Interleukin-1-mediated Acute Lung Injury and Tolerance to Oxidative Injury

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Interleukin-1 (IL-1) is a highly potent molecule that has a myriad of effects in biologic systems. This brief review describes some of our findings on the effects of IL-1 in biologic systems. On the one hand, IL-1 treatment caused a neutrophil-dependent acute edematous lung injury that resembled changes in the lungs of patients with the acute respiratory distress syndrome (ARDS). On the other hand, IL-1 pretreatment conferred a tolerance to lung oxidative lung injury and ischemia-reperfusion insults—conditions manifest in sick patients. The potential mechanisms responsible for these seemingly paradoxical influences of IL-1 are described and related to possible strategies for the treatment of patients with ARDS, ischemia-reperfusion disorders, and other oxidant-mediated conditions. — Environ Health Perspect 102(Suppl 10):75-78 (1994)

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Introduction

A delicate balance exists between oxidants and antioxidants in health and disease. This balance may be especially relevant to lung physiology and pathophysiology. This report briefly addresses the effects of interleukin-1 (IL-1) on this pivotal balance. We examine both beneficial and detrimental effects of IL-1 on oxidant-antioxidant systems. As part of the symposium on the role of oxidants and antioxidants, this article focuses largely on reviewing work that we presented at the conference; the research of other investigators will be presented first hand elsewhere.

II-1-mediated Acute Lung Injury

Acute noncardiogenic edematous lung injury (often called the adult respiratory distress syndrome or ARDS) is a highly fatal lung disorder that occurs after a variety of pulmonary and nonpulmonary insults (1). The etiology of ARDS is largely unknown and, accordingly, no specific therapy is available for this devastating and perplexing condition.

Considerable evidence has been compiled, however, that supports the hypothesis that oxidants contribute to the development of ARDS. I will briefly summarize these findings:

• ARDS patients have increased numbers of neutrophils in their lungs that are powerful generators of O$_2^.$ radicals in vitro (2).
• ARDS patients have numerous factors in their blood or lung lavages that increase neutrophil and alveolar macrophage O$_2^.$ radical release in vitro (2,3).
• Neutrophils recovered from the blood or lungs of ARDS patients are either more or less active than neutrophils from control subjects—either case suggesting neutrophil activation (4,5).
• ARDS patients have increased levels of xanthine oxidase (XO) in their blood compared to that of control subjects (6).
• ARDS patients exhale increased amounts of hydrogen peroxide (7) and have increased levels of lipid peroxidation products (8-10) and oxidized antiproteases (11) in their blood compared to control subjects.
• Finally, ARDS patients have decreased blood levels of glutathione (GSH) (12), and, for unknown reasons, increased blood levels of manganese superoxide dismutase (MnSOD) and catalase (13).

IL-1 levels are substantially increased in lung lavages from ARDS patients compared to the negligible levels of IL-1 in lavages from control subjects (14,15). In addition, cultured alveolar macrophages (AM) recovered from ARDS patients release more IL-1 than AM from control subjects (16). Combined with the effects of IL-1 on neutrophils in vitro, these observations lead us to propose that increased intracellular levels of IL-1 could cause acute lung injury by a mechanism involving neutrophils and neutrophil-derived oxidants. We proceeded to test this hypothesis by administering IL-1 intratracheally to rats. Our experimental protocol involved intratracheal insufflation of 50 ng of IL-1—a dose comparable to levels measured in lavages from ARDS patients. Approximately 4.5 hr later, $^{125}$-labeled albumin was injected iv; 30 min later (5 hr after IL-1 administration) rats were sacrificed and the lungs were removed, perfused blood-free, and lavaged. The ratio of the $^{125}$-labeled albumin recovered in lung lavages to $^{125}$-labeled albumin concentrations in blood was used as a measure of lung injury. The number of neutrophils in lung lavages was assessed by direct counts and measurement of myeloperoxidase (MPO) activity (17). For some experiments, neutrophils were depleted from the blood by prior treatment with vinblastine.

We found that rats given intratracheal IL-1 had increased lung leak and lung neutrophils compared to that of control rats (Figure 1) (18). Lung leak and lung neutrophil accumulation were both dependent on the dose of IL-1 given, with approximately 50 ng causing an optimal response. In contrast to native IL-1, administration of previously heated IL-1 did not increase lung neutrophils or lung leak. Histologic examination of lungs given IL-1 revealed an increased lung cellularity involving neutrophils and an abnormal edematous process characterized by perivascular cuffing.

To determine the contribution of neutrophils to lung leak, we made rats neutropenic by giving vinblastine 4 days before IL-1 administration (18). Rats made severely neutropenic with vinblastine and then given IL-1 intratracheally had considerably less lung leak than control rats or rats given vinblastine just 1 day before IL-1—a regimen that undoubtedly has many of the systemic effects of vinblastine but does not decrease circulating neutrophil counts.

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These studies suggest that neutrophils contribute to the development of lung leak in rats given IL-1 intratracheally.

A number of studies (19–23) were conducted using interventions to ascertain the mechanism of injury following IL-1 administration (Figures 2, 3). First, and not surprisingly, we found that pretreatment with the IL-1 receptor antagonist (IL-1ra) completely prevented lung leak and lung neutrophil influx in rats given IL-1 intratracheally (19). Second, treatment with a variety of antioxidant agents reduced lung leak and lung neutrophil accumulation. Reduction of lung leak occurred in rats treated with N-acetylcysteine (NAC), an agent that increases cellular GSH levels (20); dimethyl sulfoxide (DMSO), a scavenger of hydroxyl radical (‘OH) in vitro (18); and manganese-superoxide dismutase (MnSOD), a scavenger of O$_2^-$ in vitro (18). Third, treatment with liposomal encapsulated prostaglandin E$_1$ (PGE$_1$), but not empty liposomes or free PGE$_1$, also decreased leak in lungs of rats given IL-1 intratracheally (21). Rats given IL-1 and then treated with IL-1ra, DMSO, Lip-PGE$_1$, NAC and MnSOD also had decreased numbers of lung neutrophils compared to control rats given IL-1 intratracheally (Figure 3). Finally, vitamin E, an inhibitor of lipid peroxidation in vitro, given by supercritical fluid aerosol technique (22,23), also decreased lung leak in rats given IL-1 intratracheally. However, vitamin E treatment did not decrease lung neutrophil accumulation (as assessed by lung MPO analyses) in rats given IL-1 intratracheally. Rats given IL-1 intratracheally also exhaled more H$_2$O$_2$ and developed greater lung oxidized glutathione (GSSG) concentrations than untreated control rats. Treatment with NAC, DMSO, and Mn-SOD reduced both H$_2$O$_2$, exhalation and lung GSSG levels in rats given IL-1 intratracheally (18).

These findings in intact rats were corroborated by recent experiments in isolated saline-perfused rat lungs (24,25). Briefly, we found that intratracheal administration of native, but not heated, IL-1 (or IL-8) caused lung injury (reflected by increased lung weights and lung Ficoll concentrations) in rats perfused with purified human neutrophils compared to lungs given only IL-1 (or IL-8), lungs perfused only with neutrophils, and untreated control lungs (24,25). The contribution of neutrophil-derived O$_2^-$ radicals was substantiated when neutrophils previously heated at 48°C for 10 min—a process that specifically inactivates NADPH-oxidase activity and neutrophil O$_2^-$ release—did not damage isolated lungs given IL-1 intratracheally. In parallel, heated neutrophils adhered and chemotaxed normally but did not make O$_2^-$ in vitro (24).

Taken en toto, these findings suggest that oxygen radicals from neutrophils contribute to acute edematous lung injury in rats given IL-1 intratracheally. Thus, IL-1 can have detrimental effects.

**IL-1-mediated Tolerance to Oxidative Injury**

The aforementioned section indicates that IL-1 has negative effects on the lung, notably increasing lung neutrophils and causing lung leak. This section focuses on the possibility that IL-1 can have a beneficial influence on oxidative lung injury by increasing lung antioxidant enzyme activity. The latter phenomenon is known as tolerance.

Frank and his colleagues made a remarkable discovery when they found that rats pretreated with endotoxin survived subsequent exposure to hyperoxia (>95% O$_2$), while untreated rats exposed to hyperoxia died within 72 hr (26). Endotoxin-induced protection against O$_2$ toxicity was associated with increases in lung antioxidant enzyme activity. Subsequently, we reasoned that because many of the effects of endotoxin are mediated by cytokines, such as tumor necrosis factor α (TNFα) and IL-1, then cytokines could also provide tolerance to oxygen toxicity. We found that rats pretreated with TNFα and IL-1 were completely resistant to oxygen toxicity (27,28).

Analogous to the situation following pretreatment with endotoxin, all rats pretreated with TNFα and IL-1 survived indefinitely (>144 hr) while untreated control rats invariably died within 72 hr of exposure to 100% O$_2$ (Figure 4). Moreover, pretreatment with TNFα and IL-1 prevented lung GSSG level increases that occurred in control rats subjected to hyperoxia (27). The latter finding indicates the antioxidant-enhancing effects of cytokine pretreatment. Subsequently, other investigators have confirmed these findings and documented that treatment with either TNFα or IL-1 alone is sufficient to develop tolerance (29,30). Furthermore, administration of IL-1 intratracheally induced tolerance, which was also associated with increases in lung antioxidant enzyme activity.

Although the mechanisms responsible for tolerance, as well as the causal relationship between tolerance and increases in lung antioxidant enzyme activity, are not yet clearly established, the following observations seem relevant (29–31):

- Hearts isolated from rats previously treated with endotoxin, IL-1 or TNFα resist I/R and have increased myocardial...
antioxidant enzyme activities (34–36). This generalizes the tolerance phenomena to another organ and another oxidative insult, I/R, which appears to involve xanthine oxidase (XO) activity.

- Six hours after IL-1 pretreatment, rat hearts developed a neutrophil-dependent, oxidative stress (increased myocardial GSSG levels). This low-grade insult was a necessary precursor for the subsequent development of IL-1-induced tolerance to I/R (Figure 5, 6) (35). This finding is consistent with the premise that a smaller antecedent oxidative stress can increase antioxidant enzyme activity and stimulate resistance to a larger, subsequent oxidative insult.

- Myriad studies using a variety of cultured cells, including lung endothelial cells, support these relationships in simpler in vitro systems (37–40). Moreover, there is new evidence that synergy may exist with respect to the induction of tolerance. For example, IL-6 facilitates IL-1-induced tolerance (41).

- The initial evidence linking tolerance with increases in antioxidant enzyme was the observation that endotoxin-pretreated rats given diethylthiocarbamate (DDC), a copper chelator and an inhibitor of Cu,Zn-SOD, were not resistant to oxygen toxicity (26). This finding established a role for Cu,Zn-SOD in endotoxin-induced tolerance to hyperoxic lung injury. A second link was revealed in rats given IL-1 (35). In this case, co-treatment with aminonicotinamide reduced glucose-6-phosphate dehydrogenase (G6-PD) enzyme activity increases and abrogated the associated protection to I/R in hearts from rats pretreated with IL-1.

- In all cases, tolerance only occurs 24 hr or more following treatment. Rats given endotoxin or IL-1 just 1 hr before or during the challenge were not resistant to I/R. This suggests that tolerance depends on new protein synthesis and thus distinguishes tolerance from the preconditioning response (42). In the preconditioning situation, brief episodes of I/R rapidly made hearts resistant to subsequent I/R episodes. The preconditioning phenomenon is also distinguishable from tolerance because it does not appear to involve increases in antioxidant enzyme activity.

In summary, there is substantial evidence in animal and in vitro systems that implicates a tolerance response involving, but perhaps not limited to, increases in antioxidant enzyme activity. This tolerance response could occur in some patients with ARDS. Notably, blood Mn-SOD and catalase activities were increased in septic patients who developed ARDS compared to both septic patients who did not develop ARDS and control subjects. It is not known why these levels were increased but if they do not simply reflect cell damage, they might reflect a tolerance reaction. Even though these responses did not prevent ARDS, they might have altered the course, frequency, or severity of the disease.

Alternatively, increased catalase or SOD activities may paradoxically increase lipid peroxidation (43,44) and contribute to tissue damage. Furthermore, it remains perplexing that only a small fraction of patients with apparently similar insults develop ARDS. Could this reflect tolerance, or perhaps an ineffective tolerance response, because of genetic or acquired circumstances?

**Conclusions**

This brief article presents two cases (Figure 7). The first implicates IL-1 as a cause of a neutrophil-dependent acute oxidative lung injury. The second points to IL-1 as a potential mediator of a tolerance process that confers protection against oxidant-induced lung injury, at least in part, by increasing lung antioxidant enzyme activity. When taken at face value, these two processes predict a conflicting set of events that are likely to significantly affect the course of ARDS. Additional information is necessary before the relative merits of these apparently competing systems can be understood and appreciated fully. Needless to say, this information should be obtained and would be extremely valuable for designing and evaluating the results of clinical trials that will use various antiendotoxin and anticytokine (e.g., IL-1ra, soluble TNFα receptors and CT1501R) therapies to treat patients with ARDS.

**REFERENCES**

1. Repine JE. Scientific perspectives on adult respiratory distress syndrome. Lancet 339:466–469 (1992).
2. Tate RM, Repine JE. Neutrophils and the adult respiratory distress syndrome. Am Rev Respir Dis 128:552–559 (1983).
3. Parsons PE, Fowler AA, Hyers TM, Henson PM. Chemoattractant activity in bronchoalveolar lavage fluid from patients with adult respiratory distress syndrome. Am Rev Respir Dis 132:490–493 (1985).
4. Zimmerman GA, Renzetti AD, Hill HR. Functional and metabolic activity
of granulocytes from patients with adult respiratory distress syndrome. Am Rev Respir Dis 127:290–300 (1983).

5. Martin TR, Pistoressu BP, Hudson LD, Mauner RJ. The function of lung and blood neutrophils in patients with the adult respiratory distress syndrome: implications for the pathogenesis of lung injury. Am Rev Respir Dis 144:254–262 (1991).

6. Grun CM, Ragdale RA, Ketai LH, Simon RH. Plasma xanthine oxidase activity in patients with ARDS. J Crit Care 2:22–26 (1987).

7. Baldwin SR, Simon RH, Grun CM, Boxer LA, Simon RH, Ketai LH, Duvall LT. Oxidant activity in expired breath of patients with adult respiratory distress syndrome. Lancet 1:111–14 (1986).

8. Deby C. Differences in tocopherol-lipid ratios in ARDS and non-ARDS patients. Inten Care Med 15:877–893 (1989).

9. Richard C, Lemonnier F, Thibault M, Couturier M, Auzepy P. Vitamin E deficiency and lipoperoxidation during adult respiratory distress syndrome. Crit Care Med 18:4–9 (1990).

10. Bertrand Y. Oxigen-free radicals and lipid peroxidation in adult respiratory distress syndrome. Inten Care Med 11:56–60 (1985).

11. Cochrane CG, Spragg R, Revak SD. Pathogenesis of the adult respiratory distress syndrome: evidence of oxidant activity in bronchoalveolar lavage fluid. J Clin Invest 71:754–758 (1983).

12. Bernard GR. N-acetylcysteine in experimental and clinical acute lung injury. Am J Med 90:Suppl:6C–98C (1991).

13. Leff JA, Parsons PE, Day CE, Taniguchi N, Jochum M, Fritz H, Moore FA, Moore EE, McCord JM, Repine JE. Serum antioxidants as predictors of the adult respiratory distress syndrome in septic patients. Lancet 341:777–780 (1993).

14. Siler TM, Swierkosz JE, Hyers TM, Fowler AA, Webster RO. Immunoreactive IL-1 in bronchoalveolar lavage fluid of high-risk patients and patients with the adult respiratory distress syndrome. Exp Lung Res 15(6):881–894 (1998).

15. Suter PM, Suter S, Girardin E, Roux-Lombard P, Grau GE, Dayer JM. High bronchoalveolar levels of tumor necrosis factor and its inhibitors, interleukin-1, interferon, and elastase, in patients with adult respiratory distress syndrome after trauma, shock, or sepsis. Am Rev Respir Dis 145:1016–1022 (1992).

16. Jacobs RF, Tabor DR, Burks AW, Campbell GD. Elevated interleukin-1 release by human alveolar macrophages during the adult respiratory distress syndrome. Am Rev Respir Dis 140:1687–1692 (1989).

17. Goldblum SE, Wu KM, Jay M. Lung myeloperoxidase as a measure of pulmonary leukotriase in rabbits. J Appl Physiol 17:1978–1985 (1985).

18. Leff JA, Baer JW, Bodman ME, Kirkman JM, Shanley PF, Patton LM, Guidot DM, Beehler CJ, McCord JM, Repine JE. Interleukin-1 α-induced lung neutrophil accumulation and oxygen metabolite mediated lung leak in rats. J Appl Physiol 88:2032–2036 (1995).

19. Leff JA, Bodman ME, Cho OJ, Rohrbach S, Reiss OK, Vannice JL, Repine JE. Post-insult treatment with interleukin-1 receptor antagonist decreases oxidative lung injury in rats given interleukin-1 intratracheally. Am J Physiol (in press).

20. Leff JA, Wilke CP, Hybertson BM, Shanley PF, Beehler CJ, Repine JE. Post-treatment with N-acetylcysteine decreases interleukin-1-induced lung neutrophil sequestration and oxidative lung leak in rats. Am J Physiol (Lung Cell Mol Physiol) 9:501–506 (1993).

21. Leff JA, Baer JW, Kirkman JM, Bodman ME, Shanley PF, Cho OF, Ostro MJ, Repine JE. Post-treatment with liposome-entrapped prostaglandin E decreases interleukin-1 α-induced neutrophil accumulation and lung leak in rats. J Appl Physiol 76:151–157 (1994).

22. Hybertson BM, Repine JE, Beehler CJ, Rutledge SK, Lagalante AF, Brock JW, Sievers RE. Delivery of fine aerosol particles of drugs and other species from superspicious fluid solutions. J Aeriol Med 6:275–286 (1993).

23. Hybertson BM, Leff JA, Beehler CJ, Barry PC, Repine JE. Effect of vitamin E deficiency and supercritical fluid aerosolized vitamin E supplementation on interleukin-1 induced oxidative lung injury in rats. Free Radic Biol Med (in press).

24. Guidot DM, Stevens EE, Repine MJ, Lucca-Broco AE, Repine JE. Intratracheal but not intravascular interleukin-1 causes acute edematous injury in isolated neutrophil-perfused rat lungs through an oxygen radical mediated mechanism. J Lab Clin Med 120:605–609 (1994).

25. Repine MJ, Guidot DM, Repine JE. Neutrophil derived NADPH oxidase and lipoxynenase products mediate damage in isolated neutrophil-perfused rat lungs given interleukin-8 (IL-8) intratracheally. Clin Res 42:145A (1994).

26. Frank L, Summerville J, Massaro D. Protection from oxygen toxicity with endotoxin. Role of the endogenous antioxidant enzymes of the lung. J Clin Invest 65:1104–1110 (1980).

27. White CW, Ghezzi P, Dinarello CA, Caldwell SA, McMurtry JJ, Repine JE. Recombinant tumor necrosis factor/cachectin and interleukin-1 pretreatment decreases lung oxidized glutathione accumulation, lung injury and mortality in rats exposed to hyperoxia. J Clin Invest 79:1868–1873 (1987).

28. White CW, Ghezzi P, McManon S, Dinarello CA, Repine JE. Cytokines increase rat lung antioxidant enzymes during exposure to hyperoxia. J Appl Physiol 66:1003–1017 (1989).

29. Tsan MF, White JE. Kinetics of pulmonary superoxide dismutation in interleukin-1-induced oxygen-tolerant rats. Am J Physiol (Lung Cell Mol Physiol) 263:342–347 (1992).

30. Tsan MF, Lee CY, White JE. Interleukin-1 protects rats against oxygen toxicity. J Appl Physiol 71:680–697 (1991).

31. Gerrissen ME, Bloor J, Endothelial cell expression in response to injury. FASEB J 7:523–532 (1993).

32. Repine JE. Oxidant-antioxidant balance: some observations from studies of ischemia-reperfusion in isolated perfused rat hearts. Am J Med 91(Suppl 3C):45S–55S (1991).

33. Clerch LB, Massaro D. Tolerance of rats to hyperoxia: lung antioxidant enzyme gene expression. J Clin Invest 91:499–508 (1993).

34. Brown JM, Grosso MA, Terada LS, Whitman GJ, Banerjee A, White CW, Harken AH, Repine JE. Endotoxin pretreatment increases endogenous myocardial catalase activity and decreases ischemia-reperfusion injury of isolated rat hearts. Proc Natl Acad Sci USA 86:2516–2520 (1989).

35. Brown JM, White CW, Terada LS, Grosso MA, Shanley PF, Mulvin DW, Banerjee A, Whitmann GJ, Harken AH, Repine JE. Interleukin-1 pretreatment decreases ischemia-reperfusion injury. Proc Natl Acad Sci USA 87:5026–5030 (1990).

36. Brown JM, Anderson BO, Repine JE, Shanley PF, White CW, Grosso MA, Banerjee A, Bensard DD, Harken AH. Neutrophils contribute to TNF induced myocardial tolerance to ischemia. J Mol Cell Cardiol 24:485–495 (1992).

37. Wong GH, Elwell JH, Oberley LW, Goeddel DV. Manganese superoxide dismutase is essential for cellular resistance to cytoxicity of tumor necrosis factor. Cell 58:923–931 (1989).

38. Vinner GA, Cheshrun SE, Monnier J, Ryan US, Nick HS. Regulation of manganese superoxide dismutase IL-1 and TNF induction in pulmonary artery and microvascular endothelial cells. Biochem Biophys Res Commun 5:453–462 (1992).

39. Lu D, Maulik N, Moraru IL, Kreutzer DL, Das DK. Molecular adaptation of vascular endothelial cells to oxidative stress. Am J Physiol (Cell Physiol) 329:264:715–722 (1993).

40. Vinner GA, Block ER, Burr JM, Nick HS. Regulation of manganese superoxide dismutase in porcine pulmonary artery endothelial cells. Am J Physiol (Lung Cell Mol Physiol) 260:L444–L449 (1991).

41. Tsan MF, White JE, DelVecchio PJ, Shaffer JB. IL-6 enhances TNF α- and IL-1-induced increase of Mn superoxide dismutase mRNA and O2– tolerance. Am J Physiol (Lung Cell Mol Physiol) 263:22–26 (1992).

42. Banerjee A, Locke-Winter C, Rogers KB, Mitchell MB, Brew EC, Caia CB, Bensard DO, Harken AH. Preconditioning against myocardial dysfunction after ischemia and reperfusion by an α-adrenergic mechanism. Circ Res 7:656–670 (1993).

43. Omar BA, McCord JM. The cardioprotective effect of Mn-superoxide dismutase was lost at high doses in the post-ischemic isolated rabbit heart. Free Radic Biol Med 9:473–478 (1990).

44. Speranza MJ, Bagley AC, Lynch RE. Cells enriched for catalase are sensitized to the toxicities of bleomycin, adriamycin, and paclitaxel. J Biol Chem 268:19039–19043 (1993).