Mechanism of suppressed neutrophil mobilization in a mouse model for binge drinking: role of glucocorticoids

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Vinson, Robert B., Jennifer L. Carroll, and Stephen B. Pruett. Mechanism of suppressed neutrophil mobilization in a mouse model for binge drinking: role of glucocorticoids. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1049-R1057, 1998.—The goals of this study were to determine if suppression of neutrophil accumulation and TNF-α production in the peritoneal cavity occurs in mice exposed to a chemical stressor [ethanol (EtOH)], to evaluate the role of EtOH-induced increases in endogenous glucocorticoids in any such suppression, and to determine if decreased tumor necrosis factor-α (TNF-α) production is responsible for decreases in neutrophil accumulation in EtOH-treated mice. An inflammatory response induced in the peritoneal cavity of mice by administration of heat-killed Propionibacterium acnes (P. acnes) was suppressed by a single dose of EtOH given 1 h before administration of the bacteria, as indicated by decreased accumulation of neutrophils in the peritoneal cavity. The concentration of TNF-α in the peritoneal cavity was also decreased by EtOH, but exogenous TNF-α did not prevent the suppression of neutrophil accumulation. The glucocorticoid antagonist RU-486 did not prevent the suppression of neutrophil accumulation in mice treated with EtOH, but RU-486 did block suppression of neutrophil accumulation caused by administration of exogenous corticosterone. The suppression of neutrophil accumulation caused by exogenous corticosterone was less than produced by EtOH. These observations suggest that the increase in endogenous corticosterone induced by EtOH may explain some of the suppression of neutrophil accumulation, but other neuroendocrine mediators (or EtOH per se) are insufficient to cause the full suppressive effect when the action of corticosterone is blocked by RU-486. The results also demonstrate that EtOH decreases TNF-α production, but this is not the mechanism by which neutrophil accumulation is decreased in this model.

corticosterone; inflammation; tumor necrosis factor-α; peritoneal cavity

MANY STRESSORS INDUCE neuroendocrine responses that can be immunosuppressive. However, chemical stressors have received relatively little attention. Ethanol (EtOH) is an effective chemical stressor in humans (3, 13, 47, 57) and in rodent models (5, 43). Because a substantial number of adults and 44% of college students are reported to be binge drinkers in the United States (27, 49), many individuals are exposed to this stress. Acute exposure to EtOH suppresses the accumulation of neutrophils at inflammatory sites in the skin in humans (4, 19, 20) and rabbits (35) and in the lungs in rats (31). Acute exposure to EtOH also activates the hypothalamic-pituitary-adrenal axis, leading to elevated concentrations of glucocorticoids in rodents (5, 25, 43). This has also been noted in human subjects in almost all studies in which peak blood EtOH levels were greater than ~0.15% (3, 13, 47, 57) but not in studies in which blood EtOH levels were <0.15% (18, 39, 40, 48, 56, 57). However, it is not known if the stress response induced by acute exposure to EtOH is responsible for suppression of inflammation.

Glucocorticoids at pharmacological levels inhibit neutrophil accumulation in the peritoneal cavity (37). Elevated glucocorticoid levels in rats with experimental choledasis have been implicated in suppressed neutrophil accumulation in subcutaneous air pouches (44). There is also evidence from a particularly well-designed study that endogenous glucocorticoids, at levels that could be induced by some stressors, may regulate production of TNF-α in response to bacterial endotoxin (11). Tumor necrosis factor-α (TNF-α) is thought to be an important mediator of neutrophil accumulation at inflammatory sites in some situations (10). Therefore, it seems possible that EtOH suppresses neutrophil accumulation by increasing glucocorticoid levels, which may decrease TNF-α production or act by other mechanisms to decrease accumulation. However, it is not clear if the levels of glucocorticoids induced by EtOH would suppress TNF-α production or if this suppression alone would inhibit accumulation of neutrophils.

A mouse model for binge drinking that has been characterized and used in a number of studies in this laboratory (5, 21, 22, 50, 51, 53, 54) was also used for the study described here. In this model, EtOH doses of 3–7 g/kg administered intragastrically (by gavage) produce blood EtOH concentrations of ~0.18–0.48% Behavioral changes at these doses range from no grossly detectable behavioral effect to severe ataxia and loss of the righting reflex (in some, but not all animals). Clinical chemistry and histological studies indicate no meaningful nutritional, metabolic, or histological abnormalities, except at the 7 g/kg dose (5). At this dose, a slight, transient elevation of liver enzymes (suggesting mild liver damage) and focal necrosis and hemorrhage in the stomach or ileum were noted in some animals. The higher doses used in this model (6 and 7 g/kg) produce blood EtOH levels not typically attained in binge drinkers, except those who have developed some degree of tolerance to EtOH. However, it seems advisable to include these doses to represent the many individuals who do attain such blood levels, to compensate for the lower sensitivity of mice than humans for...
the neurological and lethal effects of EtOH (14, 36), and to compensate for the more rapid clearance of EtOH by mice than by humans (2, 14, 54).

The purpose of the study described here was to determine the role of EtOH-induced glucocorticoids in the suppression of neutrophil accumulation in the peritoneal cavity in response to heat-killed Propionibacterium acnes, a potent inflammatory stimulus. In addition, the role of glucocorticoid-induced suppression of TNF-α production in decreasing neutrophil accumulation in EtOH-treated mice was evaluated. The data indicate that glucocorticoids play only a minor role in suppression of neutrophil accumulation by EtOH. A brief period of suppressed TNF-α production occurs, but this does not explain the inhibition of neutrophil accumulation observed in EtOH-treated mice.

MATERIALS AND METHODS

Animals

Female B6C3F1 mice were purchased through the National Cancer Institute’s animal program. All mice were quarantined for 2 wk for recovery from shipping stress and adaptation to their new environment before being used for experimental purposes. The mice were 8–12 wk of age and were specific pathogen free. Sentinel mice housed in the same animal room were free of adventitious agents during the period of the study. The mice were housed in an American Association for Accreditation of Laboratory Animal Care-approved facility with temperature- and humidity-controlled conditions and a 12:12-h light-dark cycle. Mice were given Purina Lab Chow and water ad libitum. Care and use of animals followed the National Institutes Health Guide and the policies of the Animal Resources Advisory Committee of Louisiana State University Medical Center.

Administration of Test Agents

Mice were given a 32% (vol/vol) solution of EtOH (high-performance liquid chromatography grade; Quantum Chemicals, Pittsburgh, PA) in tissue culture-grade culture water. The EtOH was administered by gavage using the volume required to achieve the desired dose according to the weight of each mouse. EtOH was administered 1 h before heat-killed P. acnes. This time was selected because EtOH blood levels and the neuroendocrine response to EtOH peak ~1 h after dosing in our model (5). Mice receiving the vehicle instead of EtOH were given water by gavage, using the same volume and the same time of administration as for EtOH. Naïve control groups were left undisturbed. The binge drinking model used in this study has been characterized previously (5). EtOH doses of 5–7 g/kg produce peak blood levels of ~0.25–0.5% associated with behavioral changes ranging from mild ataxia (5 g/kg) to severe ataxia with loss of the righting reflex in a few of the animals (7 g/kg). EtOH activates the hypothalamic-pituitary-adrenal axis in mice, and doses of 5–7 g/kg induce similar peak blood corticosterone levels (~1,000 ng/ml compared with ~100 ng/ml in undisturbed mice). However, the duration of elevated corticosterone levels is dose dependent (5).

Heat-killed P. acnes from Ribi ImmunoChem Research was used in initial experiments to induce a localized inflammatory response in the peritoneal cavity. However, this product was discontinued, so P. acnes (ATCC 6919) was obtained from the American Type Culture Collection (Rockville, MD). The bacteria were grown anaerobically in reinforced clostridial medium (Difco Laboratories, Detroit, MI). The bacteria were heat killed at 63°C for 1 h. The bacterial cells were pelleted by centrifugation at 11,950 g at 4°C. The cells were washed twice with PBS, frozen at −80°C, and lyophilized using a Flexi-dry freeze drier (FTS Systems, Stoneridge, NY).

Before intraperitoneal injection, heat-killed bacteria were suspended in PBS containing 0.5% methylcellulose with 0.1% Tween 80. A vortex mixer was used to uniformly suspend the bacteria in this preparation. Mice in the vehicle groups were given the same PBS solution without P. acnes. In later experiments that involved exogenous TNF-α, heat-killed bacteria were placed in RPMI 1640 and a homogenous suspension was prepared by sonication. The optimum dose of P. acnes was determined in preliminary experiments, the results of which indicated that a dose of 0.03–0.3 mg yielded similar numbers and percentages of neutrophils in the peritoneal cavity. Therefore, a dose of 0.3 mg of heat-killed bacteria was used in the remaining experiments. Bacteria were administered 1 h after EtOH, because it had previously been shown that this is the time point at which the neuroendocrine response to EtOH and EtOH blood levels reach peak values in our model (5).

To evaluate the effects of glucocorticoids on neutrophil chemotaxis and TNF-α production, 18 mg/kg of corticosterone suspended in 10% β-cyclodextrin (Sigma) in Hanks’ balanced salt solution was injected subcutaneously 1 h before administration of P. acnes. This method produces peak blood corticosterone levels and kinetics similar to those noted in mice treated with EtOH at 7 g/kg (5).

RU-486, a glucocorticoid antagonist (obtained through the National Cancer Institute Chemical Synthesis Program from Research Biomedical, Natick, MA), was suspended in PBS with 0.5% methylcellulose and 0.1% Tween 80 and was administered 1 h before the EtOH dose. The mice were given 200 mg/kg, because our previous studies suggest that this dose completely blocks some immunologic effects of elevated levels of glucocorticoids caused by the EtOH (50, 51). Mice in the vehicle groups were given the vehicle solution without RU-486.

Recombinant murine TNF-α was obtained from Genzyme (Cambridge, MA). It was used undiluted in one experiment (1 µg·0.1 ml−1·mouse−1) and diluted in PBS with 0.1% BSA 1:10 (0.1 µg·0.1 ml−1·mouse−1) in a second experiment. The vehicle control groups received PBS with 0.1% BSA (the vehicle in which the product was supplied).

Collection of Peritoneal Fluid and Cells

Mice were euthanized by exposure to carbon dioxide, and peritoneal cells were collected by lavage using either 7 or 1 ml of ice-cold lavage fluid (PBS with 10% fetal bovine serum). The 7-ml volume was used to collect cells, and the 1-ml volume was used for TNF-α assays. Peritoneal cells were washed and resuspended in 1 ml of FACS buffer (PBS with 0.1% sodium azide and 0.1% BSA). Cell density was determined using an electronic cell counter (model Z1, Coulter Instruments, Hialeah, FL). A total of 106 cells in 150 µl of FACS buffer was placed into each well of a 96-well microtiter U-bottom plate for labeling before flow cytometry.

For TNF-α quantification, the peritoneal fluid was removed using 1 ml of lavage fluid. The fluid was then centrifuged at 250 g for 5 min at room temperature to pellet the cells and leave the soluble components in the supernatant fluid. One hundred microliters of the supernatant was used in an assay for TNF-α (described below). In the experiments involving exogenous TNF-α, lavage was done with 1 ml followed by 7 ml. The samples were centrifuged, and the supernatant from the 1-ml sample was used for analysis of
Ethanol Inhibits P. acnes-Induced Neutrophil Accumulation in the Peritoneal Cavity

Time course of the effects of EtOH on neutrophil accumulation in the peritoneal cavity. Mice were given EtOH at 6.0 g/kg, and heat-killed P. acnes was administered 1 h later. Samples were taken by peritoneal lavage. A cytogram illustrating flow cytometric analysis of peritoneal cells with the RB6–8C5 antibody is shown in Fig. 1. The distinction between labeled and unlabeled populations is clear as is the difference between control and EtOH-treated animals.

The results shown in Fig. 2 demonstrate that neutrophils progressively accumulate in the peritoneal cavity from 1 to 4 h after administration of bacteria. However, accumulation of neutrophils at 1, 1.5, and 2.25 h was significantly decreased in mice treated with EtOH at 6.0 g/kg. This effect of EtOH was no longer evident 4 or 8 h after administration of bacteria (5 or 9 h after EtOH administration). It is interesting that this dose of EtOH is mostly cleared within 4 h, and the increased concentration of corticosterone caused by EtOH also returns to near normal values within this timespan (5).

Dose-response study. The results shown in Fig. 3 demonstrate that EtOH at a dose of 5, 6, or 7 g/kg significantly suppresses the accumulation of neutrophils in the peritoneal cavity 2.25 h after administration of P. acnes.

Role of corticosterone in EtOH-induced decreases in neutrophil accumulation. Results shown in Fig. 4 demonstrate that the glucocorticoid agonist RU-486 did
not prevent the EtOH (6 g/kg)-induced decrease in neutrophil accumulation in the peritoneal cavity measured 2.25 h after administration of bacteria. It seemed possible that the effects of RU-486 might be more evident at an earlier time point, so neutrophil accumulation was also assessed 1 h after administration of P. acnes. Even at this early time point (when the concentration of RU-486 was probably greater than at 2.25 h), RU-486 did not prevent the EtOH-induced suppression of neutrophil accumulation (Fig. 4).

Direct administration of exogenous corticosterone (18 mg/kg) caused a significant decrease in neutrophil accumulation measured 2.25 h after administration of P. acnes, and this decrease was blocked by RU-486 (Fig. 5). Similar suppression of neutrophil accumulation by corticosterone was noted in a second experiment, and the relative amount of suppression was also similar when evaluated 1 h after administration of P. acnes (data not shown). Corticosterone values were determined at two time points after administration of corticosterone at 18 mg/kg to confirm that the expected levels were attained at the time this study was in progress (data not shown). The results were comparable to those noted in our previous study (50).

Data in Figs. 3 and 5 indicate that EtOH at 6 or 7 g/kg decreases neutrophil accumulation in the peritoneal cavity by 58 and 82%, respectively. Corticosterone (18 mg/kg) caused a smaller decrease (44%), although...
Corticosterone concentrations and kinetics were comparable in mice treated with corticosterone and mice treated with EtOH at 7 g/kg (5, 50). In a previous study in this laboratory, a parameter whose suppression is completely caused by increased endogenous glucocorticoid concentrations (expression of major histocompatibility complex class II molecules on B cells) was suppressed equally by EtOH and corticosterone using the same protocol as used in the present study (50).

Role of Suppressed TNF-α Production in EtOH-Induced Suppression of Neutrophil Accumulation

EtOH and corticosterone suppress P. acnes-induced TNF-α production in vivo. P. acnes induced the production of TNF-α in the peritoneal cavity at 1.00 or 2.25 h after administration of the bacteria (Fig. 6). At 2.25 h TNF-α production was suppressed by EtOH (6 g/kg) but not by exogenous corticosterone. However, TNF-α production measured 1 h after administration of bacteria was suppressed by corticosterone. In an additional experiment, EtOH (6 g/kg) also suppressed TNF-α production 1 h after administration of P. acnes (435 ± 98 pg/ml in the vehicle control group vs. 12 ± 8 pg/ml in the EtOH-treated group). As already noted, corticosterone was administered according to a protocol developed in this lab that produces peak corticosterone blood levels and kinetics that are essentially the same as observed in mice treated with EtOH at 7 g/kg. Thus administration of corticosterone in this manner results in a somewhat greater exposure than noted in mice given EtOH at 6 g/kg (the dose used in most experiments in this study) (5, 50). Even so, EtOH suppressed TNF-α production to a similar degree and for a longer period of time than did corticosterone.

Effects of EtOH on TNF-α production are primarily indirect. Although the previous experiments demonstrated that corticosterone is not completely responsible for the suppression of TNF-α production in EtOH-treated mice, it remained unclear if other indirect effects (e.g., other neuroendocrine mediators induced by EtOH) were responsible for this suppression or if EtOH acted directly on peritoneal cells to suppress TNF-α production. To address this issue, resident peritoneal cells were exposed to EtOH in vitro. The results shown in Fig. 7 demonstrate that EtOH, at concentrations achieved in our model in vivo (5), does not directly...
affect TNF-α production induced by heat-killed P. acnes in vitro. To demonstrate that suppression can be detected in this system, corticosterone was added to some wells. Concentrations of corticosterone from 75 to 200 ng/ml substantially suppressed TNF-α production measured 4 h after the addition of P. acnes. These concentrations were selected because they span the range of the peak corticosterone concentration noted in EtOH-treated mice (1,000 ng/ml), when it is taken into account that 90% of corticosterone is bound to various proteins in vivo and is not available to bind to intracellular receptors (16). Thus the concentration range used here in vitro includes the expected concentration of free corticosterone in vivo in EtOH-treated mice (1,000 ng/ml x 10% = 100 ng/ml). To confirm that the failure of EtOH to suppress TNF-α production was not caused by rapid loss to evaporation, EtOH concentrations in culture supernatants were monitored during one experiment (Fig. 7). The results indicate little evaporative loss during a 4-h period.

EtOH-induced suppression of TNF-α production does not explain suppression of neutrophil accumulation in the peritoneal cavity. The role of TNF-α in EtOH-induced suppression of neutrophil accumulation in the peritoneal cavity was evaluated by administering exogenous TNF-α (recombinant murine) at the same time (Fig. 8A) or 0.5 h after (Fig. 8B) administration of P. acnes. In the first experiment, the TNF-α dose was 1 µg/mouse, and this did not reverse the effects of EtOH on P. acnes-induced neutrophil accumulation. However, analysis of peritoneal TNF-α levels indicated that this dose produced concentrations much higher than induced by P. acnes (data not shown). Therefore, a TNF-α dose of 0.1 µg/mouse was used in the second experiment. This dose yielded TNF-α concentrations of 1,222 ± 98 pg/ml in the peritoneal cavity (determined by ELISA). However, this did not prevent the EtOH-induced decrease in neutrophil accumulation (Fig. 8). It should be noted that the dose of TNF-α used in this study is comparable to doses shown to induce accumulation of neutrophils in the peritoneal cavity of mice and rats (38, 46). The number of neutrophils per peritoneal cavity is lower in Fig. 8 than in the other experiments shown. This may have been caused by the use of a

Fig. 7. EtOH does not directly suppress TNF-α production by peritoneal macrophages in vitro, but corticosterone does suppress TNF-α production. Resident peritoneal cells were cultured with EtOH or corticosterone (Cort) at the indicated initial concentrations for 1 h before addition of heat-killed P. acnes (A). After a 4-h incubation at 37°C, supernatant samples were removed and analyzed for TNF-α concentration by ELISA. Results shown represent means ± SE for triplicate samples. Results significantly different from control (EtOH at 0%) are indicated by *** (P < 0.001). Two other experiments yielded similar results (data not shown). A replicate set of cultures was sampled to determine the rate of EtOH loss by evaporation (B).

Fig. 8. Exogenous TNF-α does not reverse the effects of EtOH on neutrophil accumulation in the peritoneal cavity. Mice were treated with EtOH or corticosterone 1 h before administration of heat-killed P. acnes. TNF-α was administered immediately after P. acnes. Mice in all groups received either the noted agent(s) or the corresponding vehicle. Vh, groups that received only the vehicles for each agent or P. acnes plus the vehicles for the other agents. All groups were treated with the appropriate vehicle for each agent they did not receive. Neutrophil percentages in the peritoneal cavity were evaluated 2.25 h after administration of P. acnes. Values shown are means ± SE (n = 5 mice/group). A: dose of TNF-α 1 µg/mouse. B: dose of TNF-α 0.1 µg/mouse.
different preparation of \( P. \) \( acnes \) than used for previous experiments. In the experiments shown in Fig. 8, heat-killed \( P. \) \( acnes \) was sonicated in RPMI 1640, not suspended in a methylcellulose-Tween 80 solution as in previous experiments. This change was made to allow more direct comparison of these data with data from in vitro studies (such as those in Fig. 7), which are continuing. In any case, the sonicated \( P. \) \( acnes \) preparation induced significant accumulation of neutrophils in the peritoneal cavity and this was inhibited by EtOH. The inhibition was not reversed by TNF-\( \alpha \).

**DISCUSSION**

The results presented here demonstrate that acute administration of EtOH suppresses accumulation of neutrophils in response to an inflammatory stimulus in a mouse model for binge drinking. In conjunction with previous reports from studies with rats (31) and humans (4, 19, 20), this suggests that suppression of neutrophil accumulation at inflammatory sites may be a common feature of acute EtOH toxicity. Because attraction of phagocytic cells to the site of infection is a key feature of resistance to the infections most often associated with excessive alcohol consumption (pneumonia and tuberculosis) (29, 32), it seems possible that decreased accumulation of neutrophils could be an important mechanism for the increased incidence of such infections in alcoholics. In addition, it has been noted that the incidence of infections in victims of penetrating abdominal trauma is increased by EtOH (17). Another study failed to note such an association (24), but it should be noted that all victims of penetrating abdominal trauma are given antibiotics, which would be expected to obscure the immunosuppressive effects of EtOH.

The results of the present study demonstrate a dramatic but transient decrease in neutrophil accumulation in the peritoneal cavity. This occurs at blood EtOH concentrations that are relevant for humans. EtOH at 5 g/kg produces peak blood EtOH concentrations of 0.25–0.30\% (5, 54), and concentrations in this range are not unusual in human binge drinkers (9). It is interesting that the essentially normal numbers of neutrophils in EtOH-treated mice 4 h after \( P. \) \( acnes \) correspond to the clearance of EtOH from the blood and with the cessation of the EtOH-induced neuroendocrine stress response (5). This suggests that EtOH or the neuroendocrine mediators induced by EtOH must be present to decrease neutrophil accumulation and that this effect does not persist after EtOH has been cleared or the EtOH-induced stress response has subsided. Although the duration of this effect is just a few hours in mice, it should be noted that humans clear EtOH two to three times more slowly than mice (5, 14, 36) and that blood levels of 0.23\% (which are less than the \( \sim 0.4\% \) expected in our mice dosed with EtOH at 6 g/kg) (5) require \( \sim 8 \) h to be cleared in humans (26). In addition, human binge drinkers frequently continue to ingest EtOH for a period of several hours.

Further studies are needed to determine the potential impact of transient decreases in neutrophil accumulation on host resistance to infection in our mouse model. Previous studies in other models suggest that the early stages of an infection are often critical in determining the outcome (7, 55), and in some cases the role of neutrophils early in the infection is critical (7, 8).

It has previously been documented that acute administration of EtOH suppresses inflammation and TNF-\( \alpha \) production in the lungs in a rat model (31) and that acute administration of EtOH induces a neuroendocrine stress response in rats (43) as well as mice (5, 6) and humans (3, 13, 47, 57). Although it is known that mediators of stress responses, such as glucocorticoids, can suppress inflammation (37) and TNF-\( \alpha \) production (11), the role of glucocorticoids in EtOH-induced suppression of inflammation had not previously been determined.

The data presented here are open to at least two different interpretations. It should be noted that RU-486 failed to block the suppression of neutrophil accumulation in EtOH-treated mice and there was considerably less suppression in mice treated with exogenous corticosterone than in mice treated with EtOH. This is the case although the cumulative corticosterone exposure was greater in mice treated with exogenous corticosterone than in mice treated with EtOH. This might be interpreted as indicating that corticosterone plays only a very minor role in the effects of EtOH on neutrophil accumulation in the peritoneal cavity. However, it is interesting that the experiments with RU-486 actually suggest that glucocorticoids play no role, whereas the experiments with exogenous corticosterone suggest that corticosterone should contribute substantially to the overall suppression, although it cannot account for all of it. This apparent conflict can be resolved if one assumes that EtOH suppresses neutrophil accumulation by at least two independent mechanisms. In this case the elimination of one mechanism (e.g., elimination of the effects of corticosterone by administration of RU-486) would not be expected to block the suppression, because the other mechanism(s) would still be operating. However, administration of corticosterone alone would reveal its effects. The stress response induced by EtOH leads to increases in the levels of a number of neuroendocrine mediators that have been shown to affect various components of the immune system (1, 34, 43). For example, we have noted that suppression of natural killer (NK) cell activity in this mouse model for binge drinking is mediated by corticosterone and catecholamines as well as a minor direct effect of EtOH (51–53). The mediators responsible for EtOH-induced suppression of neutrophil accumulation by EtOH remain to be elucidated, and this matter is currently under investigation using an in vitro system with \( P. \) \( acnes \)-activated peritoneal cells. It should also be noted that it remains possible that EtOH (or one of its metabolites) could act directly on relevant cell types in the peritoneal cavity or blood to prevent neutrophil accumulation. These matters are currently under investigation.

It has been shown that TNF-\( \alpha \) can be involved in the accumulation of neutrophils at sites of inflammation.
within or near the time frame during which EtOH affects this process (30, 45, 46). Thus it seemed possible that EtOH-induced decreases in TNF-α production could explain the decreased neutrophil accumulation in the peritoneal cavity noted in EtOH-treated mice. However, exogenous TNF-α did not prevent the suppression of neutrophil accumulation by EtOH. The intraperitoneal TNF-α levels achieved were within a range of physiological values reported in inflammatory responses (12) and were greater than those induced by P. acnes in the present study. Therefore, it seems very unlikely that EtOH-induced suppression of TNF-α production is responsible for the decrease in neutrophil accumulation noted in EtOH-treated mice. It remains possible that EtOH acts by suppressing the response to TNF-α or that suppression of the production of or response to other chemotactic factors (e.g., leukotriene B4, interleukin-8, or cytokine-induced neutrophil chemoattractant) is involved. These mediators can apparently induce neutrophil accumulation in some cases, even in the absence of TNF-α (28, 41, 46). It has also been shown that macrophage inflammatory protein 2 can cause an influx of neutrophils to an inflammatory site, and production of this mediator is not inhibited by glucocorticoids (33). It should also be noted that the profound suppression of TNF-α production noted in EtOH-treated mice would be expected to affect other host resistance mechanisms known to be mediated by this important cytokine. Further studies are needed to more carefully examine the role of glucocorticoids in suppression of TNF-α production by EtOH.

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