Augmented inducible nitric oxide synthase expression and increased NO production reduce sepsis-induced lung injury and mortality in myeloperoxidase-null mice

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The myeloperoxidase (MPO)-hydrogen peroxide-halide system is an efficient oxygen-dependent antimicrobial component of polymorphonuclear leukocyte (PMN)-mediated host defense. However, MPO deficiency results in few clinical consequences indicating the activation of compensatory mechanisms. Here, we determined possible mechanisms protecting the host using MPO−/− mice challenged with live gram-negative bacterium Escherichia coli. We observed that MPO−/− mice unexpectedly had improved survival compared with wild-type (WT) mice within 5–12 h after intraperitoneal E. coli challenge. Lungs of MPO−/− mice also demonstrated lower bacterial colonization and markedly attenuated increases in microvascular permeability and edema formation after E. coli challenge compared with WT. However, PMN sequestration in lungs of both groups was similar. Basal inducible nitric oxide synthase (iNOS) expression was significantly elevated in lungs and PMNs of MPO−/− mice, and NO production was increased two- to sixfold compared with WT. Nitrotyrosine levels doubled in lungs of WT mice within 1 h after E. coli challenge but did not change in MPO−/− mice. Inhibition of iNOS in MPO−/− mice significantly increased lung edema and reduced their survival after E. coli challenge, but iNOS inhibitor had the opposite effect in WT mice. Thus augmented iNOS expression and NO production in MPO−/− mice compensate for the lack of HOCl-mediated bacterial killing, and the absence of MPO-deficient oxidants mitigates E. coli sepsis-induced lung inflammation and injury.

inflammation; endotoxin shock; rodent; host defense

MATERIALS AND METHODS

Animals. Studies were made using MPO−/− mice (5), and wild-type C57BL/6 mice (WT; The Jackson Laboratory, Bar Harbor, ME) were used as controls. Mice (22–26 g, 9–12 wk old) were housed in specific pathogen-free conditions with free access to food and water in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
the University of Illinois Animal Care Facility. All experimental procedures complied with institutional and National Institutes of Health guidelines for animal use, and approvals were obtained from Animal Care Committee of the University of Illinois at Chicago.

*E. coli* infection and survival studies in mice. Live *E. coli* were obtained from American Type Culture Collection, Manassas, VA (ATCC 25992). Mice were challenged intraperitoneally with 1 × 10⁹ live *E. coli* for survival studies and with 0.5 to 2 × 10⁹ live *E. coli* for other experiments as indicated. Control mice were injected intraperitoneally with an equal volume of PBS.

**Pulmonary microvascular permeability.** Pulmonary capillary filtration coefficient (*K<sub>P</sub>*<sub>P</sub>) was measured to determine pulmonary microvascular permeability to liquid as previously described (28). After the standard 20-min equilibration perfusion, the outflow pressure was rapidly elevated by 10 cmH₂O for 2 min, and the lung wet weight was monitored. Lung dry weight was determined, and *K<sub>P</sub>*<sub>P</sub> (ml·min⁻¹·cmH₂O⁻¹·dry g⁻¹) was calculated from the slope of the recorded weight change normalized to the pressure change and lung dry weight. Pulmonary edema formation was measured by continuously monitoring the lung wet weight change for 90 min (36). Because perfusate albumin concentration was constant, and pulmonary arterial pressure did not change, the rate and magnitude of the increase in lung wet weight provided another index of pulmonary microvascular permeability.

The effect of iNOS inhibitor L-N<sup>-<sub>A</sub></sup>-(1-iminoethyl)lysine (L-NIL) on lung edema in vivo was similar to that of MPO<sup>−/−</sup> mice treated with *E. coli* determined by measuring excess lung water. Lungs were excised, weighed, and then homogenized after the addition of 1 ml of double-distilled H₂O. The homogenate was centrifuged at 12,000 rpm for 10 min to obtain the supernatant, and the hemoglobin content was determined. Hemoglobin content and hematocrit were determined in a blood sample from the right ventricle. Fractions of homogenate, supernatant, and blood were weighed and then placed in a drying oven at 60°C for 24 h after which the dry weights were determined. The lung wet-to-dry ratio and final excess lung water were calculated as previously described (37).

**Lung PMN sequestration.** Lung PMN sequestration was assessed by determining MPO activity and also morphometrically quantifying PMN infiltration as previously described (28). Mouse lungs were infiltrated with 10% formalin and embedding in paraffin. Hematoxylin and eosin-stained tissue sections were visualized using a high magnification (>100) objective with an oil-immersion numerical aperture. Middle region (~30 mm²) of the lung upper lobe was outlined at low magnification (~1.25). At least 5% of the outlined region was measured with a systematic random design of counting frames. The total number of PMNs in the outlined region of lung was determined using the formula

\[ n = \Sigma Q^- \times \text{section sampling fraction (SSF)}/\text{area sampling fraction (ASF)}, \]

where \( \Sigma Q^- \) is the total number of PMN counted by optical evaluation using a random design procedure. The ASF is the counting frame (6,400 µm²), and SSF is the fraction of section sampled in the region of the lung.

**PMN isolation.** PMNs from WT or MPO<sup>−/−</sup> mice were purified from peripheral venous blood after collection into EDTA anticoagulant and 1:1 dilution into Ca²⁺/Mg²⁺-free HBSS-BSA, followed by a discontinuous Percoll gradient as previously described (14, 27). This procedure yielded PMN purity of 90–95% and >95% viability assessed by trypan blue exclusion.

**Measurement of nitrite concentration.** PMNs from peripheral blood of WT or MPO<sup>−/−</sup> mice were incubated in arginine (Arg)-free DMEM for 1 h. Fresh DMEM was added containing 1 mM Arg, and cells were then incubated for 3 h at 37°C. Accumulated nitrite + nitrate in the medium of PMNs or in plasma isolated from WT or MPO<sup>−/−</sup> mice was measured after reduction of nitrate to nitrite using a Cd-Cu Reducer (Nitralyzer II kit, World Precision Instruments) following the manufacturer’s instructions. Nitrite was measured in 50-µl aliquots using the Griess reagent (nitrite detection kit; Promega).

**Measurement of lung tissue iNOS activity.** Lung slices (1-mm thick) were washed and placed in HBSS with or without 1 mM Arg at 37°C. NO production was measured in real-time using a three-electrode system (6, 36); a porphyrinic microsensor supercoated with Nafion polymer, a platinum counter electrode, and a silver-silver chloride reference electrode. A micromanipulator was used to place the microsensor on the surface of the lung slice, and the baseline was recorded. To determine constitutive iNOS (cNOS) activity, lung slices in HBSS containing 1 mM Arg were stimulated with 10 µM calcium ionophore A-23187. To measure the lung iNOS activity, slices were incubated in HBSS without L-arginine (l-Arg) for 60 min, and NO generation was initiated by application of 1 mM l-Arg.

**Measurement of lung tissue nitrotyrosine.** Nitrotyrosine content was determined by stable isotope dilution gas chromatography-mass spectrometry (GC/MS) on a Finnigan Voyager quadrupole GC/MS equipped with a chemical ionization probe as previously described (17). Reactions were terminated by forming cell pellets and adding butyraldehyde hydroxylamine. Lipids and salts were removed by extraction with a single-phase solvent mixture of H₂O-water-washed diethyl ether-methanol (1:3:7 vol/vol/vol). Internal standards of 3-¹³C₆-nitrotyrosine were then added, samples were hydrolyzed with HCl, and the nitrotyrosine content in amino acid hydrolysates was determined after reduction to amino tyrosine as an n-propyl per heptfluorobutyl derivative. Nitrotyrosine content was normalized to the content of tyrosine determined by stable isotope dilution GC/MS using ¹⁵N labeled tyrosine as the standard. No significant intra-preparative formation of nitrotyrosine occurred as assessed in each sample by determining the 3-¹⁵N₅₆-nitrotyrosine present.

**Bacterial colony counts.** Lungs were removed aseptically and placed in 1 ml of sterile saline and then homogenized in a tissue homogenizer in a vented hood under sterile conditions. Lung homogenates (0.1 ml) were plated in soy base blood agar plates (Difco), the plates were incubated for 18 h at 37°C, and the number of colonies was counted.

**Western blotting.** Lungs were homogenized in 0.05 M Tris, 0.138 M NaCl, 0.0027 M KCl (pH 8) containing a protease inhibitor mixture (Sigma-Aldrich). Neutrophils were purified from mouse bone marrow using a discontinuous Percoll gradient. Protein concentration was measured, and equal amounts of total protein were loaded per lane. Proteins were separated by SDS-PAGE gradient gels (5–20%), transferred to nitrocellulose, blocked with 5% nonfat milk, and probed using antibody specific for endothelial NOS (eNOS) or iNOS (BD Transduction Laboratories, Lexington, KY).

**Statistical analysis.** Data are expressed as the means ± SE or SD as indicated in the figure legends. Statistical analysis was performed using the two-way ANOVA and Newman-Keuls test for multiple comparisons. The value of *P* < 0.05 was considered significant. Kaplan-Meier survival curves (Figs. 1A and 5) were compared using a log-rank test to determine significance.

**RESULTS**

**Mortality studies.** WT and MPO<sup>−/−</sup> mice were challenged with intraperitoneal injection of live *E. coli* (1 × 10⁹ cells). Approximately 50% of WT mice died within 5 h after *E. coli* challenge, whereas all MPO<sup>−/−</sup> mice were alive at this time (Fig. 1A). All WT mice died within 8 h after challenge; in contrast, 20% of the MPO<sup>−/−</sup> mice were still alive at 12 h.

**Lung microvascular permeability and edema formation.** Water content was continuously monitored in perfused lungs from control animals or mice challenged for 6 h with *E. coli* (1 × 10⁹ cells). All mice were alive at 6 h after this dosage. The weight of lungs from control WT or MPO<sup>−/−</sup> mice remained stable over the 90-min perfusion, whereas lungs from WT mice treated with *E. coli* had significantly increased water accumulation after 60 min of monitoring, which continued to
rise over 90 min (Fig. 1B). In contrast, lungs from MPO<sup>−/−</sup> mice showed almost no wet weight gain (Fig. 1B). This difference was particularly striking at 90 min. We determined pulmonary capillary filtration coefficient (K<sub>vc</sub>), a measure of vascular permeability. WT and MPO<sup>−/−</sup> mice demonstrated similar lung microvascular permeability values under basal condition (Fig. 1C). However, at 6 h after E. coli challenge, K<sub>vc</sub> increased significantly in both groups, but the response was threefold greater in lungs from WT than MPO<sup>−/−</sup> mice (Fig. 1C).

**PMN sequestration in lungs.** After E. coli challenge, lung tissue MPO activity (a measure of PMN sequestration) in WT mice increased sixfold at 6 h; predictably, MPO activity was not detected in lungs from MPO<sup>−/−</sup> mice (data not shown). Thus we determined PMN sequestration in lungs of MPO<sup>−/−</sup> mice by morphometric analysis (Fig. 1D). PMN uptake in

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**Fig. 2.** A: plasma nitrite + nitrate accumulation in WT and MPO<sup>−/−</sup> mice after E. coli challenge. Plasma nitrite + nitrate levels were measured before or 6 h after E. coli challenge (10<sup>9</sup> E. coli). Bars indicate means ± SE (n = 5), *P < 0.05 compared with WT before E. coli challenge. B: NO production from isolated peripheral blood PMN before and after E. coli challenge. PMNs, equilibrated for 60 min in L-arginine (L-Arg)-free medium, were incubated for 3 h in medium with 1 mM L-Arg, and nitrite + nitrate accumulation was measured. Bars indicate means ± SE (n = 5). *P < 0.05 compared with WT before E. coli challenge; **P < 0.05 compared with WT before E. coli challenge. C: E. coli-induced inducible nitric oxide synthase (iNOS) expression in mouse PMNs was measured by Western blot analysis. Right shows blot representative of 3 experiments. Left shows quantitation by densitometry, normalized for protein loading (β-actin staining). Bars indicate means ± SE (n = 3 each), *P < 0.05 compared with WT before E. coli challenge; **P < 0.05 compared with WT before E. coli challenge.
lungs of MPO<sup>−/−</sup> mice increased ninefold at 6 h after E. coli challenge, which was similar to the response in lungs of WT mice (Fig. 1D).

**NO production.** Blood plasma nitrite + nitrate concentration, the stable NO breakdown products, in MPO<sup>−/−</sup> mice before E. coli challenge was twofold greater than WT mice (Fig. 2A). At 6 h after E. coli challenge, plasma nitrite + nitrate concentration in WT mice increased twofold. E. coli challenge of MPO<sup>−/−</sup> mice slightly increased the nitrite + nitrate concentration, and it rose to the same final level as in WT mice (Fig. 2A). Because plasma nitrite + nitrate concentration only provides a general index of overall NO production that can be derived from several sources, we directly evaluated NO production and iNOS protein expression in two important targets, peripheral blood PMNs and lungs of WT and MPO<sup>−/−</sup> mice before and after E. coli challenge. To measure iNOS, which, once expressed, has calcium-independent constitutive activity before and after E. coli challenge, NO production was measured in real-time using a porphyrinic microsensor as described in MATERIALS AND METHODS. Bars indicate means ± SE of area under the NO curve for 20 min (n = 3 each). **P < 0.05 vs. WT control; ***P < 0.05 vs. mice before E. coli challenge; *P < 0.05 vs. WT control mice. E: effects of E. coli challenge on lung nitrotyrosine in WT and MPO<sup>−/−</sup> mice. NO2-Tyr/Tyr ratio was measured by stable isotope dilution gas chromatography-mass spectrometry as described in MATERIALS AND METHODS. Bars indicate means ± SE (n = 5). *P < 0.05 vs. WT control; **P < 0.05 vs. MPO<sup>−/−</sup> 1 h after E. coli challenge.
followed by a plateau at 45–60 min and then slowly declining to baseline by 90 min (data not shown). Although the peak concentration of NO achieved by eNOS activity was similar to that derived from iNOS-dependent activity at 20 min, the total output of iNOS-derived NO was much greater because of the prolonged time course compared with the short, 20-s burst provided by eNOS (Fig. 3). eNOS activity in lungs of WT or MPO−/− mice was similar and did not change after E. coli challenge (Fig. 3A). In contrast, basal iNOS activity was twofold greater in lungs from MPO−/− mice compared with WT, and E. coli challenge in both cases resulted in significant increases in iNOS-dependent NO production (Fig. 3, B and C). Western blot analysis (Fig. 3D) showed that the basal iNOS protein level in lung tissue from MPO−/− mice was greater than WT and that E. coli challenge induced similar final levels of iNOS protein in lungs of WT and MPO−/− mice. In contrast, eNOS protein levels were similar in WT and MPO−/− lungs and did not increase after E. coli (data not shown), similar to our previous findings (36).

Nitrotyrosine formation in lungs. Nitrotyrosine is generated by peroxynitrite (ONOO−) and NO2−, an oxidant produced by MPO conversion of NO3− (12, 17). We evaluated nitrotyrosine production in lungs from WT and MPO−/− mice. The ratio of NO2-Tyr/Tyr increased from 35 ± 7 to 66 ± 8 1 h after E. coli challenge in WT lungs (Fig. 3E). In contrast, basal NO2-Tyr/Tyr ratio in MPO−/− mice did not differ from WT and did not increase 1 h after E. coli (Fig. 3E) indicating that MPO plays an important role in nitrosyrosine formation.

Effects of iNOS inhibition. Treatment of WT mice with different doses of the iNOS-specific inhibitor L-NIL 20 min before E. coli challenge induced concentration-dependent decrease in sepsis-induced accumulation of plasma nitrite + nitrate (Fig. 4A) with a maximum effect seen at 5 mg/kg. Pretreatment with 5 mg/kg L-NIL did not change basal nitrite + nitrate levels in WT mice but reduced the elevated plasma nitrite + nitrate concentration in MPO−/− mice to that of WT mice (Fig. 4B), indicating that high basal nitrite + nitrate concentration in MPO−/− mice is derived from iNOS activity. L-NIL pretreatment also blocked nitrite + nitrate accumulation in response to E. coli in both WT and MPO−/− mice (Fig. 4B). Treatment with l-NIL also blocked iNOS activity in lungs of control MPO−/− mice and in WT and MPO−/− mice after E. coli challenge (Fig. 4C).

Next, we compared the bacterial clearance capacity of WT and MPO−/− mice. The bacterial burden in lungs at 1 or 6 h after E. coli challenge was significantly less in MPO−/− mice compared with WT (Fig. 4D). Pretreatment with iNOS-specific inhibitor L-NIL significantly reduced bacterial colony counts 6 h after E. coli in WT mice, whereas in MPO−/− mice, the same pretreatment increased bacterial colony counts at 1 and 6 h after E. coli challenge.

Fig. 4. A: nitrite + nitrate production in blood plasma of WT mice after E. coli challenge. Different doses of L-N-(1-iminoethyl)lysine (L-NIL) were administrated to WT mice 20 min before E. coli challenge, and blood plasma levels were measured at 6 h after E. coli. Bars indicate means ± SE (n = 3). **P < 0.05 vs. control; *P < 0.05 vs. 0 mg/kg L-NIL. B: effects of iNOS inhibition on nitrite + nitrate levels in plasma of WT and MPO−/− mice after E. coli challenge. WT and MPO−/− mice were pretreated without or with 5 mg/kg L-NIL for 20 min, and nitrite + nitrate levels were measured before or 6 h after E. coli challenge. L-NIL blocked the increase in plasma nitrite + nitrate in WT mice after E. coli and reduced basal and post-E. coli nitrite + nitrate levels in MPO−/− mice. Bars indicate means ± SE (n = 4–6). *P < 0.05 vs. without L-NIL pretreatment; **P < 0.05 vs. WT, no E. coli. C: iNOS activity in lungs from mice treated as in B was measured in real-time using a porphyrinic microsensor as described in MATERIALS AND METHODS. Data are presented as relative area under NO curve for 20 min. Bars indicate means ± SE (n = 4–6). *P < 0.05 vs. without L-NIL pretreatment; **P < 0.05 vs. WT, no E. coli. D: lung bacterial clearance at 1 and 6 h following intraperitoneal administration of E. coli [2 × 109 colony-forming units (CFU)] in WT and MPO−/− mice were measured as described in MATERIALS AND METHODS. Bars indicate means ± SE (n = 5). *P < 0.05 vs. corresponding point without L-NIL treatment; #P < 0.05 vs. corresponding point in WT mice.
6 h compared with mice without iNOS inhibitor (Fig. 4D). These data suggest a role for iNOS-derived NO in bacterial clearance in MPO<sup>−/−</sup> mice.

To determine the role of iNOS-derived NO on lung injury, we measured the effect of E. coli challenge on excess lung water in animals treated with the iNOS inhibitor L-NIL. E. coli treatment resulted in increased lung edema in WT mice but did not cause a significant increase in MPO<sup>−/−</sup> mice (Fig. 5A). Interestingly, inhibition of iNOS led to a decrease in lung edema in WT mice after E. coli treatment, whereas in MPO-null mice, L-NIL resulted in increased edema (Fig. 5A), consistent with the effects of iNOS inhibition on bacterial clearance (Fig. 4D).

Because MPO deletion conferred a significant survival advantage to lethal E. coli challenge (Fig. 1A), we investigated the effect of iNOS inhibition on the response. Pretreatment of WT mice with L-NIL resulted in an increase in survival, whereas pretreatment of MPO<sup>−/−</sup> mice with L-NIL resulted in decreased survival (Fig. 5B). Taken together, these data suggest a role for iNOS-derived NO in MPO<sup>−/−</sup> mice that results in increased bacterial clearance, reduced lung injury, and increased survival in this model of E. coli-induced sepsis.

**DISCUSSION**

NADPH oxidase, MPO, and iNOS are important enzymes regulating antimicrobial host defense (see Refs. 22, 31, 41). MPO produces HOCl, a potent bactericidal agent that is also a proinflammatory oxidant capable of inducing tissue injury and inflammation (15, 22, 42). MPO also catalyzes nitrotyrosine formation and lipid peroxidation by converting NO<sub>2</sub> to the potent oxidant ·NO<sub>2</sub> (22, 30, 43). Despite the presumed importance of MPO in host defense, patients with MPO deficiency exhibit almost normal resistance to infection (15, 26). Although this led to claims that MPO does not play a crucial role in host defense, a more likely explanation is that compensatory mechanisms substitute for its loss (16, 22). However, the nature and relative importance of these compensatory effects remain controversial (16, 22).

In the present study, we used MPO-null mice to investigate mechanisms responsible for protection of the host in the absence of a functional peroxide-halide pathway. We observed, surprisingly, that MPO<sup>−/−</sup> mice had enhanced bacterial clearance, reduced lung injury and edema formation, as well as greater survival following E. coli challenge compared with WT mice. The results show that increased iNOS expression and NO production in MPO<sup>−/−</sup> mice were crucial in mitigating the effects of MPO deficiency. Several lines of evidence support this contention. First, basal expression of iNOS and NO production were significantly enhanced in PMNs and lungs of MPO<sup>−/−</sup> mice as reflected in the higher iNOS protein levels, iNOS activity in lung tissue and isolated PMNs, and plasma NO<sub>2</sub> levels. Second, bacterial colony counts in lungs of MPO<sup>−/−</sup> mice were significantly lower than in WT mice at both 1 and 6 h after E. coli administration. Third, inhibition of iNOS significantly increased bacterial colony counts in lungs of MPO<sup>−/−</sup> mice after E. coli administration. Fourth, inhibition of iNOS significantly reduced the survival of MPO<sup>−/−</sup> mice in response to E. coli challenge.

High basal expression of iNOS in MPO-null mice could help to explain differences in survival seen in MPO<sup>−/−</sup> and WT mice after E. coli challenge. Lungs and PMNs of MPO<sup>−/−</sup> mice constitutively produced higher levels of NO than WT mice. The preexisting higher level of NO, which has antimicrobial activity (29, 40), may act to kill or slow the replication of the E. coli from the time of administration compared with WT mice in which iNOS upregulation was not maximal until 6 h. NO itself is generally not toxic to host tissue but may become damaging in combination with ROS (30), which would...
become prominent at later times during sepsis. Thus the high NO existing before E. coli challenge in MPO−/− mice in this case would be protective at the early stage of sepsis, whereas in WT mice, high NO generated later in the response could combine with ROS that are also present to cause host tissue damage (30). This possibility is consistent with our present finding that inhibition of iNOS results in less lung injury and increased survival in WT mice as well as our previous study (36) showing that NO inhibition reduced macrophage inflammatory protein-2 (MIP-2) expression and lung edema associated with E. coli-induced sepsis in WT mice. Reactive oxygen and nitrogen species were also shown to impair the ability of the lung to clear alveolar fluid by inhibiting epithelial Na+ channels in mycoplasma-infected mice (19). Interestingly, MPO−/− mice maintained their ability to clear alveolar fluid after mycoplasma infection, likely due to lack of generation of reactive oxidants (19), consistent with our findings of reduced lung injury and edema in response to E. coli infection in MPO-deficient mice.

MPO-derived ·NO2 causes tyrosine nitration as well as lipid peroxidation, resulting in alteration of protein function, cell membrane injury, and generation of proinflammatory mediators (30, 43). Because NO terminates lipid peroxidation (20), and MPO scavenges and inactivates NO (1), MPO−/− mice are expected to have reduced lipid peroxidation. In our studies, we observed that E. coli challenge resulted in a marked increase in nitrotyrosine production in lungs of WT mice, but no increase was seen in MPO−/− mice. In addition to MPO-derived ·NO2, nitrotyrosine is generated from reaction of tyrosine residues with ONOO− derived from NO and superoxide (30). It was shown that MPO−/− mice injected intraperitoneally with thioglycolate or zymosan produced significantly less nitrotyrosine compared with WT mice (43). Thus, in some inflammatory models, MPO appears to play a dominant role in nitrotyrosine formation. In the E. coli sepsis model in the present study, however, ONOO− did not appear to contribute to nitration of tyrosine in MPO−/− lungs at 1 h after E. coli challenge despite high basal expression of iNOS and NO production. This finding helps to explain the reduction in lung inflammatory injury and mortality in MPO−/− mice compared with WT mice. In addition, the lack of HOCl production during sepsis in MPO−/− mice could reduce tissue injury compared with WT (22). These data are consistent with a previous study showing no difference in lung nitrotyrosine formation between WT and iNOS−/− mice after mycoplasma infection and a reduction in nitrotyrosine to uninfected levels in mice depleted of PMNs by cyclophosphamide, indicating the importance of PMN-derived MPO in this process (18). MPO also oxidizes glutathione (39), an important cellular antioxidant; thus augmentation of this antioxidant defense in MPO−/− mice could be another factor contributing to their protection.

We considered the possibility that alterations in eNOS could have contributed to the higher NO production and protection seen in MPO−/− mice. Studies have reported varying effects of inflammation on eNOS expression (7). For example, eNOS promoter activity and mRNA expression were reduced by TNF-α in cultured bovine endothelial cells (3) and human endothelial cells (25), whereas IFN/LPS treatment of bovine endothelial cells resulted in increased expression of eNOS mRNA (21). However, in our previous study (36) and the present study, we observed that eNOS immunoreactivity in mouse lungs remained constant for up to 6 h after in vivo E. coli challenge, indicating that eNOS expression is not altered in response to gram-negative septicemia. In addition, the iNOS-specific inhibitor, L-NIL, blocked the excess NO production in MPO−/− mice. Thus the present results cannot be explained by alterations in the activity of eNOS.

A key unresolved question is the mechanism of upregulation of iNOS expression in the MPO−/− mice. Studies showed that MPO inhibited iNOS expression in IFN-γ/LPS-treated monocye macrophages, and it was proposed that MPO scavenges the low levels of NO that are necessary for the induction of iNOS (24). It is thus possible that iNOS is upregulated in MPO−/− mice secondary to absence of such an inhibitory effect of MPO on iNOS expression. However, this question has not been addressed in the MPO-null mouse model.

In summary, our data show that augmentation of iNOS-dependent NO production in the absence of MPO is a crucial compensatory mechanism regulating host defense and thus prevents lung inflammation and injury induced by E. coli septicemia in MPO-null mice.

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Enhanced iNOS Activation in MPO Deficiency


