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Ethanol Blocks Leukocyte Recruitment and Endothelial Cell Activation In Vivo and In Vitro

Rubina W. Saeed,* Santosh Varma,* Tina Peng,* Kevin J. Tracey, † Barbara Sherry,‡ and Christine N. Metz2*

Immune system impairment and increased susceptibility to infection among alcohol abusers is a significant but not well-understood problem. We hypothesized that acute ethanol administration would inhibit leukocyte recruitment and endothelial cell activation during inflammation and infection. Using LPS and carrageenan air pouch models in mice, we found that physiological concentrations of ethanol (1–5 g/kg) significantly blocked leukocyte recruitment (50–90%). Because endothelial cell activation and immune cell-endothelial cell interactions are critical regulators of leukocyte recruitment, we analyzed the effect of acute ethanol exposure on endothelial cell activation in vivo using the localized Shwartzman reaction model. In this model, ethanol markedly suppressed leukocyte accumulation and endothelial cell adhesion molecule expression in a dose-dependent manner. Finally, we examined the direct effects of ethanol on endothelial cell activation and leukocyte-endothelial cell interactions in vitro. Ethanol, at concentrations within the range found in human blood after acute exposure and below the levels that induce cytotoxicity (0.1–0.5%), did not induce endothelial cell activation, but significantly inhibited TNF-mediated endothelial cell activation, as measured by adhesion molecule (E-selectin, ICAM-1, VCAM-1) expression and chemokine (IL-8, MCP-1, RANTES) production and leukocyte adhesion in vitro. Studies exploring the potential mechanism by which ethanol suppresses endothelial cell activation revealed that ethanol blocked NF-κB nuclear entry in an IκBα-dependent manner. These findings support the hypothesis that acute ethanol overexposure may increase the risk of infection and inhibit the host inflammatory response, in part, by blocking endothelial cell activation and subsequent immune cell-endothelial cell interactions required for efficient immune cell recruitment. The Journal of Immunology, 2004, 173: 6376–6383.

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1 Abbreviations used in this paper: BAC, blood alcohol concentration; EBM, endothelial cell basal media; HuMVEC, human microvascular endothelial cell.

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inhibited TNF-induced endothelial cell activation in vitro, as assayed by adhesion molecule expression, leucocyte-endothelial cell binding, and chemokine production. Further studies show that ethanol reduces NF-κB nuclear localization in TNF-treated endothelial cells. Thus, the immunosuppressive effects of acute ethanol exposure on leucocyte recruitment are mediated, in part, by suppression of endothelial cell activation and immune cell-endothelial cell interactions required for efficient leucocyte trafficking during inflammation and infection.

Materials and Methods

**Abs and reagents**

Human VCAM-1 and E-selectin Abs were purchased from BD Pharmingen (San Diego, CA). Human ICAM-1 Abs were purchased from Chemicon International (Temecula, CA). Mouse VCAM-1 and E-selectin Abs were purchased from BD Pharmingen. NF-κB (p65) and IκBα Abs were purchased from Cell Signaling Technology (Beverly, MA) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. Control Abs for β-actin (cytoplasmic) and total p44/p42 MAPK (nuclear) were purchased from Chemicon International and New England Biolabs (Beverly, MA), respectively. Anhydrous ethyl alcohol (100% v/v) was purchased from Pharmco-Aaper (Oxford, MS). Antileukinate, a CXC chemokine receptor inhibitor that blocks neutrophil recruitment in vivo (24, 25), was a gift from Dr. E. Miller (North Shore-Long Island Jewish Research Institute, Manhasset, NY).

**Animal models**

All experimental procedures using animals were approved by the Institutional Animal Care and Use Committee (IACUC) of the North Shore-Long Island Jewish Research Institute. Animals were housed in a pathogen-free room under standard light and dark cycles with free access to food and water. All experiments were performed at least twice.

**Carrageenan and LPS air pouch models.** The carrageenan and LPS air pouch models were performed as previously described by Garcia-Ramalho et al. (26) and Nakamura et al. (27), respectively. Female Swiss Webster mice weighing 24–33 g (Taconic Farms, Germantown, NY) were used. To generate dorsal air pouches, mice were anesthetized (ketamine/xylazine) on day 0, and 6 ml of sterile air was injected subcutaneously to form a cavity on the dorsum, and then 3 days later, 3 ml of air were injected into the previously formed cavity. On day 6, animals received either vehicle (saline, i.p.) or ethanol (1, 2.5, or 5 g/kg) i.p. To determine ethanol concentrations in animals following i.p. injection (31) they were sacrificed by CO2 asphyxiation. Blood was collected by cardiac puncture using a heparinized 25-gauge needle and ethanol concentrations were determined using the ethanol UV method kit (R-Biopharm, Darmstadt, Germany) according to the manufacturer’s directions.

**Analysis of BAC**

To determine BAC, ethanol (1, 2.5, or 5 g/kg) was administered to mice i.p. at 30 min after ethanol injection, and then 5 and 30 min later (preparatory dose). For determination of BAC by ear exudate in animals following i.p. injection (31) they were sacrificed by CO2 asphyxiation. Blood was collected by cardiac puncture using a heparinized 25-gauge needle and ethanol concentrations were determined using the ethanol UV method kit (R-Biopharm, Darmstadt, Germany) according to the manufacturer’s directions.

**Cells and cell culture**

Primary cultures of adult human dermal microvascular endothelial cells (HuMVECs) were obtained from Clonetics (San Diego, CA). Endothelial cells were maintained in complete growth medium (endothelial growth medium for microvascular cells; Clonetics). Experiments were initiated using synchronized confluent cells (passages 4–8). Because synchronization by serum deprivation induces apoptosis in endothelial cells (32), confluent endothelial monolayers were G0-G1 synchronized (as determined by flow cytometry methods) by contact inhibition (i.e., cells were left in their medium for 2 days postconfluence or left in basal medium (endothelial cell basal medium (ECBM)) 5% FCS for 1–2 days) as previously described (33). For cytotoxicity studies, parallel sets of cells (treated and untreated) were assessed using MIT and neutral red staining performed as previously described (34, 35).

Endothelial cell apoptosis was analyzed by flow cytometry with the Apoptosis Detection kit (R&D Systems) using FITC-Annexin V and propidium iodide staining.

**Analysis of adhesion molecule expression by HuMVECs**

Endothelial cell surface expression of human ICAM (CD54), VCAM-1 (CD106), and E-selectin (CD62E) was determined using a cell-based ELISA technique according to previously published methods (36). Briefly, G2/G1-synchronized endothelial cells (described earlier) were preincubated with basal medium containing 5% FCS alone or basal medium containing 5% FCS plus ethanol (as indicated in the figures) for 1 h before the addition of TNF (1 ng/ml). The cultures were then incubated for 18–20 h before analysis of ICAM-1, VCAM-1, and E-selectin surface expression by cell-based ELISA (n = 4 per condition). Each experiment was repeated at least three times; representative data are shown. Data are presented as percentage of control ± SD, with TNF-treated endothelial cells as controls.

**Cytokine and chemokine expression by HuMVECs**

Culture supernatants from the adhesion molecule expression assays (earlier) were harvested, centrifuged to remove debris, and frozen at −80°C until analysis (n = 4 per condition). IL-8 secretion was analyzed using the Duoset IL-8 ELISA kit (R&D Systems). MIP-1α and MIP-1β were analyzed by ELISA as previously described (37). RANTES and MCP-1 levels were analyzed by ELISA using conditions optimized for each specific recombinant chemokine (purchased from R&D Systems) with Abs purchased from R&D Systems (RANTES, MAB678, and AF478; MCP-1, MAB679, respectively). Data are expressed as percentage of control ± SD, with TNF-treated endothelial cells as controls.
and AF279). Each experiment was repeated at least three times; representative data are shown. The chemokine data are shown as percentage of control chemokine expression ± SD, with the control as chemokine production by endothelial cells treated with TNF (1 ng/ml) for 18–20 h in basal medium containing 5% FCS.

**Western blotting to assess NF-κB nuclear translocation**

Endothelial cells were grown until >90% confluent and incubated in EBM 5% FCS overnight. Cells were treated with ethanol (0.5%) or left untreated for 0.5 h before the addition of TNF (1 ng/ml). After 0.25 h, nuclear and cytoplasmic extracts were prepared using the NE-PER kit (Pierce, Rockford, IL) for Western blot analysis of NF-κB (p65, nuclear) and IκBα (cytoplasmic) expression (p44/p42 and β-actin were as controls for nuclear and cytoplasmic proteins, respectively). Equal amounts of cell lysates (~20 μg/lane, as determined using the BCA assay; Pierce) were electrophoresed using NuPAGE Novex Tris-acetate gels (Invitrogen Life Technologies). Separated proteins were transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA), and then probed with primary Ab followed by incubation with HRP-conjugated secondary Ab. Specific proteins were revealed using the ECL detection system (Amer- sham Pharmacia, Piscataway, NJ). Band densities were determined using the NIH Image program and the ratios of the bands are shown.

**Semiquantitative RT-PCR of HuMVEC cultures**

G0-G1 synchronized endothelial cells were either left untreated or preincubated with ethanol for 1 h before the addition of TNF (1 ng/ml), or treated with TNF alone in EBM containing 5% FCS. Four hours post-TNF addition, the cells were harvested and total RNA was extracted using RNAzol (Tel-Test, Friendswood, TX) according to the manufacturer’s protocol. The cDNA was prepared from 1.0 μg of RNA using 0.25 ng of oligo(dT)12-18 and Moloney murine leukemia virus reverse transcriptase (2). The cDNA was amplified using Supermix (Invitrogen Life Technologies) by PCR in a PerkinElmer model 9600 thermal cycler using specific primers for IL-8 and GAPDH, as previously described (38).

**In vitro leukocyte-endothelial cell adhesion assays**

**Monocyte isolation.** Buffy coats were prepared from human peripheral blood collected from healthy individual donors to the Long Island Blood Bank Services (Melville, NY). Primary blood mononuclear cells were isolated by density-gradient centrifugation through Ficoll/Hypaque (Amer- sham Pharmacia), suspended at 2 × 10^6 cells/ml RPMI 1640 medium with 10% heat inactivated human serum, and seeded onto flasks. After a 2 h incubation at 37°C, the flask was aspirated, gently washed with medium to remove nonadherent cells, and then incubated overnight. Adherent monocytes were washed once with PBS, once with PBS 2 mM EDTA, and gently lifted after adding PBS. Lifted monocytes were washed with HBSS 0.2% BSA and labeled with calcein AM according to the manufacturer’s directions (Molecular Probes, Eugene, OR).

**Neutrophil isolation.** Human neutrophils were isolated from human blood following the sedimentation of erythrocytes using dextran, followed by centrifugation through Ficoll/Hypaque (Amersham Pharmacia), as previously described (39). Remaining RBCs were removed by hypotonic lysis. Neutrophils were washed and labeled with calcein AM according to the manufacturer’s directions (Molecular Probes).

**Endothelial cell adhesion assays.** Synchronized confluent monolayers of HuMVECs grown in 96-well plates (as earlier described) were either untreated or treated for 1 h with ethanol (0.5%) before stimulation with TNF (1 ng/ml) (or no stimulation). Approximately 18 h later (or 5 h later), monocytes (or neutrophils) previously labeled with calcein AM were added (2 × 10^5 cells/well) to washed endothelial cell monolayers (n = 4–6 replicates per sample). After a 1.5 h incubation period, nonadherent leukocytes were removed by gently washing the monolayer three times with RPMI 1640. Leukocyte adhesion was determined by cytofluorescence using a microplate reader (CytoFluor II: Perspective Biosystems, Framingham, MA) with excitation/emission set to 485/530 (± 20) SD. Control adhesion (100%) is reported as average percentage of control (number of inflammatory cells) ± SEM, with saline-treated animals as control.

**Statistics**

The statistical significance between experimental groups (animals and cell cultures) was determined by ANOVA followed by Tukey HSD post hoc analysis to make pairwise comparisons, except for the LPS air pouch model, which was analyzed using a two level ANOVA.

**Results**

**Acute ethanol blocks leukocyte recruitment in vivo**

The efficient elimination of invading pathogens by the host requires the rapid emigration of leukocytes across the endothelial cell barrier to the site of invasion. To test the effect of acute ethanol on leukocyte trafficking we chose the mouse dorsal air pouch model using carrageenan (inflammatory agent) or LPS (bacterial product) as stimuli. In these studies, saline or ethanol (1, 2.5, or 5 g/kg) was administered i.p. 15 min before carrageenan or LPS intrapouch injection. Analysis of the pouch exudative inflammatory cells revealed that acute ethanol treatment significantly reduced cellular recruitment in response to carrageenan in a dose-dependent manner, with up to 90% inhibition at the highest dose (5 g/kg) (Fig. 1A). Mice treated with 1, 2.5, or 5 g/kg ethanol (i.p.) had BACs of 0.074%, 0.186%, and 0.37%, respectively (as a reference, the legal limit in most states is 0.08%). Using this model, neutrophils are the predominant cell type found in the pouch (>90%), with monocytes contributing <10% to the total cell number (26). Flow cytometric analyses using cell-specific Abs showed...
that acute ethanol treatment significantly reduced both neutrophil and monocyte accumulation in the pouch (data not shown). For comparison, we treated animals with antileukikate (20 mg/kg), a hexapeptide CXC chemokine receptor inhibitor that blocks neutrophil chemotaxis in vivo (24, 25) before carrageenan challenge. Ethanol, at 2.5 and 5 g/kg, blocked leukocyte recruitment more effectively than antileukikate (Fig. 1A, inset). The inhibitory effect of alcohol on leukocyte recruitment in vivo was time-dependent (Fig. 1B). Alcohol, when administered to the animals up to 7 h before carrageenan, significantly reduced leukocyte accumulation in the pouch when compared with saline-treated animals. By contrast, ethanol given 20 h before carrageenan had no significant effect on leukocyte trafficking to the pouch when compared with saline-treated animals (Fig. 1B). The dose-dependent reduction in cellular accumulation within the pouch in response to ethanol treatment was reflected by a significant dose-dependent decrease in the carrageenan-induced cellular infiltrate and inflammation in the skin surrounding the pouch when compared with saline-treated animals (Fig. 1C).

Although the carrageenan model is an established model for studying cellular recruitment in response to inflammation, this model does not emulate the process of immune cell trafficking during infection. To examine the effect of acute ethanol administration on immune cell trafficking in response to a bacterial product, we injected LPS into the preformed pouches. We observed that immune cell accumulation in response to LPS within the pouch was significantly (50–60%) blunted when ethanol (5 g/kg) was administered 15 min before LPS challenge (Fig. 1D) when compared with saline (vehicle) treatment. These data suggest that ethanol can inhibit leukocyte recruitment in response to both inflammatory and bacterial product stimuli.

Local exudative fluids generated in response to the injection of carrageenan into the pouch were collected and assayed for TNF, PGE₂, and several chemokines including, MCP-1, MIP-2, MIP-1α, and MIP-1β (Table I). Ethanol treatment (5 g/kg) reduced TNF and PGE₂ levels by ~2.5- and 1.8-fold, respectively, whereas MCP-1, MIP-1α, and MIP-1β levels were more significantly decreased (~3.7- to 6-fold) by ethanol administration (5 g/kg) (Table I).

**Ethanol blocks endothelial cell activation in vivo**

Based on the critical role of the endothelium in mediating immune cell migration, we next examined the effect of acute ethanol treatment on endothelial cell adhesion molecule expression in vivo using the Shwartzman reaction model, a localized cutaneous vasculitis model. Proinflammatory mediators elicit potent proinflammatory and immunomodulatory responses by microvascular endothelial cells resulting in cellular activation. Increased expression of cell surface adhesion molecules by the endothelium is a hallmark feature of cellular activation. When ethanol was administered 15 min before the challenge dose of LPS, we observed a dose-dependent decrease in both VCAM-1 and E-selectin mRNA expression, as determined by RT-PCR (Fig. 2A) and VCAM-1 and E-selectin protein expression by the endothelium, as determined by immunostaining of the tissue (Fig. 2B). By contrast, we were unable to detect VCAM-1 or E-selectin expression on skin tissue obtained from the carrageenan air pouch model. H&E staining of the ear tissue confirmed the reduction in LPS-induced leukocyte accumulation by acute ethanol when compared with saline controls (Fig. 2C). These findings suggest that acute ethanol treatment attenuates endothelial cell activation and leukocyte recruitment in response to LPS in vivo using the Shwartzman reaction model system.

**Ethanol inhibits TNF-mediated adhesion molecule expression by endothelial cells in vitro**

To directly assess the effect of ethanol on endothelial cell activation, we treated HuMVECs with proinflammatory mediators in the absence and presence of an ethanol pretreatment and analyzed endothelial cell adhesion molecule expression in vitro. Treatment of endothelial cells with TNF (1 ng/ml) significantly enhanced ICAM expression, and induced both VCAM and E-selectin expression (Fig. 3A). Preincubation (1 h) of endothelial cultures with ethanol (>0.1%) significantly abrogated TNF-induced expression of these adhesion molecules in a dose-dependent manner, with the most

### Table 1. Analysis of pouch exudates for proinflammatory mediators

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mean (% control) ± SEM</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>TNF-α</td>
<td>100 ± 8.2</td>
</tr>
<tr>
<td>PGE₂</td>
<td>100 ± 7.2</td>
</tr>
<tr>
<td>MCP-1</td>
<td>100 ± 20</td>
</tr>
<tr>
<td>MIP-2</td>
<td>100 ± 25</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>100 ± 22</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>100 ± 27</td>
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</tbody>
</table>

*Pouch exudates were collected from saline (control) and ethanol (EtOH, 5 g/kg) mice and analyzed for soluble inflammatory mediators by ELISA. *p < 0.05 and **p < 0.01, comparing saline-treated vs EtOH-treated.
significant effects (50–60% inhibition) observed at 0.5% ethanol in the presence of 1 ng/ml TNF. Similarly, ethanol (≥0.1%) inhibited the expression of E-selectin, ICAM, and VCAM induced by IL-1β (1 ng/ml) (Fig. 3B). Using our experimental system, incubation of endothelial cells with ethanol alone (0.1–1%) did not significantly alter basal adhesion molecule expression by the cultured endothelial cells (data not shown).

Ethanol reduces TNF-induced chemokine production by endothelial cells

The endothelium is not only a target of proinflammatory mediators, the endothelium is also a source of proinflammatory molecules that when secreted amplify the host inflammatory response. Such molecules (including, but not limited to IL-6, IL-8, MIP-1α, MIP-1β, MCP-1, and RANTES) play an important role in mediating the different aspects of the host response to infection and inflammation. Treatment of cultured HuMVECs with TNF (1 ng/ml) induced the production of IL-8, MCP-1, and RANTES (Fig. 4A). By contrast, TNF-treated HuMVECs did not secrete detectable levels of either MIP-1α or MIP-1β (data not shown). Based on these observations, we next examined whether ethanol suppressed TNF-mediated chemokine (IL-8, MCP-1, RANTES) production by endothelial cells. Ethanol (>0.1%) significantly decreased MCP-1, RANTES, and IL-8 production (∼50–60%) by TNF-induced endothelial cells (Fig. 4A). Correspondingly, ethanol reduced IL-8 mRNA expression, as determined by RT-PCR (data not shown). Similar to its effects on adhesion molecule expression ethanol, when added alone, ethanol had no effect on chemokine expression by endothelial cells (data not shown).

Ethanol (≤0.8%) is not cytotoxic to endothelial cells

To rule out the possibility that the observed effects of ethanol suppression of endothelial cell activation reflected cellular toxicity induced by ethanol and not ethanol-mediated immunosuppression, we assessed cell viability following ethanol treatment. We found that incubation of endothelial cells for 18–20 h with ethanol (≤0.5%), irrespective of TNF addition, was not significantly cytotoxic as determined by MTT staining (Fig. 4B). Studies performed in parallel showed that ethanol (up to 0.8%) did not affect endothelial cell proliferation over 42 h (data not shown). Cytotoxicity was observed at ethanol concentrations ≥0.8%, irrespective of the presence of TNF. Consistent with these findings, we found that ethanol treatment (1% for 24 h; when kept in basal medium containing 5% FCS) increased endothelial cell apoptosis by 2-fold.
Discussion

Alcohol abuse is the third leading preventable cause of death in the United States (41). Alcohol abusers are at increased risk for both trauma-related injuries and subsequent postinjury infections. One significant, but not well-studied problem among adult alcoholics and binge-drinkers is suppression of the immune system. Although the incidence of binge drinking (five or more drinks on one occasion) reported among adults and adolescents is significantly greater than chronic drinking (42), our understanding of the immunosuppressive effects of acute, high dose alcohol exposure on host defense responses is limited. In this study, we show that acute alcohol administration blocks leukocyte recruitment in vivo and endothelial cell activation in vivo and in vitro. To our knowledge, these studies are the first to show the suppressive effects of acute alcohol on endothelial cell inflammatory responses related to immune cell recruitment.

The concentrations of ethanol (1, 2.5, and 5 g/kg) used in these studies represent physiological BACs of ~0.08% (from one to two drinks; the legal limit in many states), 0.19% (from three to four drinks), and 0.37% (over five drinks), respectively. Obviously, the range of BACs among drinkers varies widely depending on many factors. The average BAC observed in admitted trauma patients in the United States was ~200 mg/dL or 0.2% (43), whereas studies conducted in Sweden and France report BACs as high as 0.4–0.5% in many detained drunk drivers (44, 45). Using in vivo air pouch models of localized inflammation (carrageenan) and infection (LPS), we observed that acute ethanol administration significantly reduced leukocyte trafficking in response to inflammatory and bacterial agents (Fig. 1, A and D, respectively).

Interestingly, the time-course data show that a single high dose of ethanol (5 g/kg), given up to 7 h before carrageenan (Fig. 1B), significantly attenuates leukocyte recruitment and this effect is lost by 20 h. These data raise the question of whether ethanol or its metabolite, acetaldehyde, blocks leukocyte trafficking. Given the approximate alcohol clearance time in mice (~0.026% w/v per hour) (31), a 5 g/kg dose of ethanol (which produces a BAC of 0.37%) would require at least 10 h to completely metabolize, suggesting that ethanol may be the mediator. Additional preliminary studies in our laboratory using acetaldehyde treatment and alcohol and acetaldehyde dehydrogenase inhibitors suggest that ethanol, itself, is the responsible mediator (data not shown). Similarly, the clearance time of ethanol in humans is similar to rodents (~0.023% w/v per hour) (44), suggesting that an acute ethanol binge might suppress host immune responses for several hours.

Analysis of the carrageenan pouch exudates revealed that ethanol treatment (at 5 g/kg) reduced TNF and PGE2 levels by ~2- to 2.5-fold and chemokines (MCP-1, MIP-1α, and MIP-1β) by >3.5-fold (Table 1). These variations in mediator levels may be associated with the differential effects of variable doses of ethanol on the source of the mediators (endothelial cells, monocytes, neutrophils, lymphocytes), with their location (within the surrounding tissue area vs within the pouch) and whether ethanol directly or indirectly regulates their synthesis.

Based on our finding that leukocyte accumulation in the pouch was blocked by acute ethanol pretreatment, we hypothesized that endothelial cell activation and subsequent leukocyte-endothelial cell interactions might be targets of the immunosuppressive actions of acute ethanol. We examined the skin surrounding the pouch for the expression (mRNA and protein) of markers associated with endothelial cell activation (E-selectin, ICAM-1, VCAM-1), but were unable to detect significant expression. However, we did observe reduced leukocyte infiltration within the tissue surrounding the pouch with ethanol (Fig. 1C). Using the localized Shwartzman reaction model to assess endothelial cell activation in vivo, we observed that acute ethanol exposure significantly reduced VCAM-1 and E-selectin expression (mRNA and RT-PCR) when given 15 min before LPS challenge (Fig. 2, A and B), and suppressed leukocyte infiltration (Fig. 2C). VCAM-1 binds to lymphocytes and monocytes (but not neutrophils), whereas E-selectin binds to neutrophils, monocytes, and some subsets of T cells (reviewed in Ref. 46), suggesting that acute ethanol exposure could result in a global reduction in leukocyte adhesion and transmigration.

Our findings that acute ethanol exposure suppresses leukocyte migration and alters endothelial cell inflammatory responses in vivo are consistent with previous studies showing the effects of alcohol on numerous cell types in vivo. Acute ethanol intoxication suppressed neutrophil recruitment following an intratracheal LPS challenge (47). Similarly, acute ethanol exposure in mice is associated with decreased macrophage phagocytosis (48) and reduced NK activity (49). Both
human and murine systems show that acute alcohol administration blunts host inflammatory responses to bacterial challenge (reviewed in Ref. 50). The effects of ethanol on host immune responses are variable depending on the type of exposure (acute vs chronic) and whether LPS (vs TNF) or infection is present. Although acute alcohol exposure appears to suppress host responses induced by LPS (51), chronic alcohol administration in animals and human subjects (in the absence of LPS challenge) is associated with 1 enhanced leukocyte recruitment into the liver (52) and 2) elevated cytokine and chemo-

tokine levels (53–57).

To further examine the direct effects of ethanol on endothelial cell activation in response to inflammatory stimuli, we used an in vitro model system of HuMECs treated with TNF. Ethanol (at 0.1–0.5%; representing noncytotoxic concentrations) (Fig. 4B) similar to or less than those used in previous in vitro studies ex-
aminining the effects of ethanol on immune cell function (15, 20, 58–62), when incubated with endothelial cells before TNF admin-
istration significantly reduced cellular activation, as determined by adhesion molecule (E-selectin, ICAM-1, and VCAM-1) expres-
sion (Fig. 3), chemokine (IL-8, MCP-1, RANTES) production (Fig. 4A), and leukocyte (monocyte and neutrophil) binding (Fig. 5). By contrast, ethanol alone (0.1–0.5%) did not induce signif-
cant endothelial cell activation in vitro. Furthermore, ethanol had no effect on TNF expression by HuMECs (data not shown). Our findings are consistent with a previous report showing that treatment of HuVECs with ethanol (0.01–1.0%) significantly re-
duced GM-CSF and G-CSF and IL-8 expression induced by LPS (63). Numerous studies show that ethanol affects immune cell function in response to bacterial or proinflammatory cytokine stim-
ulation in vitro. Alcohol (0.1–2.0%) inhibits TNF secretion by LPS-treated monocytes (58–60), IL-8 release by LPS-treated pe-

eripheral blood monocytes (15); TNF release by LPS-treated human PBLs (61); superoxide production, elastase release, and CD11b expression by neutrophils (20); and IL-8 release by human neu-

trophils following LPS/IFN-y stimulation (62).

To identify the molecular mechanism by which ethanol exerts its immunosuppressive effect on stimulated endothelial cells, we examined the expression and localization of NF-kB, a transcription factor that regulates TNF-induced adhesion molecule expression and cytokine and chemokine production by activated endothelial cells. We found that ethanol pretreatment blocked TNF-induced NF-kB nuclear translocation in an IkB-dependent manner (Fig. 6). Although several studies link ethanol exposure to altered NF-kB activation and localization, the results of these studies are somewhat inconsistent. Ethanol has been shown to activate NF-kB in hepatocytes (64), hepatocellular carcinoma cells (65), Kupffer cells (52), and smooth muscle cells (66). By contrast, ethanol has been reported to decrease NF-kB binding activity in acinar cells (67), LPS-treated macrophages or monocytes and TNF- or IL-1B-treated human peripheral blood monocytes (59, 60); LPS-treated human vascular endothelial cells (63), and T lymphocytes (61). In some cell types (e.g., monocytes or macrophages), the effects of ethanol on NF-kB activation are dependent on IkB degradation, whereas, in other cell types (lymphocytes), the effects of ethanol on NF-kB activation are IkB-independent (61). These variable effects of ethanol on NF-kB activation are likely to be associated with differences in alcohol exposure (acute, semichronic, chronic), dose (low, moderate, high), the model system used (in vitro vs in vivo), the type of cells used (Kupffer, monocytes, macrophages, lymphocytes, and others), the source of cells used (liver, spleen, circulation), and the stimulus (none, LPS, TNF, others) for NF-kB activation.

Together, our data suggest that excessive acute ethanol intake suppresses the host immune response as observed by decreased leukocyte recruitment, chemokine production, and endothelial cell adhesion molecule expression in vivo. Further studies demonstrate that ethanol directly suppresses endothelial cell activation and re-
duces leukocyte-endothelial cell interactions in vitro. By contrast, the effects of chronic alcohol exposure on leukocyte recruitment and endothelial cell activation in vivo may be quite different from those observed in this study using acute ethanol administration. A comparison of acute vs chronic alcohol administration on leuko-

cyte-endothelial cell interactions and endothelial cell activation are the focus of current investigations in our laboratory.

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References


anol in C57BL/6 mice. Life Sci. 43:965.


22. Alon, R., and S. Feigelson. 2002. From rolling to arrest on blood vessels: leu-


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