pulmonary cell apoptosis is underscored by the observation that systemic administration of a pancaspase inhibitor in septic mice reduced pulmonary apoptosis, improved lung function, and significantly improved animal survival (19). We should emphasize that not all pulmonary cells undergo significant apoptosis in acute lung injury. In fact, it has recently been shown that a low number of apoptotic neutrophils are detected in the bronchoalveolar lavage fluid of patients with acute lung injury, and that incubation of bronchoalveolar lavage fluid of these patients with normal neutrophils leads to reduction in apoptosis of these cells (26).

Apoptosis is induced by two main pathways: 1) the mitochondrial pathway, which involves cytochrome c release from the mitochondria and the activation of caspase-9, which then cleaves and activates caspase-3, -7, and -6; and 2) the death-domain receptor pathway that involves activation of Fas or tumor necrosis factor receptors followed by activation of caspase-8 and subsequent activation of downstream caspase. Cytochrome c release is determined by the ratio of anti-apoptotic (Bcl2, Bcl-xL, XIAP, and survivin) and proapoptotic (Bax, Bad, Bak, and Bid) members of the Bcl-2 family proteins (1), whereas caspase-3, -9, and -7 activities are regulated by IAPs that include survivin, XIAP, cIAP1, and cIAP2 (7). Our study confirms that LPS-induced apoptosis was not associated with significant alterations in the expression of antiapoptotic pro-
teins such as Bcl-2, Bcl-xL, XIAP, and survivin, which thus excludes the possibility that downregulation of these antiapoptotic proteins triggers pulmonary apoptosis after LPS injection. We should emphasize that the involvement of other protein members of the Bcl-2 family or IAPs could not be excluded in our study.

NO and Acute Lung Injury

Although evidence of enhanced NO production has been documented in the lungs of patients with ARDS (14, 21), the functional significance of NO in general and iNOS expression in particular in the pathogenesis of sepsis-induced lung injury is still controversial. Pharmacological inhibition of iNOS activity attenuates (29), intensifies (31), or has little effect (30) on the progression of sepsis-induced acute lung injury. Numata et al. (29) reported that aminoguanidine (a selective iNOS inhibitor) attenuates acute lung injury induced by LPS injection. We reported that many of the indices of LPS-induced acute lung injury were attenuated in iNOS−/− compared with iNOS+/+ mice (22), thereby suggesting that iNOS plays a deleterious role in promoting acute lung injury. Wang Le et al. (36) and Razavi et al. (32) used bone marrow-transplanted chimeric mice and reported that pulmonary microvascular protein leak and pulmonary lipid peroxidation are mediated by inflammatory cell derived iNOS activity but not by pulmonary parenchymal cell localized iNOS activity. These results are suggestive of cell source-dependent compartmentalization of the effects of iNOS derived from NO.

We report here for the first time that NO derived from iNOS but not from nNOS plays an important role in attenuating LPS-induced apoptosis of alveolar and airway epithelial cells. This finding is based on rat experiments in which iNOS activity was inhibited by 1400W. We should emphasize that 1400W is the most selective iNOS inhibitor currently available commercially, and its selectivity toward inhibition of the activity of this isoform has been verified in vivo and in vitro (10). The strong antiapoptotic effect of iNOS in rat experiments is also validated in mice experiments in which mice deficient in the iNOS gene showed significant more pulmonary apoptosis than wild-type mice in response to LPS injection. This antiapoptotic effect of NO in pulmonary cells is in agreement with previous findings of an antiapoptotic effect of NO in endothelial cells, hepatocytes, eosinophils, and ovarian follicles (4, 5). There are several mechanisms through which NO inhibits apoptosis, the most important of which is S-nitrosylation of critical cysteine residues in caspase-3, -8, and -9, which leads to inhibition of these important caspases (24). This mechanism could explain the increase in pulmonary caspase-3 and -7 cleavage and activity in response to NO inhibition in this study. NO could also inhibit apoptosis by restoration of mitochondrial membrane potential, inhibition of the opening of mitochondrial permeability transition pores, attenuation of cytochrome c release from the mitochondria, and induction of the expression of antiapoptotic heat-shock protein 70 and heme oxygenases (20).

Implications

We demonstrate in this study a new and important role for endogenous pulmonary iNOS expression, namely, regulation of pulmonary parenchymal cell apoptosis in animal models of LPS-induced acute lung injury. Although this protective role of iNOS may appear to contradict results of previous studies including our own, which documented deleterious effects of iNOS in the pathogenesis of acute lung injury (22), we should emphasize two important points. First, the timing of experimental observations is very important when one compares various studies. For instance, although our current study focused on the role of iNOS in pulmonary apoptosis during the late phase of sepsis (24 h after LPS administration), other studies evaluated the involvement of iNOS in acute lung injury during the early phases of sepsis. For instance, Kristof et al. (22) measured pulmonary wet/dry ratios after 12 h of LPS injection in iNOS−/− and iNOS+/+ mice because lung iNOS expression in their experiments peaked after 12 h of LPS injection. Similarly, Wang Le et al. (36) studied the role of iNOS in sepsis-induced pulmonary protein leak by measuring Evans blue dye levels after 4 h of cecal ligation and perforation. Second, the nature of the biological roles of iNOS in lung function is highly dependent on the cellular localization of this enzyme. In our study, LPS-induced pulmonary apoptosis was located in specific sites including endothelial cells and alveolar and airway epithelial cells. These cells, particularly airway epithelial cells, are known to have abundant iNOS expression not only under normal conditions, as in the case of large airway epithelial cells, but in response to sepsis or endotoxemia. We propose, therefore, that the antiapoptotic role of NO in acute lung injury is cell source dependent, may be limited to parenchymal pulmonary cells, and differs from deleterious effects of inflammatory cell derived iNOS. We should also point out that our present conclusion regarding the role of iNOS and pulmonary apoptosis applies only to the relatively late phase (24 h) of sepsis.

Our results may also explain why iNOS inhibition has not resulted in significant improvement of mortality of patients with severe sepsis. In fact, human studies with NOS inhibitors have been disappointing and have resulted in increased mortality leading to the termination of a phase III randomized-placebo controlled study of septic shock patients (12, 35). We speculate that increased mortality of these patients in response to NOS inhibitors may be mediated in part through augmentation of pulmonary apoptosis as a result of inhibition of iNOS activity.

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DISCLOSURE

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