**ExoU-induced redox imbalance and oxidative stress in airway epithelial cells during \textit{Pseudomonas aeruginosa} pneumosepsis**

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**Abstract** ExoU is a potent proinflammatory toxin produced by \textit{Pseudomonas aeruginosa}, a major agent of severe lung infection and sepsis. Because inflammation is usually associated with oxidative stress, we investigated the effect of ExoU on free radical production and antioxidant defense mechanisms during the course of \textit{P. aeruginosa} infection. In an experimental model of acute pneumonia, ExoU accounted for increased lipid peroxidation in mice lungs as soon as 3 h after intratracheal instillation of PA103 \textit{P. aeruginosa} strain. The contribution of airway cells to the generation of a redox imbalance was assessed by in vitro tests carried out with A549 airway epithelial cells. Cultures infected with the ExoU-producing PA103 \textit{P. aeruginosa} strain produced significantly increased concentrations of lipid hydroperoxides, 8-isoprostane, reactive oxygen intermediates, peroxynitrite and nitric oxide (NO), when compared to cells infected with \textit{exoU}-deficient mutants. Overproduction of NO by PA103-infected cells likely resulted from overexpression of both inducible and endothelial NO synthase isoforms. PA103 infection was also associated with a significantly increased activity of superoxide dismutase (SOD) and decreased levels of reduced glutathione (GSH), a major antioxidant compound. Our findings unveil another potential mechanism of tissue damage during infection by ExoU-producing \textit{P. aeruginosa} strains.

**Keywords** ExoU · Oxidative stress · Lipid peroxidation · NO synthase · Pneumosepsis · \textit{Pseudomonas aeruginosa}

**Introduction**

\textit{Pseudomonas aeruginosa} is a major agent of life-threatening pneumonia in hospitalized patients. The poor outcome of these individuals usually correlates with the development of sepsis, a clinical syndrome characterized by the excessive release of proinflammatory mediators, activation of different cell types and increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1]. In lungs, excessive neutrophil recruitment and accumulation are associated with increased permeability of the capillary/alveolar barrier, lung tissue injury and impaired oxygenation [2].

Under physiological conditions, a homeostatic balance exists between the formation of ROS and RNS, and their removal by endogenous antioxidant scavenging compounds [3]. Oxidative stress occurs when this balance is disrupted by excessive production of oxidative agents and/or by overcoming antioxidant defense mechanisms. ROS and RNS are major mediators of cellular injury via peroxidation of polyunsaturated lipids from biomembranes and subcellular organelles. Breakdown products of lipid peroxides, mostly reactive aldehydes, may serve as oxidative second messengers due to their prolonged half-life and ability to diffuse from their site of formation, compared to free radicals. Lipid breakdown products can also make covalent modifications on nucleic acids and proteins and dramatically alter cell integrity [4]. Among other effects, peroxidation of membrane...
lipids represents a major threat to cellular integrity, once protein oxidation can alter metabolic function, and oxidative damage to DNA can impair cell division and repair.

The pathogenesis of *P. aeruginosa* infections involves a number of virulence factors, including ExoU, a potent cytotoxin with phospholipase A₂ (PLA₂) activity that is injected into host cytosol through the type III secretion system. Upon injection, ExoU is activated by host cell cofactors [5–7] and targeted to the plasma membranes, where it cleaves membrane phospholipids, resulting in rapid lysis of a variety of cell types [8]. ExoU PLA₂ activity also accounts for a potent stimulation of inflammatory response and increased release of free arachidonic acid, cytokines, eicosanoids and PAF from infected cells [9–11], as well as for the activation of the NF-κB transcription factor via a PAFR signaling pathway [12, 13]. The proinflammatory effect of ExoU has been extensively confirmed in experimental models of pneumosepsis in which *P. aeruginosa* inoculation into mice airways resulted in a rapid influx of large numbers of inflammatory cells, primarily neutrophils, into the lungs [9, 11–16].

Activated phagocytic cells form the majority of ROS and RNS produced during the inflammatory response. Furthermore, endothelial and airway epithelial cells may also contribute to the generation of a local state of oxidative stress in an inflammatory environment, as documented for the respiratory tract exposed to different stimuli [17–19]. However, there are no studies demonstrating that ExoU is capable to induce oxidative stress in airway epithelial cells.

In the present study, we addressed the question of whether ExoU would induce a state of oxidative stress in lungs of *P. aeruginosa*-infected mice and whether airway cells would contribute to such redox imbalance.

### Materials and methods

#### Bacterial strains and culture conditions

The ExoU-producing PA103 *P. aeruginosa* strain and the *exoU*-deficient PA103ΔexoU mutant [9] were used throughout this study. In some assays, the PA103ΔUT/S142A strain was used to confirm the contribution of the PLA₂ activity in the phenomenon under evaluation. PA103ΔUT/S142A, an *exoU*-depleted mutant complemented with an *exoU* gene with a site-specific mutation in the PLA₂ catalytic site, was kindly furnished by Dr. A. Hauser (Northwestern University, USA). Bacteria were grown in Luria-Bertani broth at 37 °C for 14–16 h under moderate agitation, harvested by centrifugation and resuspended in lipopolysaccharide-free saline or in F12 nutrient culture medium (Invitrogen) supplemented with 10 % fetal calf serum and glutamine (complete culture medium).

#### Mice infection

Female Swiss mice (8–12 weeks old) anesthetized with a mixture of ketamine (65 mg/kg) and xylazine (13 mg/kg) were infected intratracheally with 10⁴ colony-forming units of PA103 or PA103ΔexoU in 50 μL of lipopolysaccharide-free saline. As control, mice were instilled with saline only. At 3 h post-infection, animals were euthanized and their lungs were removed and frozen in liquid nitrogen. All animal experiments were approved by the Animal Ethics Research Committee of the State University of Rio de Janeiro (CEUA/022/2011) and performed in accordance with the guidelines of this committee.

#### Assessment of lipid peroxidation in mice lungs

Lipid peroxidation in mice lungs was assessed by the determination of their content of thiobarbituric acid reactive species (TBARS). Briefly, mice lungs were homogenized in cold phosphate buffer, pH 7.4 with 0.2 % BHT (2,6-di-tert-butyl-4-methylphenol). Samples (0.5 ml) were mixed with equal volume of 0.67 % thiobarbituric acid (Sigma-Aldrich) and then heated at 96 °C for 30 min. TBARS were determined by the absorbance at 535 nm.

#### Cell culture and infection

Human airway epithelial cells from the A549 line (purchased from the Rio de Janeiro Cell Bank) were cultured in F12 nutrient complete culture medium containing antibiotics. Confluent cultures were trypsinized, and cells were suspended in complete medium with antibiotics, seeded in 6-well (8.2 × 10⁵ cells/well), 24-well (1.7 × 10⁵ cells/well), 96-well (3.0 × 10⁴ cells/well) tissue culture plates or 25-cm² tissue culture flask (1.12 × 10⁶ cells/flask) and incubated at 37 °C for 48 h. Cells were then infected with *P. aeruginosa* strains at a multiplicity of infection of about 100. Since translocation of effector proteins from the type III secretory system depends on close contact between bacteria and host cells, bacteria were centrifuged (1,000×g for 10 min at 4 °C) onto the cultured cells. Control cultures were exposed to culture medium only. After incubation for 1 h at 37 °C, cell cultures were treated with gentamicin at 300 μg/ml in complete culture medium to kill extracellular bacteria and incubated for the remaining time needed to complete 6, 24 or 48 h.

#### Analysis of lipid peroxidation, catalase activity and SOD activity in cell lysates

Cells were cultured in 6-well or 24-well tissue culture plates or in 25-cm² tissue culture flasks to assess lipid peroxidation, catalase and superoxide dismutase (SOD) activity,
respectively, and infected with the different bacterial strains for 6, 24 or 48 h. Infected and noninfected control cells were washed with phosphate-buffered saline pH 7.4 (PBS), scraped from the culture plates, centrifuged at 1260×g for 10 min at 15 °C, resuspended in chilled ultrapure water (LPO), SOD buffer (20 mM Hepes pH 7.2, 1 mM EGTA, 210 mM mannitol, 70 mM sucrose) or catalase buffer (50 mM potassium phosphate pH 7.0, 1 mM EDTA) at a final concentration of 3 × 10⁶ cells/ml (LPO) or 1.5 × 10⁷ cells/ml (SOD and catalase) and lysed by 4 short burst of 30 s with a CPX-130 Cole-Parmer sonicator. Cell lysates were centrifuged (10,000×g for 15 min at 4 °C), and the supernatants were used for the different analysis using the Calbiochem Lipid Hydroperoxide Assay Kit and the Cayman Chemical commercial kits Superoxide Dismutase Assay and Catalase Assay, according to the manufacturer instructions.

**Quantification of 8-isoprostone**

Cells cultured in 24-well tissue culture plates were infected for 6, 24 and 48 h. The supernatants from infected and noninfected control cultures were recovered and analyzed with the Cayman Chemical 8-Isoprostane EIA Kit. In parallel, the number of cells excluding the trypan blue dye in infected and noninfected cultures was determined by counting trypsinized cells in Neubauer chambers, in order to normalize the levels of 8-isoprostane by the number of viable cells.

**Quantification of glutathione (GSH)**

Cells cultured in 96-well tissue culture plates were infected and the GSH levels were measured using a luminescence-based assay (GSH-Glo™ Glutathione Assay, Promega), according to the manufacturer’s instructions.

**Determination of ROS, NO and peroxynitrite production**

Cells were seeded in black 96-well tissue culture plate, cultured overnight in complete F12 medium, washed three times with PBS, incubated with serum-free Hanks balanced salt solution for 1 h and loaded for more 1 h at 37 °C with 5 μM of the Molecular Probes products 5-(6)-chloromethyl-2,7-dichlorodihydrofluorescein (CM-H₂DCF-DA), 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM-DA) or 3'-(p-hydroxyphenyl) fluorescein (HPF) to assess ROS, NO and peroxynitrite production, respectively. After probe load, cells were washed and infected with bacterial suspensions for different periods. Control cells were treated with culture medium only. Fluorescence emitted by each probe was measured in the microplate reader Envision™ (PerkinElmer).

**Western blot**

Western blot was performed for the detection of iNOS, eNOS and nNOS in extracts from control or infected A549 cells. Briefly, 35 μg of protein from the different cell extracts was separated by SDS-polyacrylamide gel electrophoresis on 10 % acrylamide gel and transferred to nitrocellulose membranes. Membranes were blocked with 5 % fat-free milk at room temperature for 1 h and then incubated overnight at 4 °C with appropriate Santa Cruz Biotechnology primary antibodies against β-actin, iNOS (sc-651), eNOS (sc-654) and nNOS (sc-648) in 5 % milk in Tris-buffered saline (TBS). After being washed with TBS containing 0.05 % Tween 20 (TTBS), membranes were incubated for 1 h with appropriate secondary antibody at room temperature, washed and treated with chemiluminescence reagents (ECL Plus) for 5 min. The target bands were then imaged on a ChemiDoc™ XRS and analyzed with the Image Lab™ software. The expression level of the β-actin housekeeping gene was used for equal loading control in all experiments.

**Statistical analysis**

The results were expressed as the mean ± standard error of the means obtained in at least three different experiments. Statistical analysis was performed using a one-way analysis of variance (ANOVA) for multiple group analysis with a Bonferroni adjustment to determine significant statistical differences between groups. p < 0.05 was taken as statistically significant.

**Results**

**ExoU induced a state of oxidative stress in lungs of P. aeruginosa-infected mice**

Because ROS have extremely short half-lives, they are difficult to measure directly. Therefore, the existence of oxidative stress in mice lungs was assessed by the TBARS assay that measures malondialdehyde, an end product formed during lipid peroxidation [20]. As shown in Fig. 1, as soon as 3 h after bacteria inoculation, malondialdehyde concentration in lungs of PA103-infected mice was significantly increased, when compared to concentrations in lungs of control and PA103ΔexoU-infected mice.

**Airway A549 cells injected with ExoU exhibited evidences of oxidative stress**

Three different approaches were used to ascertain the ability of airway epithelial cells to produce increased amounts of oxidizing agents in response to ExoU: (1) determination
of lipid hydroperoxide concentration in cell lysates; (2) evaluation of 8-isoprostane concentration in cell culture supernatants [21] and (3) evaluation of ROS, NO and peroxynitrite levels in control and infected cell cultures.

PA103-infected cells produced increased concentration of lipid hydroperoxides (Fig. 2a) and 8-isoprostane (Fig. 2b), a prostaglandin-like compound produced by free radical-catalyzed peroxidation of fatty acids, which has been used as an accurate marker of lipid peroxidation in both animal and human models of oxidative stress [21, 22]. These effects were significantly reduced in PA103ΔexoU-infected cells.

PA103-infected cells also produced increased concentrations of ROS, NO and peroxynitrite, when compared to control noninfected cells (Fig. 3), which were also significantly higher than the increase detected in cells infected with the exoU-deficient mutant or with the mutant complemented with ExoU with a site-directed mutated catalytic motif. Significantly increased production of ROS was detected very early, at 10 min after infection, and levels remained elevated for as long as 120 min (data not shown).

NO production by PA103-infected cells is likely to result from overexpression of both eNOS and iNOS. Indeed, the eNOS level in PA103-infected cells was about 150% the one detected in control cells, while the iNOS level was increased in about 1000%. Levels of eNOS and iNOS expression in PA103ΔexoU-infected cells were similar to those found in control noninfected cells (Fig. 4). No difference was detected in the expression of nNOS in the infected cells, when compared to the expression in control noninfected cells (data not shown).

**Effect of ExoU on A549 cell antioxidant defense mechanisms**

At 48 h post-infection, PA103-infected cells exhibited a significant increase in SOD activity (Fig. 5a) and...
reduction in GSH levels (Fig. 5b) compared to noninfected or PA103 ΔexoU-infected cells. No significant difference was detected in catalase activity in control or infected cells (data not shown).

Discussion

Bacterial infection of the lungs is accompanied by an influx of leukocytes, which are stimulated to produce inflammatory mediators, superoxide and hydrogen peroxide, a more stable and membrane-permeable product. Both superoxide and hydrogen peroxide are toxic to cells in the vicinity. Myeloperoxidase is very abundant in activated leukocytes and converts hydrogen peroxide into hypochlorous acid, a more powerful oxidant agent. In this context, oxygen metabolites may lose their physiological role in the killing of pathogens, to turn into toxic effectors responsible for the damage of the pulmonary epithelium, as well as of other components of the lung parenchyma and lining fluids [23]. In consequence, leukocyte overproduction of oxidants within lungs contributes greatly to the pathophysiology of acute respiratory distress syndrome (ARDS), a life-threatening complication of severe pneumonia with high mortality rates [2]. Besides ROS production, neutrophil upregulated expression of iNOS, which accounts for enhanced production of NO, contributes also to exacerbating oxidative stress indices and to ARDS pathophysiology [24].

Lung infection by ExoU-producing P. aeruginosa is accompanied by a robust infiltration of inflammatory cells consisting primarily of neutrophils [9, 11–13]. However, this highly cytotoxic toxin kills both resident alveolar macrophages and recruited neutrophils, thereby interfering with the clearance of bacteria from infected lungs [14, 15]. Whether ExoU-injected phagocytic cells could still release reactive oxygen and nitrogen species, and potentially contribute to the damage of lung parenchyma, was yet unknown. In the present study, designed to investigate whether acute pneumonia by ExoU-producing P. aeruginosa is associated with oxidative stress in infected lungs, we show that ExoU accounted for increased lipid peroxidation in mice lungs as soon as 3 h after intratracheal instillation of bacteria, testifying the association between this P. aeruginosa toxin and the occurrence of oxidative stress.

A described consequence of oxidative stress in lungs is the enhancement of the airspace epithelial permeability, secondary to increased epithelial cell detachment, decreased cell adherence and increased cell lysis [25]. Increased epithelial permeability favors the dissemination of bacterial into host bloodstream [26, 27]. Indeed, in the course of lung infection by a cytotoxic ExoU-producing P. aeruginosa strain, bacteria were shown to cause alveolar epithelial injury and progressive dissemination from infected lungs as soon as 4 h after intratracheal instillation [28]. Interestingly, in a more recent study, only relatively few alveolar epithelial cells were shown to be injected with
ExoU [14]. Therefore, it seems unlikely that alveolar epithelial injury has resulted from a direct effect of ExoU, but it is plausible to assume that the increased epithelial permeability detected in mice with pneumonia by ExoU-producing bacteria has resulted from a disequilibrium between pro-oxidant and antioxidant molecules in infected lungs.

During pulmonary infections, the majority of oxidants in lungs arise from stimulated phagocytic cells that can generate toxic oxygen metabolites from assembly on cell surfaces of NADPH oxidase (also known as NOX2) [23]. Similar pathways leading to oxidant generation can occur in endothelial and alveolar epithelial cells [23]. A recent study revealed that, in later periods of infection, P. aeruginosa was able to induce NOX2 and NOX4 expression, but not NOX1 and NOX3, in mice lungs. NF-κB was critical to the expression of both NOX1 and NOX3, but NOX2 and NOX4 seemed to have distinct roles in P. aeruginosa pathogenesis, with NOX4 involved in apoptosis and disruption of endothelial barrier integrity and NOX2 in inflammation and oxidative response to the bacteria [29]. Furthermore, it has been shown that, in pulmonary endothelial and phagocytic cells, activation of NOX2 depends on the PLA2 activity of peroxiredoxin 6 [30, 31]. Since ExoU has PLA2 activity and activates NF-κB, we hypothesized whether this virulence factor would be able to interfere with the production of oxidants by airway epithelial cells. In agreement with our hypothesis, PA103-infected A549 cells produced increased amounts of lipid hydroperoxide, 8-isoprostane, ROS, NO and peroxynitrite, a strong oxidant that results from the reaction of superoxide and NO.

It is commonly believed that excessive NO production, critically implicated in the pathophysiology of sepsis and ARDS, results from the activity of the iNOS isoform. However, increasing evidence demonstrates the contribution of
constitutively expressed NOS isoforms to NO overproduction [32, 33]. In our study, although airway epithelial cells infected with the ExoU-producing bacteria exhibited enhanced levels of eNOS, expression of iNOS was increased in about 1000 % at as early as 1 h after infection. A similar increase in iNOS and eNOS was reported by Lange et al. [34] in a ovine model of \( P. \) aeruginosa pneumosepsis. The novelty of our study is the demonstration of the precocity of iNOS expression in cells infected with the ExoU-producing bacteria, which likely stems from the ExoU-triggered NF-\( \kappa \)B activation by the canonical pathway [13].

Besides its association with extensive injury of different targets, oxidizing agents can function as cellular signaling molecules influencing a variety of molecular and biochemical processes, including the expression of proinflammatory mediators through the activation of transcription factors [35, 36]. During \( P. \) aeruginosa pneumonia, \( gp91^{{phox}} \) mice showed significantly lower concentrations of \( H_2O_2 \), IL-6 and TNF-\( \alpha \) in the bronchoalveolar lavage than wild-type mice [29]. Although ExoU activates NF-\( \kappa \)B via the PAF–PAFR signaling [13], the increased generation of oxidizing agents is likely to further enhance the NF-\( \kappa \)B transcriptional activity and upregulate a number of genes involved in inflammation [37].

Defenses against toxic oxygen metabolites in lung include antioxidant enzymes whose intrapulmonary levels can be greatly elevated when the lung faces a large burden of oxidants that compromise its redox balance. Indeed, enzymes such as SOD can be induced in lung by hyperoxia, bacterial LPS and virus, to name just a few examples [38, 39]. In the present study, infection by ExoU-producing \( P. \) aeruginosa resulted in significant increase in the activity of SOD, a group of enzymes that convert superoxide anion to \( H_2O_2 \). Catalase and glutathione peroxidase exert their antioxidant activity by converting \( H_2O_2 \) to water and oxygen. In our study, the increase in SOD activity together with the decreased levels of GSH suggest that infection by ExoU-producing bacteria likely resulted in enhanced intracellular \( H_2O_2 \) production that was at least partially detoxified by glutathione peroxidase.

In conclusion, our findings demonstrated the ExoU potential to induce ROS production and disrupt the pro-oxidant–antioxidant balance in favor of the former. The oxidative damage of infected lungs is certainly an important contribution of ExoU to the pathogenesis of \( P. \) aeruginosa pneumonia.

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References

1. Rocha M, Herance R, Rovira S, Hernández-Mijares A, Victor VM (2012) Mitochondrial dysfunction and antioxidant therapy in sepsis. Infect Disord Drug Targets 12:161–178
2. Günther A, Walmrath D, Grimminger F, Seeger W (2001) Pathophysiology of acute lung injury. Semin Respir Crit Care Med 22:247–258
3. Gutteridge JM, Mitchell J (1999) Redox imbalance in the critically ill. Br Med Bull 55:49–75
4. Guéraud F, Atalay M, Bresgen N, Cipak A, Eckl PM, Huc L et al (2010) Chemistry and biochemistry of lipid peroxidation products. Free Radic Res 44:1098–1124
5. Sato H, Feix JB, Frank DW (2006) Identification of superoxide dismutase as a cofactor for the Pseudomonas type III toxin, ExoU. Biochemistry 45:10368–10375
6. Anderson DM, Schmalzer KM, Sato H, Casey M, Terhune SS, Tyson GH, Hauser AR (2013) Phosphatidylinositol 4,5-bisphosphate is a novel coactivator of the Pseudomonas aeruginosa cytoxin ExoU. Mol Microbiol 82:1454–1467
7. Tyson GH, Hauser AR (2013) Phosphatidylinositol 4,5-bisphosphate is a novel coactivator of the Pseudomonas aeruginosa cytoxin ExoU. Infect Immun 81:2873–2881
8. Sato H, Frank DW (2004) ExoU is a potent intracellular phospholipase. Mol Microbiol 53:1279–1290
9. Saliba AM, Nascimento DO, Silva MCA, Assis MC, Gayer B activa-
10. Rahman I, MacNee W (1998) Role of transcription factors in inflammation. Thorax 53:601–612
11. Machado GB, de Assis MC, Leão R, Saliba AM, Silva MC, Suassuna JH et al (2010) ExoU-induced vascular hyperpermeability and platelet activation in the course of experimental Pseudomonas aeruginosa pneumosepsis. Shock 33:315–321
12. Rochelle LG, Fischer BM, Adler KB (1998) Concurrent production of reactive oxygen and nitrogen species by airway epithelial cells in vitro. Free Radic Biol Med 15:863–868
13. Leonarduzzi G, Arkan MC, Başağa H, Chiarpotto E, Sevanian A, Poli G (2000) Lipid oxidation products in cell signaling. Free Radic Biol Med 28:1370–1378
14. Chanock S, Spivack S, Mossman BT (1994) Regulation of anti-
15. Xue LF, Qu JM, Li HP (2011) N-acetylcysteine modulates acute lung injury induced by Pseudomonas aeruginosa in rats. Clin Exp Pharmacol Physiol 38:345–351
16. Lange M, Connelly R, Traber DL, Hamahata A, Esechie A, Jonkam C et al (2010) Role of different nitric oxide synthase isoforms in a murine model of acute lung injury and sepsis. Biochem Biophys Res Commun 399:286–291
17. Lane M, Nakan Y, Traber DL, Hamahata A, Esechie A, Jonkam C et al (2010) Role of different nitric oxide synthase isoforms in a murine model of acute lung injury and sepsis. Biochim Biophys Acta 1800:1422–1433
18. Leau R, de Assis MC, Saliba AM, Silva MC, Suassuna JH et al (2010) ExoU-induced vascular hyperpermeability and platelet activation in the course of experimental Pseudomonas aeruginosa pneumosepsis. Shock 33:315–321
19. Rochelle LG, Fischer BM, Adler KB (1998) Concurrent production of reactive oxygen and nitrogen species by airway epithelial cells in vitro. Free Radic Biol Med 15:863–868
20. Del Rio D, Stewart AJ, Pellegrini N (2005) A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis 15:316–328
21. Janssen LJ (2001) Isoprostanes: an overview and putative roles in pulmonary pathophysiology. Am J Physiol Lung Cell Mol Physiol 280:L1067–L1082
22. Romero PV, Rodríguez B, Martínez S, Cañizares R, Sepúlveda D, Manresa F (2006) Analysis of oxidative stress in exhaled breath condensate from patients with severe pulmonary infections. Arch Bronconeumol 2:113–119
23. Ward PA (2010) Oxidative stress: acute and progressive lung injury. Ann N Y Acad Sci 1203:53–59
24. Wang L, Taneja R, Razavi HM, Law C, Gillis C, Mehta S (2012) Specific role of neutrophil inducible nitric oxide synthase in murine sepsis-induced lung injury in vivo. Shock 37:539–547
25. MacNee W (2001) Oxidative stress and lung inflammation in airways disease. Eur J Pharmacol 429:195–207
26. Zhang ZQ, Song YL, Chen ZH, Shen Y, Bai CX (2011) Deletion of aquaporin 5 aggravates acute lung injury induced by Pseudomonas aeruginosa. J Trauma 71:1305–1311
27. Junkins RD, Carrigan SO, Wu Z, Stadnyk AW, Cowley E, Issekutz T et al (2014) Mast cells protect against Pseudomonas aeruginosa-induced lung injury. Am J Pathol 184:2310–2321
28. Kurahashi K, Kajikawa O, Sawat T, Ohara M, Gropper MA, Frank DW et al (1999) Pathogenesis of septic shock in Pseudomonas aeruginosa pneumonia. J Clin Invest 104:743–750
29. Fu P, Mohan V, Mansoor S, Tiruppathi C, Sadikot RT, Natarajan V (2013) Role of nicotinamide adenine dinucleotide phosphate-reduced oxidase proteins in Pseudomonas aeruginosa-induced lung inflammation and permeability. Am J Respir Cell Biol 48:477–488
30. Chatterjee S, Feinstein SI, Dodia C, Sorokina E, Lien YC, Nguyen S et al (2011) Peroxiredoxin 6 phosphorylation and subsequent phospholipase A2 activity are required for agonist-mediated activation of NADPH oxidase in mouse pulmonary microvascular endothelium and alveolar macrophages. J Biol Chem 286:11696–11706
31. Ellison MA, Thurman GW, Ambroso DR (2012) Phox activity of differentiation of PLB-985 cells is enhanced, in an agonist specific manner, by the PL2A activity of Prdx9-PLA2. Eur J Immunol 42:1609–1617
32. Lange M, Nakano Y, Traber DL, Hamahata A, Esechie A, Jonkam C et al (2010) Role of different nitric oxide synthase isoforms in a murine model of acute lung injury and sepsis. Biochem Biophys Res Commun 399:286–291
33. Xue LF, Qu JM, Li HP (2011) N-acetylcysteine modulates acute lung injury induced by Pseudomonas aeruginosa in rats. Clin Exp Pharmacol Physiol 38:345–351
34. Lange M, Connelly R, Traber DL, Hamahata A, Nakano Y, Esechie A et al (2010) Time course of nitric oxide synthases, nitrosative stress, and poly(ADP ribosylation) in an ovine sepsis model. Crit Care 14:R129
35. Rahman I, MacNee W (1998) Role of transcription factors in inflammatory lung diseases. Thorax 53:601–612
36. Leonarduzzi G, Arkan MC, Basaga H, Chiarpotto E, Sevanian A, Poli G (2000) Lipid oxidation products in cell signaling. Free Radic Biol Med 28:1370–1378
37. Guo RF, Ward PA (2007) Role of oxidants in lung injury during sepsis. Antioxid Redox Signal 9:1991–2002
38. Quinlan T, Spivack S, Mossman BT (1994) Regulation of anti-
39. Hosakote YM, Liu T, Castro SM, Garofalo RP, Casola A (2009) Respiratory syncytial virus induces oxidative stress by modulating antioxidant enzymes. Am J Respir Cell Mol Biol 41:348–357