Granulocyte Colony-Stimulating Factor Modulates the Pulmonary Host Response to Endotoxin in the Absence and Presence of Acute Ethanol Intoxication

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Alcohol impairs neutrophil function and predisposes the host to infectious complications. Granulocyte colony-stimulating factor (G-CSF) increases both the number and functional activities of neutrophils. This study investigated the effects of G-CSF on the pulmonary response to endotoxin in rats with or without acute ethanol intoxication. Acute ethanol intoxication inhibited tumor necrosis factor (TNF)-α and macrophage inflammatory protein (MIP)-2 production in the lung and suppressed the recruitment of neutrophils into the lung. Ethanol also inhibited CD11b/c expression on recruited neutrophils and suppressed the phagocytic activity of circulating neutrophils. G-CSF pretreatment up-regulated CD11b/c expression on circulating polymorphonuclear leukocytes, augmented the recruitment of neutrophils into the lung, and enhanced the phagocytic activity of circulating and recruited neutrophils in both the absence and presence of acute ethanol intoxication. G-CSF inhibited MIP-2 but not TNF-α production in the lung. These data suggest that G-CSF may be useful in the prevention or treatment of infections in persons immunocompromised by alcohol.

Infectious diseases, particularly pneumonia, are common causes of morbidity and mortality in patients who abuse alcohol. Ethanol-induced impairment of immune cell function is considered a major risk factor for increased susceptibility to lung infection [1–3]. The normal innate immune response of the lung to bacterial invasion is primarily mediated by resident alveolar macrophages (AMs). While ingesting and killing invading microorganisms, AMs generate numerous inflammatory mediators, including cytokines and chemokines, that orchestrate the migration of polymorphonuclear leukocytes (PMNLs) from the peripheral circulation into the lower respiratory tract. These recruited PMNLs provide auxiliary phagocytic defenses to the lung that are critical for the effective eradication of bacterial infection [4, 5]. Ethanol inhibits AM phagocytic activity [6] and suppresses the production of proinflammatory cytokines by AMs [7, 8]. In addition, ethanol inhibits the delivery of PMNLs into inflammatory tissue sites [2, 7, 9, 10] and several other PMNL functions, including phagocytosis, adhesion molecule expression, and oxygen metabolism [9, 11]. These defects in normal pulmonary host defense likely contribute to the increased susceptibility of alcohol-abusing hosts to infection.

Granulocyte colony-stimulating factor (G-CSF) is a lineage-specific hematopoietic growth factor that selectively stimulates the proliferation and maturation of myeloid progenitor cells to PMNLs [12–14]. G-CSF also exerts direct effects on the functional activity of mature PMNLs [15–18]. Administration of exogenous G-CSF to animals challenged with either intratracheal endotoxin or bacteria augments the pulmonary recruitment of PMNLs, up-regulates PMNL phagocytic activity, and enhances intrapulmonary bactericidal function [10, 19]. G-CSF also attenuates the adverse effects of ethanol on PMNL delivery into the lung in response to intrapulmonary bacterial challenge and improves survival [10]. These data suggest that G-CSF may be a potentially useful immunomodulator in both the prevention and treatment of pulmonary infection in persons who abuse alcohol.

In the present study, we compared the effects of exogenous G-CSF on the functional activities of circulating and pulmonary recruited PMNLs in rats challenged with intrapulmonary endotoxin in both the absence and presence of acute ethanol intoxication in order to define the mechanisms underlying the beneficial effects of this hematopoietic growth factor on pulmonary host defenses responsible for eliminating invading microorganisms. Since the presence of a chemotactic gradient is essential for the directed migration of PMNLs from the vasculature into infected tissue sites [20], the effects of acute ethanol and G-CSF on tumor necrosis factor (TNF)-α and macrophage inflammatory protein (MIP)-2 production in the lung were also determined.
Materials and Methods

Reagents. We used recombinant human G-CSF (specific activity 10^6 U/mg; gift of Amgen, Thousand Oaks, CA). Fluorescein isothiocyanate–conjugated (FITC) polystyrene latex microspheres (1.5-μm diameter) were obtained from Polysciences (Warrington, PA). We purchased FITC-conjugated monoclonal antibody against rat CD11b/c (mouse IgG2a, clone OX-42) and CD18 (mouse IgG1, clone WT.3) and FITC-conjugated isotype-matched control antibodies (mouse IgG2a, IgG1; Pharmingen, San Diego). ELISA kits for rat MIP-2 and TNF were obtained from BioSource International (Camarillo, CA). *Escherichia coli* (O26:B6) lipopolysaccharide (LPS) was from Difco Laboratories (Detroit). 2,7'-dichlorofluorescein diacetate (DCFH-DA) was from Eastman Kodak (Rochester, NY). PBS was obtained from Life Technologies (Grand Island, NY). PMA and all other enzymes, tissue culture reagents, density gradients, and other biochemicals were from Sigma Chemical (St. Louis).

Animals. Male specific path–free Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) with a body weight of 175–200 g were maintained on a standard laboratory diet and housed in a controlled environment with a 12-h light/dark cycle. Rats were pretreated for 2 days with a subcutaneous injection of recombinant human G-CSF at 50 μg/kg or vehicle (100 μL) twice daily. On the third day, 150 min after the last dose of G-CSF or vehicle, acute ethanol intoxication was induced in rats by intraperitoneal injection of 20% ethanol (5.5 g/kg) in PBS. This alcohol dose produced a blood ethanol concentration of 300–350 mg/dL during the subsequent 4-h observation period in these animals [7, 10]. Control rats were given an equal volume of PBS. Thirty minutes after intraperitoneal injection with ethanol or PBS, endotoxin (100 μg in 0.5 mL of PBS) was administered intratracheally under ether anesthesia. Control animals were intratracheally injected with an equal volume of PBS. Depending on the experimental protocol, rats were sacrificed at either 90 min or 4 h after the intratracheal injection for bronchoalveolar lavage (BAL) and to obtain a heparinized blood sample from the abdominal aorta.

BAL and isolation of pulmonary cells. Lungs were surgically removed under anesthesia and lavaged with cold PBS containing 0.1% dextrose. Recovered lavage fluid was centrifuged at 200 g for 5 min, and the cell pellet was resuspended in PBS. Cells were quantified under a light microscope with a hemocytometer. Cell monolayers were prepared by cytocentrifugation, and Wright-Giemsa stain was used to differentiate AMs and pulmonary recruited PMNLs. The cell suspension was subjected to discontinuous ficoll-hypaque density gradient centrifugation to separate AMs and PMNLs. The isolated AMs and PMNLs were washed twice with PBS and resuspended in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal calf serum (FCS). The viability of the cells was >95% as assessed by trypan blue exclusion. Since few PMNLs were recovered from BAL fluid in saline-injected rats (0.08 ± 0.02 · 10^3/BAL), functional activities in these cells could not be measured.

Phagocytosis. Phagocytosis was determined by previously described methods [21]. In brief, 100 μL of heparinized (10 U/mL) blood or 100 μL of isolated AM or PMNL suspension containing 10^5 cells was incubated with 0.9 mL of DMEM containing 1.25 · 10^4 latex microspheres and 10% FCS in a metabolic shaker at 37°C for 60 min. To measure nonspecific adhesion of latex microspheres on the cell surface, an aliquot of these mixtures was simultaneously incubated at 4°C. At the end of incubation, red blood cells in blood samples were lysed by hypotonic shock. The recovered leukocytes were washed twice and finally resuspended in 0.5 mL of cold PBS containing 5 mM glucose and 0.1% gelatin (PBSg).

CD11b/c and CD18 expression. CD11b/c and CD18 expression on PMNLs was measured as previously reported [21, 22]. Leukocytes obtained from 100 μL of heparinized blood after hypotonic lysis of red blood cells or 10^4 isolated pulmonary recruited PMNLs or AMs were suspended in 100 μL of DMEM containing 10% FCS and 1 μg of FITC-conjugated anti-CD11b/c or anti-CD18 monoclonal antibody or the related isotype antibodies. The mixtures were incubated at 4°C in the dark for 30 min. At the end of incubation, the cells were washed twice with cold PBSg and fixed with 0.5 mL of PBS-buffered 1% paraformaldehyde.

Hydrogen peroxide generation. Intracellular hydrogen peroxide generation was determined by a modification of described protocols [23]. Heparinized blood (100 μL) or isolated AM or PMNL suspension (100 μL) containing 10^5 cells was preincubated with 1.9 mL of PBSg containing 5 mM sodium azide and 5 μL of DCFH-DA (40 μM) in dimethyl sulfoxide (DMSO) in a metabolic shaker at 37°C for 20 min. Thereafter, 0.5 mL of EDTA (25 mM) in PBSg containing 5 mM sodium azide and 100 ng of PMA in 5 μL of DMSO was added to each cell mixture. For the determination of basal intracellular hydrogen peroxide generation, 5 μL of DMSO was added without PMA. The cell mixtures were further incubated at 37°C for 30 min. At the end of incubation, red blood cells were lysed by hypotonic shock. The leukocytes were pelleted by centrifugation and resuspended in 0.5 mL of PBSg containing 3 mM EDTA.

Flow cytometry. The phagocytic activity, β2-integrin expression, and hydrogen peroxide generation of PMNLs and AMs were measured by flow cytometer (ELITE; Coulter, Hialeah, FL) using a 488-nm excitation line from an argon laser. Green fluorescence was monitored at 525 nm. Light scatter gates (using 90° light scatter vs. forward angle light scatter) were selected to identify the PMNLs and AMs. We analyzed 5000 cells within the gated regions in each sample. Results are expressed as percentage of cells that engaged in phagocytosis (percentage phagocytosis) and mean channel fluorescence intensity (MCF) for phagocytosis, CD11b/c and CD18 expression, and hydrogen peroxide generation.

TNF-α and MIP-2. TNF-α and MIP-2 concentrations in BAL fluid were measured by immunoassay kit (BioSource International). Both TNF-α and MIP-2 concentrations are expressed as nanograms per milliliter of BAL fluid.

Statistics. Data are presented as mean ± SEM. Sample size is indicated in each figure and table. Data were compared by unpaired Student’s t test or one-way analysis of variance followed by the Student-Newman Keuls test. Differences were considered statistically significant at P < .05.

Results

Effects of G-CSF on pulmonary recruitment of PMNLs in response to intratracheal LPS. G-CSF pretreatment resulted
in about a 7-fold increase in the number of peripheral PMNLs when compared to those of vehicle-pretreated rats (figure 1). This G-CSF–induced increase in peripheral PMNL counts was not seen in rats 4 h after intratracheal LPS challenge. In the presence of ethanol intoxication, intratracheal LPS did not affect the G-CSF–induced increase in peripheral PMNLs. Neither intratracheal LPS nor a combination of acute ethanol intoxication and intratracheal LPS affected the circulating PMNL counts in vehicle-pretreated animals. In rats instilled with intratracheal PBS, few PMNLs were present in BAL fluid (0.08 ± 0.02 × 10⁶ PMNL/BAL, or 1.63 ± 0.46% of the total cells recovered in BAL fluid). Intratracheal LPS challenge elicited a significant increase in PMNLs recovered by BAL at 4 h as shown in table 1. This LPS-induced pulmonary recruitment of PMNLs was markedly suppressed by acute ethanol intoxication. G-CSF pretreatment augmented the pulmonary recruitment of PMNLs in rats challenged intratracheally with LPS (4.7-fold increase) and attenuated the ethanol-induced suppression of PMNL migration into the lung in response to intratracheal LPS.

Effects of G-CSF on PMNL phagocytosis. As shown in figure 2, intratracheal LPS did not affect MCF phagocytosis by circulating PMNLs in vehicle-pretreated rats in the absence of acute ethanol intoxication (222.80 ± 9.52 vs. 257.70 ± 23.62, P > .05). Pulmonary recruited PMNLs showed a similar level of MCF phagocytosis (283.83 ± 26.15) compared with circulating PMNLs in vehicle-pretreated animals. Acute ethanol intoxication significantly inhibited MCF phagocytosis of circulating PMNLs in vehicle-pretreated rats (146.90 ± 13.54, P < .05) but had no effect on MCF phagocytosis by PMNLs recruited into the lung (250.03 ± 17.93). G-CSF pretreatment enhanced MCF phagocytosis of circulating PMNLs in rats without or with LPS challenge (412.53 ± 40.98 and 469.35 ± 32.37, respectively, P < .05) and overcame ethanol-induced inhibition of MCF phagocytosis in circulating PMNLs (283.10 ± 33.15, P < .05). G-CSF pretreatment also enhanced MCF phagocytosis of pulmonary recruited PMNLs in both the absence and presence of ethanol intoxication (402.27 ± 34.72 and 401.52 ± 39.14, respectively, P < .05) when compared with those of vehicle-pretreated animals.

In vehicle-pretreated rats, acute ethanol intoxication also inhibited percentage phagocytosis of circulating PMNLs compared with those not acutely intoxicated with ethanol (63.72% ± 4.46% vs. 78.08% ± 2.15%, P < .05). G-CSF pretreatment prevented ethanol-induced inhibition of percentage phagocytosis in circulating PMNLs (80.77% ± 3.36%). In the presence of ethanol intoxication, percentage phagocytosis in lung-recruited PMNLs was higher in the G-CSF–pretreated group than in the vehicle-pretreated group.

Effects of G-CSF on PMNL adhesion molecule expression. In vehicle-pretreated rats, CD11b/c expression on circulating PMNLs was not affected by either intratracheal LPS or by acute ethanol intoxication (6.56 ± 0.80 and 6.71 ± 0.38, respectively, vs. 5.71 ± 0.42, P > .05; figure 3). LPS-induced pulmonary recruited PMNLs showed a marked up-regulation of CD11b/c expression (58.24 ± 3.30, P < .05) in comparison with circulating PMNLs. This up-regulation of CD11b/c expression on lung-recruited PMNLs was inhibited by acute ethanol intoxication (32.61 ± 5.91, P < .05). G-CSF pretreatment up-regulated CD11b/c expression on circulating PMNLs (10.05 ± 0.44, P < .05), and this up-regulation of CD11b/c expression on circulating PMNLs was further increased in the presence of intratracheal LPS challenge (14.11 ± 1.72, P < .05). G-CSF pretreatment did not further enhance the up-regulation of CD11b/c expression on pulmonary-recruited PMNLs (56.86 ± 2.85) in response to intratracheal LPS. Ethanol-induced inhibition of CD11b/c up-regulation on pulmonary recruited PMNLs was attenuated by G-CSF pretreatment (42.16 ± 1.83). However, this effect failed to reach statistical significance.

Intratracheal LPS, ethanol intoxication, or G-CSF alone did not alter CD18 expression on circulating PMNLs. In LPS-challenged rats, G-CSF pretreatment resulted in a small but

Table 1. Polymorphonuclear leukocytes (PMNLs) recovered by bronchoalveolar lavage in rats 4 h after intratracheal lipopolysaccharide (LPS).

<table>
<thead>
<tr>
<th>Group</th>
<th>PMNL × 10⁶</th>
<th>% of PMNL in total cells</th>
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<tbody>
<tr>
<td>V/S/LPS</td>
<td>17.43 ± 2.08</td>
<td>80.40 ± 2.85</td>
</tr>
<tr>
<td>V/A/LPS</td>
<td>0.63 ± 0.16</td>
<td>20.20 ± 3.05</td>
</tr>
<tr>
<td>G/S/LPS</td>
<td>82.32 ± 14.67</td>
<td>94.10 ± 1.05</td>
</tr>
<tr>
<td>G/A/LPS</td>
<td>10.02 ± 1.37</td>
<td>76.50 ± 5.78</td>
</tr>
</tbody>
</table>

NOTE: Data are mean ± SEM (n = 10); V, pretreated with vehicle; G, pretreated with granulocyte colony-stimulating factor; S, intraperitoneal injection with PBS; A, intraperitoneal injection with ethanol (alcohol); LPS, intratracheal challenge with LPS.

a P < .05 vs. other groups.

b P < .05 vs. V/S/LPS group.

Figure 1. Circulating polymorphonuclear leukocytes (PMN) 4 h after intratracheal challenge. Data are mean ± SEM (n = 8). Vehicle, pretreated with vehicle; G-CSF, pretreated with G-CSF; S/S, intraperitoneal injection with PBS followed by intratracheal challenge with PBS; S/LPS, intraperitoneal injection with PBS followed by intratracheal challenge with lipopolysaccharide; A/LPS, intraperitoneal injection with ethanol (alcohol) followed by intratracheal challenge with LPS. Bars with different letters (a, b) are statistically different (P < .05).
Figure 2. Mean channel fluorescence intensity (MCF) phagocytosis and % phagocytosis of polymorphonuclear leukocytes (PMN) 4 h after intratracheal challenge. Data are mean ± SEM (n = 6). C-PMN, circulating PMN; L-PMN, lung recruited PMN; vehicle, pretreated with vehicle; G-CSF, pretreated with G-CSF; S/S, intraperitoneal injection with PBS followed by intratracheal challenge with PBS; S/LPS, intraperitoneal injection with PBS followed by intratracheal challenge with lipopolysaccharide; A/LPS, intraperitoneal injection with ethanol (alcohol) followed by intratracheal challenge with LPS. Bars with different letters (a–c) are statistically different (P < .05).

Figure 3. CD11b/c and CD18 expression on polymorphonuclear leukocytes (PMN) 4 h after intratracheal challenge. Data are mean ± SEM (n = 6). MCF, mean channel fluorescence intensity; C-PMN, circulating PMN; L-PMN, lung recruited PMN; vehicle, pretreated with vehicle; G-CSF, pretreated with G-CSF; S/S, intraperitoneal injection with PBS followed by intratracheal challenge with PBS; S/LPS, intraperitoneal injection with PBS followed by intratracheal challenge with lipopolysaccharide; A/LPS, intraperitoneal injection with ethanol (alcohol) followed by intratracheal challenge with LPS. Bars with different letters (a–c) are statistically different (P < .05).

statistically significant increase in CD18 expression on circulating PMNLs (11.60 ± 0.51 vs. 9.48 ± 0.66, P < .05) compared with those of vehicle-pretreated animals. In vehicle-pretreated rats, pulmonary recruited PMNLs exhibited a marked up-regulation of CD18 expression compared with circulating PMNLs (35.18 ± 3.52 vs. 9.48 ± 0.66, P < .05) following intratracheal challenge with LPS. Neither acute ethanol intoxication nor G-CSF pretreatment affected the up-regulation of CD18 expression on pulmonary recruited PMNLs.

Effects of G-CSF on hydrogen peroxide generation by PMNLs. Table 2 shows spontaneous and PMA-stimulated hydrogen peroxide generation by circulating and lung recruited PMNLs. Spontaneous hydrogen peroxide generation by circulating PMNLs was similar among all groups. Pulmonary recruited PMNLs also showed a similar level of spontaneous hydrogen peroxide generation compared with circulating PMNLs, which was not affected by either acute ethanol intoxication or G-CSF pretreatment. PMA-stimulated hydrogen peroxide generation by circulating PMNLs was lower in G-CSF-pretreated rats than in vehicle-pretreated rats (24.30 ± 2.47 vs. 37.82 ± 1.00, P < .05). In both vehicle- and G-CSF-pretreated animals, PMA-stimulated hydrogen peroxide generation by circulating PMNLs was not affected by either intratracheal LPS challenge alone or acute ethanol intoxication plus intratracheal LPS challenge. Pulmonary recruited PMNLs had a less PMA-stimulated hydrogen peroxide generation than did circulating PMNLs in both vehicle- and G-CSF-pretreated rats (11.73 ± 1.38 vs. 32.05 ± 1.49, P < .05; and 10.85 ± 1.03 vs. 21.95 ± 1.87, P < .05, respectively) after intratracheal challenge with LPS. PMA-stimulated hydrogen peroxide generation by pulmonary recruited PMNLs was not affected by acute ethanol intoxication.

AM phagocytosis, CD18 expression, and hydrogen peroxide
AM phagocytosis, CD18 expression, and hydrogen peroxide generation were determined 90 min after intratracheal LPS challenge, and results are shown in table 3 and table 4. AM phagocytosis and CD18 expression were not affected by either acute ethanol intoxication or G-CSF pretreatment (table 3). Similarly, neither ethanol nor G-CSF affected AM spontaneous hydrogen peroxide generation (table 4). However, PMA-stimulated hydrogen peroxide generation by AMs was slightly higher in the G-CSF-pretreated group than in the other groups.

**Table 2.** Hydrogen peroxide generation by polymorphonuclear leukocytes (PMNLs) in rats 4 h after intratracheal challenge.

<table>
<thead>
<tr>
<th>Condition</th>
<th>C-PMNL</th>
<th>L-PMNL</th>
<th>C-PMNL</th>
<th>L-PMNL</th>
</tr>
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<tbody>
<tr>
<td>V/S</td>
<td>3.49 ± 0.74</td>
<td>11.73 ± 1.38</td>
<td></td>
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<tr>
<td>V/S/LPS</td>
<td>3.83 ± 0.18</td>
<td>2.48 ± 0.40</td>
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<tr>
<td>V/LPS</td>
<td>2.98 ± 0.11</td>
<td>21.95 ± 1.87</td>
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<tr>
<td>G/S</td>
<td>2.96 ± 0.36</td>
<td>11.75 ± 1.21</td>
<td></td>
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</tr>
<tr>
<td>G/S/LPS</td>
<td>2.93 ± 0.14</td>
<td>1.87 ± 0.42</td>
<td></td>
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<tr>
<td>G/A/LPS</td>
<td>2.53 ± 0.13</td>
<td>11.75 ± 1.21</td>
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</table>

**Table 3.** Phagocytosis and CD18 expression by alveolar macrophages in rats 90 min after intratracheal lipopolysaccharide (LPS).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Phagocytosis</th>
<th>CD18</th>
</tr>
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<tr>
<td>V/S/LPS</td>
<td>90.55 ± 1.75</td>
<td>42.75 ± 2.43</td>
</tr>
<tr>
<td>V/A/LPS</td>
<td>92.05 ± 0.94</td>
<td>58.88 ± 5.44</td>
</tr>
<tr>
<td>V/S/LPS</td>
<td>92.73 ± 0.38</td>
<td>47.45 ± 2.84</td>
</tr>
<tr>
<td>G/A/LPS</td>
<td>91.58 ± 0.98</td>
<td>52.38 ± 8.00</td>
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**Discussion**

In response to intratracheal LPS, numerous PMNLs migrate from the vascular compartment into the alveolar space, and this LPS-induced recruitment of PMNLs into the lung is significantly suppressed by acute ethanol intoxication. The results of our current study are consistent with previous observations that ethanol impairs PMNL delivery into the intra-alveolar compartment of the lung in animals challenged with intrapulmonary bacteria or endotoxin [2, 7, 9, 10]. In vivo, the recruitment of PMNLs from the circulation into infected tissue sites is a complex phenomenon requiring PMNL margination, adhesion, and transendothelial migration. This multistep process involves an intricate interplay of multiple adhesion molecules (selectins, integrins, and proteins of the super immunoglobulin family) on the surface of both PMNLs and the endothelium of the microvasculature [24]. β2-integrin CD11b/CD18 mediates the firm attachment of PMNLs to endothelial cells and their subsequent transendothelial migration [25]. Previous studies have shown that in vitro exposure of PMNLs to ethanol inhibits PMNL “hyperadherence” to endothelial monolayers after stimulation by FMLP [26]. Ethanol intoxication in vivo does not affect baseline expression of CD11b/c and CD18 on circulating PMNLs but significantly inhibits endotoxin-induced up-regulation of these adhesion molecules on circulating PMNLs [11].

In the present study, pulmonary recruited PMNLs exhibited a marked up-regulation of CD11b/c and CD18 expression as compared with their counterparts remaining in the circulation of rats challenged intratracheally with LPS. These data support the critical role of β2-integrins in mediating PMNL transendothelial migration. Acute ethanol intoxication did not affect CD11b/c and CD18 expression on the circulating PMNLs. However, it suppressed the up-regulation of CD11b/c expression on pulmonary recruited PMNLs. The mechanisms underlying this inhibition of CD11b/c up-regulation in pulmonary recruited PMNLs potentially involve both the direct and indirect effects of ethanol intoxication. In vitro, ethanol at concentrations of 250–1000 mg/dL inhibits FMLP-induced up-regulation of CD11b and CD18 expression on human peripheral PMNLs [26, 27].
Table 4. Hydrogen peroxide generation by alveolar macrophages in rats 90 min after intratracheal lipopolysaccharide (LPS).

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous</th>
<th>PMA (100 ng)-stimulated</th>
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<tr>
<td>V/S/LPS</td>
<td>6.05 ± 0.89</td>
<td>8.70 ± 0.62</td>
</tr>
<tr>
<td>V/A/LPS</td>
<td>7.28 ± 1.13</td>
<td>8.45 ± 0.13</td>
</tr>
<tr>
<td>G/S/LPS</td>
<td>6.68 ± 1.14</td>
<td>11.55 ± 0.70</td>
</tr>
<tr>
<td>G/A/LPS</td>
<td>5.10 ± 0.79</td>
<td>9.08 ± 0.71</td>
</tr>
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NOTE. Data are mean ± SEM (n = 4). V, pretreated with vehicle; G, pretreated with granulocyte colony-stimulating factor; S, intraperitoneal injection with PBS; A, intraperitoneal injection with ethanol (alcohol); LPS, intratracheal challenge with LPS.

*P < .05 vs. other groups of PMA-stimulated hydrogen peroxide generation.

In the present study, intratracheal LPS-induced TNF-α was significantly inhibited in ethanol-intoxicated rats. Our previous studies also showed that the pulmonary TNF-α response to LPS is suppressed by acute or chronic plus acute ethanol intoxication as determined by a cytotoxicity assay using L929 cells [7, 8]. TNF-α is a proinflammatory cytokine that rapidly up-regulates β2-integrin expression on PMNLs in vivo and in vitro [28, 29]. Therefore, alcohol-induced suppression of TNF-α may be one mechanism that impairs the up-regulation of CD11b/c expression on lung-recruited PMNLs in acutely ethanol-intoxicated rats.

Generation of spatial concentration gradients of chemotactic factors in tissue sites of infection is essential in directing the migration of PMNLs to these sites. MIP-2, a member of the C-X-C chemokine family, is a potent chemoattractant in the airways. Instillation of MIP-2 into the alveolar space results in a significant inflammatory response involving PMNLs in both the vasculature and alveolar spaces in rats [30]. Neutralization of MIP-2 with anti–MIP-2 serum causes a 60% decrease in lung PMNL influx in mice with bacterial pneumonia [31]. It is estimated that >75% of the chemotactic activity for PMNLs in BAL fluid from rats challenged intratracheally with LPS can be attributed to MIP-2 [32]. In the current study, intrapulmonary LPS induced a significant increase in MIP-2 production in the lower respiratory tract. Four hours after intratracheal LPS challenge, MIP-2 concentration in BAL fluid increased >75-fold compared with that in control animals challenged intratracheally with PBS. These data support the hypothesis that MIP-2 contributes to the recruitment of PMNLs into the lung in response to local LPS stimulation. Acute ethanol intoxication significantly inhibited the MIP-2 response to intratracheal LPS challenge, thereby suppressing a key signal for the delivery of PMNLs to infected tissue sites.

G-CSF, a lineage-specific hematopoietic growth factor, stimulates PMNL release from the bone marrow and augments PMNL recruitment into the lung in response to infection and inflammatory stimuli [10, 19]. In the present study, rats pretreated with G-CSF had a 7-fold increase in circulating PMNLs. G-CSF also augmented the recruitment of PMNLs into the lung in response to intrapulmonary LPS. The number of lung PMNLs recovered by BAL at 4 h in rats pretreated with G-CSF increased about 5-fold compared with those in vehicle-treated control animals following intratracheal challenge with LPS. Furthermore, G-CSF pretreatment markedly attenuated ethanol-induced suppression of PMNL recruitment in rats challenged intratracheally with LPS. This enhancement of PMNL recruitment into the lung in response to an inflammatory stimulus in the absence and presence of acute ethanol intoxication is likely to result, in part, from the increase in the number of circulating PMNLs stimulated by G-CSF. G-CSF also significantly up-regulated CD11b/c expression on circulating PMNLs, suggesting that this factor primed PMNLs for increased adhesion and facilitated the migration of these cells into the alveolar spaces. Consistent with our previous observations, CD11b/c expression on pulmonary recruited PMNLs was not affected by G-CSF, which suggests that G-CSF may not be able to further enhance the up-regulation of β2-integrin expression on recruited PMNLs in normal animals challenged with intrapulmonary LPS.

In the present study, we also determined the effect of G-CSF pretreatment on LPS-induced TNF-α and MIP-2 production in the lung. The data show that G-CSF did not affect the LPS-induced TNF-α response in the absence or presence of acute

Figure 4. Tumor necrosis factor (TNF)-α and macrophage inflammatory protein (MIP)-2 concentration in bronchoalveolar lavage (BAL) fluid 4 h after intratracheal challenge with lipopolysaccharide (LPS). Data are mean ± SEM (n = 6). Vehicle, pretreated with vehicle; G-CSF, pretreated with G-CSF; S/LPS, intraperitoneal injection with PBS followed by intratracheal challenge with LPS; A/LPS, intraperitoneal injection with ethanol (alcohol) followed by intratracheal challenge with LPS. Bars with different letters (a–c) are statistically different (P < .05).
ethanol intoxication. However, G-CSF significantly suppressed MIP-2 production in the lung in both the absence and presence of acute ethanol intoxication. The mechanism underlying G-CSF–induced suppression of MIP-2 generation remains unclear. Studies have shown that MIP-2 induces a rapid mobilization of hematopoietic progenitor cells (HPC) into peripheral blood, and this MIP-2–induced HPC mobilization is markedly enhanced by G-CSF [33]. It appears that G-CSF and MIP-2 may be functionally linked to each other in mobilizing immune effector cells during the host response to infection and inflammation. Further characterization of this relationship is currently under way. In addition, we previously reported that pulmonary production of cytokine-induced neutrophil chemoattractant, another potent C-X-C chemokine for PMNL chemotaxis, is not affected by G-CSF in rats challenged with intrapulmonary LPS [19]. These data suggest that G-CSF–enhanced tissue recruitment of PMNLs may be primarily based on the effects of G-CSF on the PMNLs themselves and not on the generation of chemotactic stimuli.

Acute ethanol intoxication suppressed the phagocytic activity of circulating PMNLs (MCF phagocytosis and percentage phagocytosis) similar to previous results [9]. G-CSF pretreatment significantly enhanced the phagocytic activity (MCF phagocytosis) of circulating PMNLs and attenuated ethanol-induced suppression of both MCF and percentage phagocytosis in circulating PMNLs. G-CSF also significantly up-regulated the phagocytic activity (MCF phagocytosis) of pulmonary recruited PMNLs regardless of the presence or absence of acute ethanol intoxication. It was previously reported that G-CSF stimulates the phagocytic activity of normal human PMNLs in vitro and corrects the defective phagocytic activity of PMNLs isolated from human immunodeficiency virus–infected patients [15]. G-CSF enhances PMNL phagocytic activity and overcomes ethanol-induced inhibition of PMNL phagocytic function, which may serve as a potential mechanism underlying the beneficial effects of G-CSF in nonneutropenic models of bacterial pneumonia [10].

Acute ethanol intoxication did not affect hydrogen peroxide generation by PMNLs in the present study, which is consistent with other studies [9, 11]. G-CSF has been reported to prime PMNLs for the respiratory burst in vitro. A short-term exposure of PMNLs to G-CSF enhances the respiratory burst in response to FMLP [16]. In the present study, G-CSF pretreatment did not affect hydrogen peroxide generation by pulmonary recruited PMNLs. In addition, PMA-stimulated hydrogen peroxide generation in circulating PMNLs was significantly lower in G-CSF–pretreated animals in both the absence or presence of acute ethanol intoxication. These data suggest that G-CSF induces a selective modulation of PMNL function.

AMs are thought to be the primary cells responsible for the generation of cytokines and chemokines in the airways during lung infection and inflammation. The results of our current study show that pulmonary production of TNF-α and MIP-2 was suppressed by acute ethanol intoxication, and MIP-2 generation in the lung was also inhibited by G-CSF. We further determined whether ethanol and G-CSF exert any direct effects on other AM activities in vivo. Since pulmonary recruitment of PMNLs typically ensues ~2 h after intratracheal challenge, AMs (≥95% pure) were collected from BAL fluid 90 min after intratracheal injection of LPS to avoid the possible effects of recruited PMNLs. In the current study, AM phagocytosis, CD18 expression, and hydrogen peroxide generation were not affected by acute ethanol intoxication. In a prior study in a mouse model, AM phagocytosis and superoxide production were reduced 1 h after ethanol intoxication [3]. In the present investigation, G-CSF did not alter AM phagocytosis and CD18 expression. Only PMA-stimulated hydrogen peroxide generation by AMs was slightly higher in G-CSF–pretreated rats than in vehicle-pretreated controls. The significance and the mechanism underlying this observation remain to be elucidated.

In summary, G-CSF augments the pulmonary recruitment of PMNLs and attenuates ethanol-induced suppression of PMNL recruitment into the lung in response to proinflammatory stimuli. Acute ethanol intoxication inhibits CD11b/c expression on lung-recruited PMNLs and suppresses the phagocytic activity of circulating PMNLs. G-CSF up-regulates CD11b/c expression on circulating PMNLs. G-CSF also enhances the phagocytic activity of both circulating and lung-recruited PMNLs in the absence and the presence of acute ethanol intoxication. These data suggest that G-CSF may be an effective immunomodulator in persons immunosuppressed by alcohol.

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References