Septic pulmonary microvascular endothelial cell injury: role of alveolar macrophage NADPH oxidase

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Farley KS, Wang L, Mehta S. Septic pulmonary microvascular endothelial cell injury: role of alveolar macrophage NADPH oxidase. Am J Physiol Lung Cell Mol Physiol 296: L480–L488, 2009. First published December 12, 2008; doi:10.1152/ajplung.90201.2008.—A significant role for alveolar macrophages (AM) in the pathophysiology of sepsis-induced acute lung injury (ALI) has been shown; however, the mechanisms behind AM-related lung injury remain relatively uncertain. We examined the role of AM nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in pulmonary endothelial cell septic injury. NADPH oxidase is one of the major sources of cellular reactive oxygen species and has been implicated in endothelial injury in ALI. Pulmonary microvascular endothelial cells (PMVEC) monolayers were grown on Transwell inserts and incubated with wild-type and NADPH oxidase-deficient AM in the presence or absence of cytomix (equimolar TNF-α, IL-1β, and IFN-γ). Injury to the monolayers was assessed by trans-PMVEC Evans blue (EB)-labeled albumin flux. We found AM under cytomix stimulation caused significant EB-albumin flux across the PMVEC monolayers, and this effect was attenuated by the genetic deletion of AM NADPH oxidase. The pharmacological inhibition of AM NADPH oxidase with apocynin and PR-39 also significantly reduced AM-dependent PMVEC injury. In the AM-PMVEC cocultures, we also assessed PMVEC injury through measurement of protein oxidation and lipid peroxidation. AM were shown to cause a significant increase in these markers of PMVEC injury, which was also attenuated by the inhibition of NADPH oxidase or through the use of NADPH oxidase-deficient AM. PMVEC NADPH oxidase was shown not to significantly contribute to PMVEC injury in our studies. From our findings we have concluded that AM NADPH oxidase is crucial for the septic increase in pulmonary vascular permeability.

alveolar macrophages; endothelial cells; oxidative stress

ACUTE LUNG INJURY (ALI) and its most severe form, the acute respiratory distress syndrome (ARDS), are characterized by increased permeability of the pulmonary microvasculature and an excessive influx of inflammatory cells into the lung tissue, both of which lead to the development of high-protein pulmonary edema, diffuse alveolar damage, and severe hypoxemia (42, 50). Injury to endothelial cells (EC), specifically in the pulmonary microvasculature (PMVEC), has been recognized as one of the key mechanisms in high-protein pulmonary edema and subsequent lung dysfunction (6, 21). Indeed, there is evidence for EC injury in humans with ALI, since plasma von Willebrand factor, a marker of EC injury, is increased in patients with ARDS and is higher in nonsurvivors vs. survivors (52). EC injury was also associated with higher pulmonary vascular permeability in a rat model of ALI (16), EC injury in sepsis and ALI is the result of the actions of multiple soluble inflammatory cytokines, as well as the effects of activated leukocytes, including macrophages and neutrophils (30, 31, 53, 54).

ALI is also characterized by significant lung oxidant stress, which can contribute to cellular injury and the pathophysiology of ALI (7). Although all cells in the lung can generate reactive oxygen species (ROS) (7), infiltrating neutrophils are thought to be the major source of ROS in ALI (18, 41). One of the major molecular sources of ROS is the multicomponent nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex (13, 14). The involvement of NADPH oxidase has been studied in many models of ALI, and it has been recognized to contribute importantly (10, 17, 18, 51). For example, in mice genetically deficient in the gp91phox or p47phox subunits of NADPH oxidase, septic lung injury and neutrophil infiltration were attenuated (17). However, the direct effects of NADPH oxidase in the septic injury of isolated PMVEC have not been addressed.

There is increasing evidence for an important role of alveolar macrophages (AM) in ALI (9, 29, 30). AM are the principal resident immune cells found in the air spaces (4, 47) and have been linked to the substantial pulmonary influx of neutrophils in ALI (28, 29). In addition, we previously showed that depletion of AM significantly reduced pulmonary protein leak and neutrophil influx in a murine model of septic ALI (12). Moreover, in vitro studies in our laboratory have shown that AM can directly induce PMVEC injury under septic conditions (11). In part, AM-dependent ALI and PMVEC injury appear to be mediated through the release of nitric oxide (NO) by inducible NO synthase (iNOS) (12). AM-derived ROS (15, 22, 27) primarily mediate the antimicrobial function of AM (17, 22, 43) but could also contribute to ALI and EC injury. Indeed, inhibition of macrophage superoxide production attenuated endotoxemic ALI (25, 45). We previously reported that AM-dependent septic PMVEC injury in vitro was attenuated by scavenging of superoxide (O2- ) (11). However, the specific contribution of AM NADPH oxidase to AM-dependent septic PMVEC injury and oxidant stress has not been addressed, nor has the role of NADPH oxidase in PMVEC.

Thus we hypothesized that AM directly mediate septic PMVEC injury through NADPH oxidase-dependent oxidant stress. To determine the contribution of AM vs. PMVEC NADPH oxidase...
oxidase to septic PMVEC injury, we isolated PMVEC and AM from wild-type as well as two distinct NADPH oxidase-deficient mouse strains (gp91phox−/− and p47phox−/−) and cocultured AM-PMVEC under “septic” conditions in vitro, modeled by treatment with cytomix, equimolar amounts of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interferon-γ (IFN-γ). In AM-PMVEC cocultures, we assessed PMVEC injury through measurement of Evans blue (EB) dye-labeled albumin flux across the PMVEC monolayers and of PMVEC oxidant stress through protein oxidation and lipid peroxidation. We confirmed our observations on NADPH oxidase-deficient cells using pharmacological inhibitors of NADPH oxidase.

METHODS

Animal preparation. Male C57BL6 mice (7–8 wk old, 20–25g; Charles River, St. Constant, QC, Canada) were used. All protocols were approved by the institutional animal ethics committee, in accordance with the guidelines of the Canadian Council on Animal Care, and were supervised by a veterinarian.

PMVEC isolation. PMVEC were isolated from three types of mice: i) wild-type mice (+/+), ii) gp91phox−/− null mice (gp91phox−/−), and iii) p47phox−/− null mice (p47phox−/−). The PMVEC were isolated from peripheral, subpleural pulmonary tissue of naive mice as previously described (37). After being minced, the tissue was incubated with the digestive enzyme collagenase before incubation with magnetic microbeads bonded to species-specific anti-platelet endothelial cell adhesion molecule (PECAM) antibody. The EC that were attached to the microbeads were magnetically captured and seeded in gelatin-coated cell culture flasks. The cells were grown to confluence and then sorted by two-channel fluorescence-activated cell sorting using anti-PECAM and acetylated-LDL fluorescence. This produced a PMVEC population with >99% homogeneity. All experiments were conducted with PMVEC at passages 3–6.

AM isolation. AM were isolated from wild-type, gp91phox−/−, or p47phox−/− mice by bronchoalveolar lavage (BAL), as previously described (12). In brief, 3 ml of 0.5 mM EDTA-PBS was instilled into the lungs of the donor mice in three separate 1-ml aliquots. Each aliquot was aspirated three times into the lung tissue, and the return fraction was pooled. The harvested AM were washed twice with Dulbecco modified Eagle medium (DMEM, based on the experiment to be conducted, before coculture was performed. The AM were incubated for 8 h in the absence or presence of cytomix (3 ng/ml) vs. PBS stimulation for 8 h.

Assessment of albumin leak across the PMVEC monolayers. PMVEC injury was assessed by the flux of Evans blue (EB) dye-labeled albumin across PMVEC monolayers. For this assay, 10^5 PMVEC (10^5) were grown to confluence on gelatin-coated 96-well plates. PMVEC monolayers were then labeled with a 10 μM concentration of the fluorescent fatty acid analog 4,4-difluoro-5-(4-phenyl-1,3-buta dienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (C11-BODIPY 581/591; Invitrogen, Burlington, ON, Canada), which incorporates into the lipid membranes of the cells. Oxidation of this dye results in a shift of its original red fluorescence to green fluorescence and serves as a marker of oxidant stress and lipid oxidation of adjacent fatty acids in the cell membrane (40, 48). A stock solution of the dye was prepared by dissolving 1 mg of the dye powder in 1 ml of dimethyl sulfoxide (DMSO) and stored at −80°C. Aliquots of the stock solution were then diluted in DMEM to a final concentration of 10 μM C11-BODIPY 581/591 at the start of each experiment; PMVEC were incubated in 200 μl for 30–45 min at 37°C and then washed twice in warm 2% BSA-PBS solution.

Assessment of PMVEC viability. PMVEC (10^4) were grown to confluence in gelatin-coated 96-well plates. The PMVEC monolayers were incubated for 8 h in the absence or presence of cytomix (3 ng/ml) and/or AM (3 × 10^5). The wells were then washed with warm PBS before incubation with 100 μl of 0.02% Trypan blue solution (Sigma-Aldrich, Oakville, ON, Canada) for 5 min. Dead cells were then identified as those that appeared blue under the microscope.

Assessment of AM vs. PMVEC NADPH oxidase-dependent PMVEC injury. To isolate the role of PMVEC NADPH oxidase in the septic injury of PMVEC, we first assessed a dose-dependent effect of cytomix stimulation on PMVEC injury. Confluent PMVEC monolayers (wild type, gp91phox−/−, or p47phox−/−) were incubated with four concentrations of cytomix (0, 3, 10, or 30 ng/ml) for 8 h, and injury was assessed as the degree of trans-PMVEC EB-albumin leak in the final hour of incubation. In addition, PMVEC monolayers were incubated with wild-type AM under cytomix vs. PBS stimulation for 8 h, and PMVEC injury was then assessed again as the degree of EB-albumin flux, as well as PMVEC lipid peroxidation as reflected by oxidation of PMVEC membrane-incorporated BODIPY dye and PMVEC protein oxidation as determined by measuring the carbonyl content of the PMVEC.

To assess the specific contribution of AM NADPH oxidase to septic PMVEC injury, we used two approaches. First, gp91phox−/− vs. p47phox−/− AM were cocultured with wild-type PMVEC monolayers under cytomix stimulation, and injury to PMVEC was assessed as

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described above. In the second approach, gp91phox or p47phox PMVEC were used, to eliminate any contribution of PMVEC NADPH oxidase, and cocultured with wild-type AM in the presence or absence of two specific inhibitors of NADPH oxidase, apocynin (1 mM; Sigma-Aldrich) and a proline- and arginine-rich peptide, PR-39 (10 μM; CS Bio, Menlo Park, CA). AM-PMVEC cocultures were pre-treated for 30 min with the inhibitors before cytomix exposure and subsequent assessment of PMVEC injury as described above.

**RESULTS**

Role of PMVEC NADPH oxidase in septic PMVEC albumin leak. We first assessed the time course of the dose-dependent effects of cytomix on EB-albumin leak across wild-type PMVEC monolayers and found maximum leak following 8-h stimulation with 10 ng/ml cytomix (Fig. 1A). However, stimulation of PMVEC with 3 ng/ml cytomix did not cause significant EB-albumin leak (Fig. 1A). We also determined the time course of cytomix effects in AM-PMVEC cocultures and again found maximum trans-PMVEC leak following 8 h of septic stimulation (Fig. 1B).

We then assessed the contribution of PMVEC NADPH oxidase to trans-PMVEC EB-albumin leak. Basal unstimulated trans-PMVEC EB-albumin flux was not significantly different among wild-type, gp91phox, or p47phox isolated PMVEC monolayers (Fig. 2). Cytomix (3 ng/ml) treatment did not induce a significant increase in trans-PMVEC EB-albumin flux across isolated PMVEC of all three genotypes, and thus this dose was used in all remaining experiments in AM-PMVEC cocultures.

Moreover, PMVEC NADPH oxidase did not appear to contribute to AM-dependent septic (cytomix) EB-albumin leak across the PMVEC monolayers. In AM-PMVEC cocultures, there was a similar, significant increase in EB-albumin flux across all three PMVEC genotypes following cytomix stimulation [175 ± 33% in wild-type, 127 ± 10% in gp91phox−/−, and 183 ± 6% in p47phox−/− PMVEC, *P < 0.01 for each; *P = not significant (NS) among the 3 PMVEC genotypes, Fig. 3] vs. respective, unstimulated AM-PMVEC cocultures.

**Effects of AM NADPH oxidase on septic PMVEC albumin leak.** We first assessed the role of AM NADPH oxidase in AM-dependent PMVEC septic albumin leak by coculturing wild-type PMVEC monolayers with either gp91phox−/− or p47phox−/− AM during cytomix stimulation. Cytomix treatment of wild-type AM-PMVEC cocultures increased trans-
PMVEC EB-albumin leak (254 ± 42% increase vs. unstimulated AM-PMVEC, P < 0.001, Fig. 4). In contrast, AM-PMVEC coculture using wild-type PMVEC with either gp91phox/−/− or p47phox/−/− AM during cytomix stimulation induced minimal, nonsignificant EB-albumin leak across PMVEC monolayers (Fig. 4).

In the second approach, we used gp91phox/−/− vs. p47phox/−/− PMVEC monolayers and pharmacological inhibitors to define the role of NADPH oxidase in wild-type AM in coculture. Coculture of gp91phox/−/− PMVEC with wild-type AM under cytomix stimulation significantly increased trans-PMVEC EB-albumin leak (172 ± 42% increase, P < 0.01, Fig. 5A; 232 ± 50% increase, P < 0.001, Fig. 5B). This significant increase in AM-dependent septic trans-PMVEC albumin leak was attenuated by pretreatment with both NADPH oxidase inhibitors, apocynin (53 ± 8% reduction, P < 0.01) and PR-39 (70 ± 5% reduction, P < 0.001).

Similar results were obtained when wild-type AM were cocultured with p47phox/−/− PMVEC monolayers (Fig. 6). Cytomix treatment increased EB-albumin leak across AM-PMVEC cocultures (170 ± 27% increase, P < 0.01, Fig. 6A; 242 ± 35% increase, P < 0.001, Fig. 6B) vs. unstimulated AM-PMVEC cocultures. This AM-dependent septic trans-PMVEC albumin leak was significantly reduced by 44 ± 7% (P < 0.05) following pretreatment with apocynin and by 72 ± 6% (P < 0.001) with PR-39 treatment.

Effects of AM NADPH oxidase on septic PMVEC oxidant stress. To determine the mechanism behind AM NADPH oxidase-dependent septic injury of the PMVEC monolayers, we assessed PMVEC protein oxidation and lipid peroxidation in cytomix-treated AM-PMVEC cocultures. The level of protein carbonyls reflects PMVEC protein oxidation, as shown in the positive control, in which we incubated PMVEC with 0.2 mM H2O2 and found a dramatic increase in PMVEC protein carbonyl levels (413 ± 30% increase vs. unstimulated PMVEC, P < 0.001). Cytomix treatment of wild-type AM-PMVEC cocultures also markedly increased PMVEC protein carbonyl levels (402 ± 37% increase vs. unstimulated, P < 0.001). In stark contrast, incubation of PMVEC with either gp91phox/−/− or p47phox/−/− AM under cytomix stimulation inhibited the septic increase in PMVEC protein carbonyl content by 48 ± 6 (P < 0.05) and 72 ± 7% (P < 0.01), respectively.
respectively (Fig. 7). Similarly, the septic increase in PMVEC protein carbonyls in coculture with wild-type AM was significantly blunted by 44\% (P < 0.05) in the presence of the NADPH oxidase inhibitor PR-39. In wild-type AM-PMVEC cocultures, we first carried out a time-course study of PMVEC lipid peroxidation under septic vs. untreated conditions. Cytomix stimulation of PMVEC alone did not cause a significant increase in PMVEC lipid peroxidation, as reflected by the increase in green fluorescence of the BODIPY dye (Fig. 8). Coculture of AM with PMVEC in the absence of cytomix was associated with significant PMVEC lipid peroxidation. However, cytomix treatment of wild-type AM-PMVEC cocultures induced significantly greater PMVEC lipid peroxidation than both cytomix-treated isolated PMVEC and unstimulated AM-PMVEC cocultures, starting at 4 h after stimulation and becoming maximal by 8 h. Subsequently, the lipid peroxidation signal was less consistent at 12 and 24 h (data not shown), possibly because of PMVEC death.

We then assessed the effects of AM NADPH oxidase genotype on AM-dependent septic PMVEC lipid peroxidation (Fig. 9). Cytomix stimulation of wild-type AM-PMVEC cocultures induced significant PMVEC lipid oxidation, which was attenuated by 36 ± 4\% (P < 0.05) by pretreatment with the specific NADPH oxidase inhibitor PR-39. Incubation of PMVEC with gp91<sup>phox</sup>−/− or p47<sup>phox</sup>−/− AM under cytomix stimulation also resulted in levels of lipid peroxidation that were significantly lower (by 32 ± 9 and 50 ± 9\%, respectively, P < 0.05 for each) than with wild-type AM.

**Effect of AM on PMVEC viability.** Incubation of PMVEC with 3 ng/ml cytomix over 8 h did not cause a significant reduction of PMVEC viability vs. PBS incubation (99.3 ± 0.3 vs. 99.4 ± 0.08\%, P = NS). In addition, coculture with AM, in the absence or presence of cytomix, did not reduce PMVEC viability (97.2 ± 0.9% without cytomix vs. 97.7 ± 0.6% with...
cytomix, \( P = \text{NS} \)). In contrast, exposure of PMVEC to 10 \( \mu \text{M} \) \( \text{H}_2\text{O}_2 \) greatly reduced PMVEC viability to 11.7 \( \pm \) 1.6%.

**DISCUSSION**

In this study, we assessed the role of murine AM in septic PMVEC injury and oxidant stress, and specifically the role of AM NADPH oxidase in this injury. PMVEC NADPH oxidase did not contribute to either the septic injury of isolated PMVEC (in the absence of AM) or AM-dependent PMVEC injury during cytomix stimulation. Wild-type AM significantly enhanced PMVEC injury under cytomix stimulation, as reflected by trans-PMVEC EB-albumin flux and PMVEC oxidant stress, as assessed by increased protein carbonyls and lipid peroxidation. AM-dependent septic PMVEC injury and oxidant stress were completely attenuated when AM from \( \text{p47phox}^{-/-} \) or \( \text{gp91phox}^{-/-} \) mice were cocultured with PMVEC under septic conditions. Moreover, results with NADPH oxidase null AM in septic AM-PMVEC coculture were confirmed by similar effects of pharmacological NADPH oxidase inhibition in septic coculture of wild-type AM-PMVEC. These results suggest that AM contribute directly to septic PMVEC injury and oxidant stress in vitro and that this AM-dependent injurious effect is mediated, at least in part, through AM NADPH oxidase.

AM are thought to contribute importantly to ALI (4, 9, 29, 30, 47), including potentially mediating pulmonary tissue injury as well as the pulmonary influx of neutrophils (28, 29). Depletion of AM attenuates the severity of key pathophysiological features of ALI, including microvascular permeability, neutrophil influx, and pulmonary cytokine and chemokine production (12, 28, 29, 55). It has been suggested that the role of AM in ALI may be an indirect one, mediated through enhanced neutrophil influx and neutrophil-dependent injury. However, in the present study, we confirmed our previous observations of a direct effect of AM in PMVEC barrier dysfunction under septic conditions (11).

The potential mechanisms of AM-dependent ALI and PMVEC injury include the release of inflammatory mediators such as reactive nitrogen species, ROS, cytokines, and chemokines. We previously showed in vivo that pulmonary protein leak in septic mice was dependent on AM iNOS (12) and confirmed in vitro that AM-dependent PMVEC injury under septic conditions, reflected by trans-PMVEC albumin flux, was also dependent on AM iNOS (11).

In the present study, PMVEC injury and barrier dysfunction in AM-PMVEC cocultures under septic conditions were not simply a result of loss of PMVEC viability. AM-dependent septic PMVEC injury was associated with increases in PMVEC protein carbonyls and lipid peroxidation, which suggested an important contribution of AM-dependent oxidant stress in septic PMVEC injury. This AM-dependent oxidant stress is thought to contribute importantly to septic ALI (8, 20). Indeed, infusion of gadolinium chloride, an inhibitor of macrophage activation (38), has been shown to abrogate ALI (19, 35, 41).
Lipid peroxidation has been suggested as a contributory mechanism of ALI (2), since both animal and clinical studies have shown increased lipid peroxidation products in lung tissue in ALI (23, 24, 44). Mechanistically, EC membrane lipid peroxidation can lead to changes in membrane fluidity and increased EC permeability (3). Moreover, pharmacological reduction of pulmonary protein leak in ALI is associated with a reduction in lipid peroxidation (32). There also is evidence of protein oxidation in ALI, including accumulation of protein carbonyl groups in the BAL of patients with ALI (26). ROS, such as H2O2, also have been shown to directly induce EC barrier dysfunction through the modification of various cytoskeletal and junction proteins, including VE-cadherin, and formation of inter-EC gaps (5, 39, 49, 56).

Our studies suggest that AM-dependent septic PMVEC injury and protein and lipid peroxidation were mediated through NADPH oxidase, specifically in AM. NADPH oxidase is a major source of injurious ROS and appears to be an important contributor to the development of pulmonary oxidant stress and ALI (10, 17). For example, NADPH oxidase null mice have significantly lower levels of septic pulmonary protein leak and free radical production compared with wild-type mice (17, 41). In addition, intratracheal LPS-induced pulmonary lipid peroxidation was significantly reduced in NADPH oxidase null mice (41). Although published studies support a contributory role for NADPH oxidase in ALI, the specific role of AM vs. PMVEC NADPH oxidase in septic ALI or EC injury has not been previously addressed. This study demonstrates the first evidence of specific AM NADPH oxidase-dependent PMVEC injury in vitro under septic conditions. Moreover, in contrast to this effect of AM NADPH oxidase in PMVEC septic injury, PMVEC NADPH oxidase did not appear to contribute to PMVEC septic injury or oxidant stress in isolated PMVEC (absence of AM) or in AM-PMVEC coculture. In contrast to our findings, it has been reported that increased human umbilical vein EC (HUVEC) ROS production did result in oxidative modification of either EC junctional proteins or other proteins that regulate the function of junctional proteins (33). The use of HUVEC, a macrovascular type of EC, as well as limited pharmacological studies with NADPH oxidase inhibitors, may explain the different outcomes compared with the present study.

Our current results indicate an unrecognized differential effect of the NADPH oxidase system from AM vs. PMVEC. This observation is consistent with our previous findings of similar cell source-specific effects of iNOS-derived NO specifically from neutrophils and AM in both septic ALI in vivo and septic PMVEC injury in vitro, with no obvious contribution of iNOS-derived NO from pulmonary parenchymal cells, such as EC, to septic ALI (36, 50). In the present study, the observed differential effects of NADPH oxidase in AM vs. PMVEC in coculture in vitro suggest biological importance, but the actual relevance in a disease model in vivo needs to be further studied. Moreover, this cell-source differential effect of NADPH oxidase may reflect differences in the localization of subcellular ROS release and physicochemical microenvironmental variations in our coculture system.

Although our data supports the hypothesis that AM-dependent PMVEC oxidant stress and injury under septic conditions were largely dependent on NADPH oxidase, other AM sources of ROS, such as xanthine oxidase and the mitochondrial electron transfer chain (13), also may contribute. Indeed, some degree of PMVEC lipid peroxidation was still observed in septic AM-PMVEC cocultures in the presence of p47phox−/− or gp91phox−/− AM, suggesting an NADPH oxidase-independent oxidant effect of AM. However, these other sources of oxidant stress did not appear to contribute significantly to PMVEC injury, as reflected by septic PMVEC barrier dysfunction, which was completely AM NADPH oxidase dependent.
We recognize the limitation of our in vitro model to mimic sepsis, specifically a fixed-dose combination of cytomix, which cannot capture the in vivo complexity of timing and levels of the wide array of cytokines present during human sepsis. However, the three cytokines we used contribute importantly to the pathogenesis of sepsis and septic ALI and are widely used for the in vitro modeling of sepsis (34). Moreover, we have focused on microvascular and EC injury as key features of septic ALI but recognize that our findings do not necessarily relate to the pathophysiology of other types of direct ALI, such as acid aspiration. Indeed, indirect and direct ALI are increasingly recognized to differ importantly pathophysiologically and likely therapeutically (1, 46). Another limitation is the lack of data from direct measurement of AM-dependent ROS production to support our contention of a role for AM NADPH oxidase. However, in pilot studies, we were unable to consistently observe a cytomix-induced murine AM-dependent ROS signal using several different published approaches, despite clear positive controls. Of relevance, there are few reliable data to support ROS production by murine AM, although significant ROS release has been described clearly for other monocyte/macrophage populations, including from peripheral blood and peritoneum.

In summary, through the use of pharmacological inhibitors and NADPH oxidase null cells, we have shown that AM-dependent PMVEC injury under septic conditions is specifically mediated through AM NADPH oxidase, with a negligible contribution from PMVEC NADPH oxidase. In addition, AM-dependent PMVEC injury is associated with significant oxidant stress, including protein oxidation and lipid peroxidation, which is also AM NADPH oxidase dependent. Thus our studies suggest that AM NADPH oxidase may contribute directly to the oxidant stress and pulmonary microvascular injury characteristic of septic ALI. As such, inhibition of AM NADPH oxidase may be a potential therapeutic option in the treatment of septic ALI in humans.

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