Acute alcohol intoxication increases interleukin-18-mediated neutrophil infiltration and lung inflammation following burn injury in rats

Xiaoling Li,1 Elizabeth J. Kovacs,2 Martin G. Schwacha,1 Irshad H. Chaudry,1 and Mashkoor A. Choudhry1

1Center for Surgical Research and Department of Surgery, University of Alabama at Birmingham, Birmingham, Alabama; and 2Alcohol Research Program, Burn and Shock Trauma Institute, Departments of Surgery and Cell Biology, Neurobiology, and Anatomy, Loyola University Chicago Medical Center, Maywood, Illinois

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Acute alcohol intoxication increases interleukin-18-mediated neutrophil infiltration and lung inflammation following burn injury in rats. Am J Physiol Lung Cell Mol Physiol 292: L1193–L1201, 2007. First published January 12, 2007; doi:10.1152/ajplung.00408.2006.—In this study, we examined whether IL-18 plays a role in lung inflammation following alcohol (EtOH) and burn injury. Male rats (225–250 g) were gavaged with EtOH to achieve a blood EtOH level of ~100 mg/dl before burn or sham injury (~12.5% total body surface area). Immediately after injury, rats were treated with vehicle, caspase-1 inhibitor AC-YVAD-CHO to block IL-18 production or with IL-18 neutralizing anti-IL-18 antibodies. In another group, rats were treated with anti-neutrophil antiserum ~16 h before injury to deplete neutrophils. On day 1 after injury, lung tissue IL-18, neutrophil chemokines (CINC-1/CINC-3), ICAM-1, neutrophil infiltration, MPO activity, and water content (i.e., edema) were significantly increased in rats receiving a combined insult of EtOH and burn injury compared with rats receiving either EtOH intoxication or burn injury alone. Treatment of rats with caspase-1 inhibitor prevented the increase in lung tissue IL-18, CINC-1, CINC-3, ICAM-1, MPO activity, and edema significantly increased in rats receiving either EtOH intoxication or burn injury alone. These findings suggest that acute EtOH intoxication before burn injury upregulates IL-18, which in turn contributes to increased neutrophil infiltration. Furthermore, the presence of neutrophils appears to be critical for IL-18-mediated increased lung tissue edema following a combined insult of EtOH and burn injury.

Several lines of evidence suggest that injury perturbs the host immune defense, leading to increased susceptibility to nosocomial infections and the development of multiple organ failure, which is the leading cause of death in patients who survive the initial injury (7, 24, 30, 37, 42, 58). According to an estimate, nearly one million people are affected with burn injuries every year in the United States, and nearly one-half of these injuries occur under the influence of alcohol (EtOH) intoxication (20, 33–35). Although the overall impact of EtOH intoxication on postburn pathogenesis remains to be investigated, a few studies have suggested that the patients who consumed EtOH before injury are more susceptible to infection, exhibit higher morbidity, and are more likely to die than patients who have not consumed EtOH (18, 33–35, 54). Similarly, findings from experimental settings suggest that EtOH intoxication before burn injury exacerbates the suppression of host immune defense, deteriorates intestinal barrier functions, and increases intestinal bacterial translocation (4, 6, 13, 26, 28, 35, 36).

A common cause of deaths in burn patients is pulmonary failure. Furthermore, studies have also shown that both chronic and acute EtOH exposure dramatically increases the risk of acute lung injury. The onset of lung injury is induced by a series of events and is characterized by inflammatory cell infiltration and extracellular fluid accumulation into the lung interstitium (3, 7, 8, 31, 32, 57). In addition, the uncontrolled production of inflammatory mediators, such as TNF-α, IL-1, IL-6, and IL-10 (2, 5, 19, 24, 29, 35, 38, 46) are also implicated in the onset of inflammation and lung pathology. Recent findings from our laboratory have shown that alterations in intestinal barrier function following a combined insult of EtOH and burn injury was accompanied with an increase in IL-18 production (27, 49). IL-18, like IL-12, was discovered initially to be a factor that drives the T cell toward T helper 1 cell subtype and thus was referred as IFN-γ-inducing factor (43, 44). However, later studies have implicated IL-18 in inducing tissue damage in patients and experimental models of arthritis and sepsis (12, 21–23, 40, 51, 53). Although some of these studies have suggested IL-18-induced IFN-γ as the cause for tissue damage, others have indicated the role of neutrophils recruited by IL-18 (21, 25). In the present study, we examined the effect of acute EtOH intoxication before burn injury on lung tissue inflammation. Furthermore, we determined whether IL-18 plays a role in lung inflammation following a combined insult of EtOH intoxication and burn injury. Our findings suggest that IL-18 recruits neutrophils by upregulating the production of neutrophil chemokines and expression of adhesion molecules. This in turn may cause lung tissue edema, which is a marker of lung injury.

MATERIALS AND METHODS

Animals and reagents. Male Sprague-Dawley rats (225–250 g) were obtained from Charles River Laboratories (Wilmington, MA). Caspase-1 inhibitor (Ac-YVAD-CHO) was obtained from Axxora (San Diego, CA). Rabbit anti-rat neutrophil antiserum was obtained...
from Accurate Chemicals and Scientific (Westbury, NY). Anti-rat IL-18 antibody was obtained from R&D Systems (Minneapolis, MN).

**Rat model of acute EtOH and burn injury.** As described previously (4, 28), rats were randomly divided into four groups: saline + sham, EtOH + sham, saline + burn, and EtOH + burn. In EtOH-treated groups, the levels of blood EtOH equivalent to 90–100 mg/dL were achieved by gavage feeding of 5 ml of 20% EtOH in saline. In saline groups, animals were gavaged with 5 ml of saline. Four hours after gavage with saline or EtOH, all animals were anesthetized and transferred into a template, which was fabricated to expose ~12.5% of the total body surface area. Animals were then immersed in a boiling water bath (95–97°C) for 10–12 s. Sham-injured rats were subjected to identical anesthesia and immersed in lukewarm water. The animals were dried immediately and resuscitated intraperitoneally with 10 ml of physiological saline. After recovery from anesthesia, the animals were returned to their cages and allowed food and water ad libitum. Depending on the type of experiment, rats were treated intraperitoneally with IL-18 inhibitor (caspase-1 inhibitor) Ac-YVAD-CHO (5 mg/kg body wt) or anti-IL-18 antibody (50 μg/kg body wt) immediately after burn injury. On day 1 after injury, rats were killed.

All the experiments were carried out in adherence to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee.

**Neutrophil depletion.** Rabbit anti-rat neutrophil antiserum (0.3 ml diluted in 1 ml of physiological saline) was injected intraperitoneally into the rats ~16 h before injury (52). On day 1 after injury, blood samples were drawn from rats via cardiac puncture, and neutrophils in blood were counted by using Hemavet-850 (Scientific, Oxford, CT). The results suggest a significant increase in neutrophil counts in circulation on day 1 following a combined insult of EtOH intoxication and burn injury (2,370 ± 313, neutrophils/μl; mean ± SE) compared with sham animals (360 ± 182, neutrophils/μl). Treatment of rats resulted in a substantial decrease in the neutrophil counts (638 ± 138 neutrophils/μl) following a combined insult of EtOH intoxication and burn injury. These findings suggest that the dose of anti-neutrophil antiserum given to the rats does not completely remove neutrophils from the circulation, but rather prevents the increase in their numbers following a combined insult of EtOH intoxication and burn injury.

**Histological analysis of lung.** On day 1 after injury, rats were anesthetized, lungs were removed, and 0.5-cubic cm pieces of lung tissue were fixed in 10% formalin (Sigma Chemical, St. Louis, MO) anesthetized, lungs were removed, and 0.5-cubic cm pieces of lung tissue were fixed in 10% formalin (Sigma Chemical, St. Louis, MO) and were sonicated at 30 cycles, twice, for 30 s on ice (27, 49, 60). Homogenates were cleared by centrifuging at 12,000 rpm at 4°C, and the supernatants were stored at −70°C. Protein levels in the homogenates were determined using the Bio-Rad (Hercules, CA) assay kit.

**Preparation of lung homogenates.** Lungs were removed and snap-frozen. Equal weights (100 mg wet weight) of lung from various groups were suspended in 1 ml of buffer containing 0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer at pH 6.0 and were sonicated at 30 cycles, twice, for 30 s on ice (27, 49, 60). Homogenates were cleared by centrifuging at 12,000 rpm at 4°C, and the supernatants were stored at −70°C. Protein levels in the homogenates were determined using the Bio-Rad (Hercules, CA) assay kit.

**Measurement of lung tissue MPO levels.** MPO activity in the lung homogenates was measured using the procedure described previously (27, 49, 60). Briefly, samples were incubated with the substrate o-dianisidine hydrochloride. This reaction was carried out in a 96-well plate by adding 290 μl of 50 mM phosphate buffer, 3 μl of substrate solution containing 20 mg/ml o-dianisidine hydrochloride, and 3 μl of 20 mM H2O2. Sample (10 μl) was added to each well to start the reaction. Standard MPO (Sigma Chemical) was used in parallel to determine MPO activity in the sample. The reaction was stopped by adding 3 μl of 30% sodium azide. Plates were read at 460 nm. MPO activity was determined by using the curve obtained from the standard MPO, which was purchased from Sigma Chemical.

**Measurement of lung tissue edema.** Lungs were removed, weighed, and dried for 48 h at 80°C. Water content (%) of lung tissue was calculated as (wt−dry wt)/wt × 100 and was used as a measure of tissue edema (27, 49, 60).

**Measurement of lung tissue IL-1β, IL-18, CINC-1, CINC-3, and ICAM-1.** IL-1β, IL-18, CINC-1, CINC-3, and ICAM-1 level in the lung homogenates were measured by using the ELISA kits (IL-1β and mature IL-18 from Biosource International, Camarillo, CA, and CINC-1, CINC-3, and ICAM-1 from R&D Systems) following the manufacturers’ instructions. The concentrations in the samples were determined by using the standard curve and by normalizing with protein.

**Statistical analysis.** Results are presented as means ± SE and were analyzed using ANOVA. The significance between the groups was determined using Tukey’s test (Statistical Package for Social Sciences Software program, version 2.0, SigmaStat). P < 0.05 between two groups was considered statistically significant.

**RESULTS**

**Lung histology, MPO activity, and edema.** We first examined the effect of acute EtOH intoxication before burn injury on lung tissue inflammation: histology, MPO activity, and edema. As shown in Fig. 1, there was no evidence of damage to the lungs of rats on day 1 after gavage with saline (Fig. 1A). However, lungs harvested from rats on day 1 after EtOH intoxication (Fig. 1B) or burn injury in the absence of EtOH intoxication (Fig. 1C) exhibited a moderate histological change compared with sham-injured rats gavaged with saline. These histological changes, as evidenced by engorgement of the lung interstitial space with infiltration of neutrophils, were more prominent in rats receiving a combined insult of EtOH intoxication and burn injury (Fig. 1D) compared with rats receiving either burn injury alone without prior EtOH intoxication or sham injury regardless of their EtOH exposure. The number of neutrophils in hematoxylin-eosin-stained lung sections was determined using simple microscopy at ×1,000 magnification. The results as shown in Fig. 1E suggest no difference in neutrophil counts in sham animals regardless of EtOH intoxication. A slight increase in neutrophil number was observed following burn injury alone in the absence of EtOH intoxication. However, a severalfold increase in neutrophil counts was observed in lung tissues of rats receiving a combined insult of EtOH intoxication and burn injury compared with rats receiving either EtOH intoxication or burn injury alone.

To further quantitate the neutrophil infiltration and tissue edema, we measured lung tissue MPO activity and water accumulation (an index of edema). Results from these analyses as shown in Fig. 2 suggest no significant difference in lung MPO activity (Fig. 2A) and tissue edema (Fig. 2B) in rats receiving either EtOH or burn injury alone compared with sham animals gavaged with saline. However, a significant increase in lung MPO activity and edema was observed in rats receiving a combined insult of EtOH intoxication and burn injury.
Fig. 1. Lung histology following alcohol (EtOH) intoxication and burn injury. Representative photomicrographs of lung of sham and burn rats with and without EtOH exposure are shown. On day 1 after EtOH and burn injury, lungs were removed and sectioned. Sections were stained with hemotoxylin-eosin, examined using a magnification of ×100, and are presented at left of A (saline + sham), B (EtOH + sham), C (saline + burn), and D (EtOH + burn). Photomicrographs of the corresponding sections at higher magnification (×400) are shown at the right of each panel. With the use of high power magnification (×1,000), sections were also screened for the presence of neutrophils. Neutrophils were identified by their distinct morphological nature such as small, pale granular cytoplasm with a multilobed nucleus. Each section was scanned for 3–4 microscopic fields, and the data thus obtained from more than 4 animals in each group were pooled and presented in E as means ± SE. *P < 0.05 compared with other groups.
injury (P < 0.05) compared with burn-injured rats gavaged with saline or sham rats regardless of their EtOH intoxication.

**Lung IL-1β and IL-18 levels.** In addition to lung histology and edema, we also measured lung IL-1β and IL-18 levels on day 1 after injury. No significant difference in lung tissue IL-1β levels was observed following a combined insult of EtOH and burn injury compared with sham-injured rats gavaged with EtOH or with saline (Fig. 3A). Furthermore, IL-18 levels were also not significantly different in homogenates prepared from lung of burn-injured rats gavaged with saline compared with sham-injured rats gavaged with EtOH or saline (Fig. 3B). However, a significant increase (P < 0.05) in IL-18 levels was observed in the lung homogenates of rats following a combined insult of EtOH intoxication and burn injury compared with rats receiving either sham or burn injury alone. Together, these findings suggest that EtOH intoxication combined with burn injury increases lung IL-18 and not IL-1β levels.

We next determined whether IL-18 upregulation plays a role in lung inflammation following a combined insult of EtOH and burn injury. To perform this study, a group of rats was treated with caspase-1 inhibitor to block endogenous IL-18 levels. The effects of IL-18 blockade were assessed on lung inflammatory markers. In these assessments, we did not include the sham + EtOH group since there were no significant differences in lung tissue IL-18, MPO activity, and edema in sham-injured rats gavaged with saline or EtOH.

**Caspase-1 inhibitor prevents the injury-induced increase in lung tissue IL-18 levels and MPO activity.** Results presented in Fig. 4 show that the administration of caspase-1 inhibitor significantly prevented the increase in lung tissue IL-18 levels (Fig. 4A) and MPO activity (Fig. 4B) following a combined insult of EtOH intoxication and burn injury. Furthermore, compared with vehicle, administration of caspase-1 inhibitor in sham animals significantly reduced lung IL-18 levels but did not influence MPO activity (Fig. 4B). In contrast, both IL-18 and IL-1β levels remained unaltered in these animals.

**Fig. 2.** Lung tissue MPO activity (A) and edema (B) following EtOH intoxication and burn injury. On day 1 after injury, lung tissues were collected from rats subjected to sham or burn injury with and without EtOH. Equal weights of lung tissue from various groups were homogenized. MPO activity was normalized to the protein contents. For edema, lung tissue water content was determined by drying the tissue for 48 h at 80°C. Water content (%) of lung tissue was calculated as (wet wt – dry wt)/wet wt × 100. Data are means ± SE from at least 6 animals in each group. *P < 0.05 compared with other groups.

**Fig. 3.** Lung tissue IL-1β and IL-18 levels following EtOH intoxication and burn injury. One day after injury, lung tissues were collected from all 4 treatment groups, and equal weights of tissue were homogenized. IL-1β and IL-18 levels were measured using ELISA kits and normalized to the protein. Data are means ± SE from at least 6 animals in each group. *P < 0.05 compared with other groups.
and MPO activity were significantly decreased in EtOH + burn-injured rats treated with caspase-1 inhibitor.

**Caspase-1 inhibitor prevents the injury-induced increase in neutrophil chemokines and adhesion molecules.** Since it is possible that IL-18 upregulates neutrophil chemokine and adhesion molecule expression and thereby indirectly causes an increase in neutrophil infiltration into the lungs after injury, we measured CINC-1, CINC-3, and ICAM-1 expression. There was no significant difference in the lung CINC-1 and CINC-3 levels between sham animals gavaged with EtOH or saline (data not shown). Furthermore, no significant difference in CINC-1 and CINC-3 (Fig. 5, A and B) levels was observed in the lungs of rats receiving burn injury alone in the absence of EtOH intoxication compared with sham-injured rats gavaged with saline. However, a significant increase in CINC-1 and CINC-3 levels was observed in lung of burn-injured rats gavaged with EtOH compared with rats receiving sham or burn injury alone. Treatment of rats with the caspase-1 inhibitor prevented the increase in CINC-1 expression following EtOH intoxication and burn injury (Fig. 5C). Similar treatment did not influence ICAM-1 expression in rats receiving either sham or burn injury alone.

Like the expression of neutrophil chemokines CINC-1 and CINC-3, there was no significant difference in lung ICAM-1 expression between sham animals gavaged with saline or EtOH (data not shown). Moreover, there was no change in ICAM-1 levels in lung tissue of rats receiving burn injury alone in the absence of EtOH intoxication compared with sham-injured rats gavaged with saline (Fig. 5C). However, a significant increase in the lung ICAM-1 expression was observed in burn-injured rats gavaged with EtOH relative to sham or burn-injured rats gavaged with saline. Treatment of rats with the caspase-1 inhibitor prevented the increase in ICAM-1 expression following EtOH intoxication and burn injury (Fig. 5C). Similar treatment did not influence ICAM-1 expression in rats receiving either sham or burn injury alone.

Fig. 4. Effect of caspase-1 inhibitor on lung tissue IL-18 level (A) and MPO activity (B) following EtOH intoxication and burn injury. Animals were treated intraperitoneally with vehicle (DMSO) or caspase-1 inhibitor (Ac-YVAD-CHO, 5 mg/kg) immediately after burn injury. One day later, lung tissues were collected and homogenized. IL-18 level and MPO activity were measured and normalized to the protein. Data are means ± SE from at least 6 animals in each group. *P < 0.05 compared with other groups; #P < 0.05 compared with corresponding vehicle-treated groups.

Fig. 5. Effect of caspase-1 inhibitor on lung tissue CINC-1 (A), CINC-3 (B), and ICAM-1 (C) levels following EtOH intoxication and burn injury. Animals were treated intraperitoneally with vehicle (DMSO) or caspase-1 inhibitor (Ac-YVAD-CHO, 5 mg/kg) immediately after burn injury. At one day after injury, lung tissues were collected and homogenized for assessment of CINC-1, CINC-3, and ICAM-1 levels using ELISA kits. Values were normalized to the protein. Data are means ± SE from at least 6 animals in each group. *P < 0.05 compared with other groups; #P < 0.05 compared with corresponding vehicle-treated groups.
**Anti-IL-18 antibody prevents the injury-induced increase on MPO activity.** To further ascertain the role of IL-18 in increased MPO activity following EtOH and burn injury, a group of rats was treated with neutralizing anti-IL-18 antibody (50 μg/kg) immediately after burn injury. Results presented in Fig. 6 show that treatment of rats with anti-IL-18 antibody significantly attenuated the increase in lung MPO activity (Fig. 6B). The efficacy of anti-IL-18 antibody was assessed by measuring IL-18 level, and the results presented in Fig. 6A show that treatment of rats with anti-IL-18 antibody prevented the increase in IL-18 levels in lungs of rats receiving a combined insult of EtOH intoxication and burn injury.

**IL-18 inhibition prevents the development of injury-induced lung tissue edema.** The development of edema as determined by lung tissue water content in various experimental groups is shown in Fig. 7. The results clearly suggest that treatment of rats with caspase-1 inhibitor or with IL-18 neutralizing anti-IL-18 antibody immediately after injury significantly prevented the development of edema following EtOH intoxication combined with burn injury. These findings suggest that an increase in IL-18 is likely the cause for the development of lung tissues edema following EtOH and burn injury.

**Neutrophil presence is critical to IL-18-mediated lung tissue edema.** To further delineate whether the increased lung tissue edema is a direct effect of IL-18 or is mediated via IL-18-dependent neutrophil infiltration, animals were treated intraperitoneally with rabbit anti-rat neutrophil antiserum 12–16 h before EtOH and burn injury. The effect of neutrophil deple- tion on lung edema was then determined. The results shown in Fig. 7 reveal that treatment of rats with anti-neutrophil antiserum prevented the increase in lung edema. The efficacy of this antibody on neutrophil accumulation in the lung was then confirmed by evaluating the MPO activity followed by assessment of pulmonary IL-18 levels. The results from these experiments are summarized in Fig. 8 and show that administration of anti-neutrophil antiserum in rats completely prevented the increase in lung MPO activity (Fig. 8B) without having an effect on IL-18 levels (Fig. 8A) in rats given EtOH before burn injury.

**DISCUSSION**

In the current study, we observed a significant increase in lung IL-18 levels after a combined insult of EtOH intoxication and burn injury relative to either EtOH or burn alone. This was accompanied by an increase in lung neutrophil chemokines (CINC-1 and CINC3), an adhesion molecule (ICAM-1), neutrophil infiltration, MPO activity, and edema (water content). Treatment of rats with IL-18 inhibitor immediately after the injury prevented the increase in pulmonary IL-18, CINC-1, CINC-3, ICAM-1, and MPO activity, as well as in lung edema after EtOH intoxication and burn injury. The increase in IL-18, MPO activity, and lung edema was also prevented when rats were treated with IL-18 neutralizing antibodies. To ascertain whether the increase in neutrophil accumulation in lung tissue following EtOH and burn injury is critical to IL-18-mediated lung tissue edema, rats were depleted of neutrophils before receiving EtOH and burn injury. The results from this experiment as shown in Figs. 7 and 8 suggest that neutrophils are required for IL-18-mediated lung tissue edema. Together, these findings suggest that EtOH intoxication and burn injury up-

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**Fig. 6.** Effect of anti-IL-18 antibody on lung tissue IL-18 level (A) and MPO activity (B) following EtOH intoxication and burn injury. Animals were treated intraperitoneally with neutralizing anti-IL-18 antibody (50 μg/kg) immediately after burn injury. One day later, lung tissues were collected and assayed for levels of IL-18 and MPO activity and normalized to the protein. Data are means ± SE from 4–6 animals in each group. *P < 0.05 compared with other groups.

**Fig. 7.** Effect of caspase-1 inhibitor, anti-IL-18 antibody, and anti-neutrophil antiserum on lung tissue edema as determined by tissue water content following EtOH intoxication and burn injury. Immediately after burn injury, rats subjected to EtOH + burn injury were divided into subgroups to receive intraperitoneally, caspase-1 inhibitor (Ac-YVAD-CHO, 5 mg/kg) or anti-IL-18 antibody (50 μg/kg). In another set of animals, ~16 h before receiving EtOH + burn injury animals, rats were treated with rabbit anti-rat neutrophil antiserum (0.3 ml diluted in 1 ml of physiological saline). One day after injury, lung tissues were collected and dried for 48 h at 80°C. Water content (%) of lung tissue was calculated as (wet wt − dry wt)/wet wt × 100. Data are means ± SE from 4–6 animals in each group. *P < 0.05 compared with other groups. Casp, caspase-1 inhibitor.
regulate IL-18 production, which, in turn, may cause lung injury by upregulation of neutrophil infiltration following EtOH and burn injury.

IL-18 is synthesized as a precursor protein (pro-IL-18), and in the presence of IL-1β-converting enzyme (ICE, or caspase-1), it matures into an 18-kDa active protein (9, 16, 39). The biological activity of IL-18 can also be regulated after its release by IL-18-binding protein (IL-18bp), which can bind IL-18 and block its bioavailability and subsequent function (9, 16, 39, 41). Since IL-18 has been shown to enhance IL-12-dependent IFN-γ production, it appears that IL-18 promotes cell-mediated immunity (9, 16, 39, 41) and is thus essential to host defenses against a variety of infections. However, later studies found that like many other proinflammatory cytokines, IL-18 possesses broad immunomodulatory properties including a role in tissue damage. We found that as a result of EtOH and burn injury, IL-12 production is downregulated (unpublished observation). Furthermore, our studies suggest that the decrease in IFN-γ following a combined insult of EtOH intoxication and burn injury is likely the result of a decrease in IL-12 and is independent of IL-18 (28). These observations are further supported by a recent study in which IL-12 was found to be reduced significantly, and IL-18 levels were significantly higher in patients with sepsis compared with control and surgical patients without sepsis (11). Furthermore, they observed that IL-18 levels were increased significantly in patients with lethal sepsis compared with sepsis survivors at all time points studied. Findings from this patient study concluded that IL-12 may contribute to protective immune reactions against a septic challenge, whereas IL-18 may preferentially promote organ injury and lethal shock (11). Consistent with this observation, our findings included in this paper suggest that IL-18 helps in the recruitment of neutrophils, which in turn may contribute to lung injury following EtOH and burn injury.

Neutrophils are well recognized for their role in producing tissue damage. However, in this study, we have not determined lung tissue damage, but several lines of evidence suggest that neutrophil infiltration and subsequent development of edema are among the early markers of tissue damage. Thus it is likely that IL-18-mediated neutrophil infiltration ultimately leads to lung tissue damage following EtOH and burn injury. Studies have shown that EtOH intoxication or burn injury results in neutrophil activation and release of proteases and free oxygen radicals (O2•-) (14, 19, 50). IL-18 receptors (IL-18Rα and β) are present on neutrophil and can directly activate neutrophil to release proteases and O2•-. Although such release of O2•- is critical to their role in pathogen killing and thus to host defense, excessive O2•- release in the environment outside neutrophils can cause tissue damage. Neutrophil-mediated oxidant injury is demonstrated in pathological conditions of acute and chronic EtOH intoxication, acute respiratory distress syndrome, and tissue ischemia, as well as in experimental models of sepsis and trauma (2, 15, 17, 19, 47, 50, 52, 60).

Although our findings suggest a role of ICAM-1 in increased neutrophil infiltration, there are other studies suggesting the role of ICAM-1-independent mechanism in neutrophil recruitment (10). Since we have not measured adhesion molecules other than ICAM-1, it is likely that those ICAM-1-independent mechanisms may also be involved in neutrophil recruitment following a combined insult of EtOH and burn injury. Our findings also suggest that IL-18 upregulates chemokines (CINC-1/CINC-3). CINC is a member of the α (CXC) subfamily of chemokines and is classified as CINC-1, CINC-2α, CINC-2β, and CINC-3. CINC-3 is also referred to as macrophage inflammatory protein-2 (17). The protein sequence of CINC-1 is 63–67% identical to that of CINC-2 and CINC-3. Although all three CINCs are likely to play roles in the neutrophil recruitment, many studies supported the suggestion that CINC-1 and CINC-3 play a predominant role in neutrophil recruitment (17, 48, 61). Studies have also shown that interaction of ICAM-1 with its counter receptor expressed on neutrophil activates the cell surface integrins and allows a strong adhesion to endothelium (15, 17, 59). The increase in chemokines, on the other hand, will promote the migration of neutrophil across endothelium and through the extracellular matrix. Studies have shown that blocking of CINC and ICAM-1 with antibody resulted in reduction in vascular permeability and a decrease in lung MPO activity. Thus these findings suggest that IL-18 promotes neutrophil recruitment by influencing the process of neutrophil transmigration at multiple points, including the increased chemokine production and the expression of adhesion molecules.

In our studies, we used an inhibitor of caspase-1, the activation of which is critical to IL-18 maturation. Furthermore, the same enzyme also plays a role in the maturation of IL-1β (9, 22, 51). Although we have not determined caspase-1

![Image](http://ajplung.physiology.org/ by 10.220.32.247 on April 19, 2017)
activity, the findings of a decrease in IL-18 levels in rats treated with caspase-1 inhibitor suggest that EtOH combined with burn injury results in increased caspase-1 activity. Thus if there is an increase in caspase-1 activity, it should also enhance the production of IL-1β following EtOH intoxication and burn injury. However, no significant difference in lung tissue IL-1β levels was observed on day 1 following a combined insult of EtOH intoxication and burn injury. The mechanism of such a differential role of caspase-1 in regulation of IL-1β and IL-18 remains to be established. Previous studies have shown that IL-18 is constitutively expressed in many cell types, whereas IL-1β is not expressed constitutively (51). It is likely that caspase-1 may not regulate immature forms of IL-1β and IL-18; rather, it participates only in the maturation process of available biologically immature IL-1β or IL-18. Furthermore, it is possible that IL-1β follows a kinetic that is different from IL-18 production following EtOH intoxication and burn injury. 

In the present study, we did not identify the source of IL-18 and CINC in lung tissues. However, based on the previous findings (21), it can be suggested that alveolar macrophages are the major producer of IL-18 and CINC in the lung following EtOH and burn injury. Nevertheless, more studies are needed to confirm this. Furthermore, studies are also needed to determine the mechanism by which EtOH exposure upregulates IL-18 levels following burn injury. In a recent study from our laboratory, we have shown a role of corticosterone in increased IL-18 levels following EtOH and burn injury (27), but the mechanism as to how corticosterone upregulates IL-18 levels remains to be investigated. One possibility is that the EtOH combined with burn injury directly or via elevated corticosterone increases caspase-1 activity, which in turn amplifies the conversion of immature IL-18 to mature IL-18.

We recognize that burn injury alone, in the absence of EtOH intoxication, did not influence any of the studied parameters. Other studies have shown that burn injury alone in the absence of EtOH exposure may cause organ dysfunction, including lung injury (3, 7, 8, 24, 31, 32, 56, 57). The reason for the differences in the observed results could be due to different experimental models used in the studies. It is also possible that neutrophil infiltration following burn injury alone in the absence of prior EtOH exposure occurs within the first few hours and is resolved by 24 h, as shown in a previous study (45, 56). Furthermore, studies have also shown that postburn injury complications are very much dependent on the size of the burn injury as well as on the additional complications associated with the burn injury, such as smoke inhalation or wound infection, etc. (1, 6, 55). It is likely that similar to these factors, EtOH intoxication before burn injury serves as an additional insult and thus exacerbates the postburn alterations.

We also recognize that measurements were performed at a single time point (i.e., day 1 after injury). The suppression in immune cell function after burn injury in the absence of EtOH intoxication is observed up to 7–10 days after injury (1, 24). We had shown in a previous study that on day 2 after injury, there was a decrease in intestinal immunity following burn injury alone in the absence of EtOH intoxication; however, the suppression was greater in the group of rats receiving the combined insult of EtOH intoxication and burn injury compared with rats receiving either burn or sham injury alone (4). These findings support the suggestion that EtOH consumption before injury may have a synergistic effect on organ function.

In summary, our findings suggest that acute EtOH intoxication before burn injury increases lung tissue IL-18 levels. This, in turn, upregulates neutrophil adhesion and chemokine expression and promotes neutrophil infiltration. Furthermore, we found that neutrophils are critical for IL-18-mediated increase in lung tissue edema following a combined insult of EtOH and burn injury.

GRANTS

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REFERENCES

IL-18 AND LUNG INJURY


