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The role of glucocorticoids in the immediate vs. delayed effects of acute ethanol exposure on cytokine production in a binge drinking model

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Abstract

Background—Acute ethanol administration just prior to a stimulus, such as the viral mimic polyinosinic polycytidylic acid (poly I:C), results in decreased proinflammatory cytokine production. Studies have indicated that this suppression is not primarily mediated by glucocorticoids (corticosterone in mice) released in the ethanol-induced stress response. Fewer studies have been done on the effects of acute ethanol administration 12 or more hours prior to a stimulus. The purpose of this study was to determine the role of corticosterone on the relatively persistent immunosuppressive effects of acute ethanol exposure. Also, since gender differences occur in immune responses and stress responses, separate experiments were performed using male and female mice.

Methods—Mice were treated with ethanol 15 minutes or 12 hours before stimulation by poly I:C to demonstrate whether acute ethanol exhibited immunosuppressive effects on cytokine production when administered at these time points. A glucocorticoid synthesis inhibitor was used to manipulate corticosterone levels.

Results—Short-term and persistent effects of acute ethanol exposure on corticosterone and cytokine levels were nearly identical in males and females. Administration of ethanol at 15 minutes and 12 hours before poly I:C yielded significant inhibition of several cytokines. Blocking glucocorticoid synthesis did not alter this inhibition in males, however in females, some or all of the persistent inhibition by ethanol was decreased for some cytokines, particularly in the case of IL-6.

Conclusion—These results indicate that the short-term effects of acute ethanol on poly I:C-induced cytokine production are not primarily mediated by corticosterone in male or female mice. In female mice, however, corticosterone does appear to mediate the persistent effects of acute ethanol administration on poly I:C-induced IL-6 levels. Since many IL-6 related disorders such as autoimmune diseases and osteoporosis are also gender associated, further research into the bidirectional effects of the HPG and HPA axes on alterations in cytokine production mediated by ethanol is warranted.

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Keywords

alcohol; corticosterone; gender differences; IL-6

INTRODUCTION

Results from many studies indicate that acute ethanol exposure suppresses proinflammatory cytokine production when administered just before a stimulus, such as TLR ligands or bacteria [1-4]. Research in our lab has indicated that this suppression is not primarily mediated by glucocorticoids produced as a result of the neuroendocrine stress response [2]. However, fewer studies have investigated the effects of acute ethanol exposure on cytokine production when administered 12 or more hours before stimulation [5]. Preliminary experiments in mice demonstrated that acute ethanol administered as long as 24 hours prior to poly I:C stimulation yielded some inhibition of at least one cytokine, IL-6, and that this inhibition may be mediated by corticosterone (data not published).

Some researchers have demonstrated that direct effects of ethanol such as increased membrane fluidity [6] as well as alterations in receptor recruitment and clustering within lipid rafts (TLR4) [6, 7] play a role in immunosuppression by ethanol. Others have indicated that indirect effects of ethanol such as activation of a neuroendocrine stress response [2, 8] also play a role in immunosuppression. Short-term effects, logically, would include those mediated through direct and indirect mechanisms while the alcohol is still detectable in the blood. Prolonged effects, which are evident after the alcohol has been cleared from the bloodstream, should include those mediated through indirect mechanisms along with any persistent alterations from direct contact with ethanol and its metabolites. In our mouse model, ethanol is cleared from the bloodstream by 8 hours after dosing [9]. Investigation of the prolonged effects of acute ethanol administration may therefore provide useful insight into the mechanisms of immunosuppression, which could aid in developing preventive measures to avoid increased susceptibility to infections.

Gender differences in immune responses are known to occur. Females generally display a stronger response than males, including greater antibody response to foreign antigens, greater allograft rejection, and greater secretion of IFN- γ and IL-1. Additionally, females are more likely to suffer from autoimmune disorders [10-12] such as systemic lupus erythematosus (SLE), which afflicts women at a much higher rate than men (~ 9:1) [13]. Osteoporosis, characterized by decreased bone mass and increased bone fragility, is also more common in women than men, and the pathogenesis of this disease has been linked to cytokines, particularly IL-6 [14]. Human studies comparing mortality rates in male *vs.* female sepsis patients have given conflicting results, likely due to uncontrollable confounding factors [15, 16], but experimental research in mice has given more consistent results. Female mice have greater survival rates than male mice in cecal ligation puncture-induced sepsis [17]. On the contrary, female mice have lower survival rates than males following burn injury, with elevated levels of IL-6 associated with a suppressed cellular immune response in females [18]. Studies have demonstrated that IL-6 production can be inhibited by glucocorticoids [19-21], and increased IL-6 has been linked to SLE [22] and to postmenopausal osteoporosis in women [20, 21]. Therefore, a comparison study of the role of corticosterone in the alterations in poly I:C-induced cytokine production following acute ethanol exposure in male *vs.* female mice could provide insight into the mechanisms for the gender-association of some immune disorders.

MATERIALS AND METHODS

Mice

Female and male C57Bl/6 x C3H (B6C3F1) mice obtained from Jackson Labs (Bar Harbor, Maine) were used in separate experiments. These mice were chosen based on their prior use by the National Toxicology program for immunotoxicology testing. They have been used in our laboratory for years, and were used to develop our mouse model for binge drinking [23]. The mice were allowed to acclimate for at least two weeks after arrival. They were maintained on a 12 hour light-dark cycle in a temperature and humidity controlled animal facility that is accredited by the American Association for Accreditation of Laboratory Animal Care. Animal care and use was in accord with the NIH Guide and LSUHSC policies. Animals were 8-12 weeks of age when experiments were performed.

Administration of test agents and sampling

Mice (5 mice per group) were treated with ethanol (absolute, Pharmco-AAPER, Shelbyville, KY) by gavage (6 g/kg) using a 32% solution in tissue culture grade water (Sigma Chemical Co.). This dosage in mice produces a peak blood ethanol concentration of ~ 0.4% (w/v), which would be extremely high, and usually lethal in humans. However, mice tolerate this level better than humans, and mice clear ethanol 2-3 times faster than humans [23], and therefore, a higher peak level must be attained to achieve the equivalent total exposure. Aminoglutethimide (Sigma Aldrich, St. Louis) was dissolved in DMSO (ThermoScientific, Rockford, IL) to prepare a 500 µg/ml stock solution, and this solution was diluted in filtered corn oil and administered subcutaneously (in 0.2 ml/mouse) to achieve a dosage of 30 mg/kg. The final concentration of DMSO in the corn oil was 2%. Toll-like receptor 3 ligand, poly I:C (Invivogen, San Diego), was administered at a dosage of 100 µg/mouse intravenously (in 0.1 ml/mouse). Aminoglutethimide was administered either 13 hours or 1 hour before poly I:C, and ethanol was administered at 15 minutes or 12 hours before poly I:C. Dosing at 12 and 13 hours before poly I:C administration was performed between 8 and 9 pm, with all other dosing performed the following morning. Blood was collected by retro-orbital bleeding 2 hours after poly I:C treatment. This time was chosen using data from a previous experiment which indicated that peak levels for most cytokines tested occurred at 2 hours after poly I:C administration. This time is not, however, optimal for TNF-α production, which peaks earlier, by 1 hour after poly I:C administration [2]. Blood was allowed to coagulate for 30 min at room temperature and was centrifuged to obtain serum.

Corticosterone and cytokine assays

Serum corticosterone levels were measured using an EIA kit (Assay Designs, Ann Arbor, MI). Interleukin 6 (IL-6) and IFN-α were measured using ELISA kits (BD Pharmingen, San Diego, CA, and PBL Interferon Source, Piscataway, N.J., respectively). Other cytokines (IL-12 (p40), IL-12 (p70), IL-10, IL-1β, IFN-γ, GM-CSF, KC, MIP-1α, MIP-1β, and TNF-α) were measured using a Bio-plex Pro multiplex cytokine assay (Bio Rad, Hercules, CA). Graphing and statistical analysis was performed using Prism GraphPad software, (San Diego, CA). Results were analyzed by ANOVA followed by Newman-Keuls *post-hoc* test.

RESULTS

Acute vs. persistent effects of acute ethanol administration on poly I:C-induced cytokine and chemokine production

Figures 1 and 2 demonstrate the serum corticosterone levels in naive vs. treated female and male mice, respectively. As expected, corticosterone levels were elevated in all groups receiving poly I:C, ethanol, or both. The corticosterone level after aminoglutethimide (AM) treatment will be discussed later. Results shown in Table 1 indicate that acute ethanol

administration shortly (15 min.) before poly I:C treatment decreased the levels of proinflammatory cytokines IFN- α , IL-6, IL-12 (p40), TNF- α , MIP-1 α , and MIP-1 β in serum in both males and females. Tables 3 and 4 illustrate that ethanol administration 12 hours before poly I:C treatment also decreased the levels of these cytokines, although in most cases the decreases were not as great. Interleukin-1- β , IFN- γ , IL-12 (p70), and GM-CSF were below levels of detection in all groups. The anti-inflammatory cytokine IL-10 as well as the chemokine KC did not demonstrate decreased serum levels following ethanol treatment at either administration time. In fact, ethanol treatment just prior to poly I:C resulted in slight increases in serum IL-10, although these were not significant in male mice. Overall, the alterations in cytokine levels due to ethanol administration were evident in both males and females.

The role of corticosterone in the acute effects of ethanol administration on cytokine production

Results shown in Fig. 1 (top) and Fig. 2 (top) demonstrate that, as expected, ethanol treatment shortly before poly I:C or poly I:C treatment alone induced increases in serum corticosterone. The treatment of mice with the glucocorticoid synthesis inhibitor aminoglutethimide prevented the increase in serum corticosterone. Poly I:C-induced increases in serum IFN- α , IL-6, IL-12 (p40), TNF- α , MIP-1 α , and MIP-1 β levels were inhibited by ethanol, and although aminoglutethimide treatment partially abrogated the inhibition of IL-6, MIP-1 α , and MIP-1 β in females, concentrations of these cytokines in the aminoglutethimide group were not increased to the same concentrations as in the poly I:C group (Table 1). This indicates that corticosterone mediates some but not all of the short-term inhibition of these proinflammatory proteins by ethanol in females. Males demonstrated the same pattern of cytokine/chemokine inhibition by ethanol. However, aminoglutethimide treatment prior to ethanol administration did not alter the levels of any of the serum cytokines assayed (Table 1).

The role of corticosterone in the persistent effects of acute ethanol administration on cytokine production

Results shown in Fig. 1 (bottom) and Fig. 2 (bottom) demonstrate that aminoglutethimide treatment 1 hour or 13 hours before poly I:C treatment blocked the increase in corticosterone from poly I:C or poly I:C + ethanol treatment. The poly I:C induced increases in serum IL-6, TNF- α , MIP-1 α , and MIP-1 β levels in females (Table 2) were inhibited by ethanol dosing 12 hours before poly I:C stimulation, and aminoglutethimide treatment 1 hour before poly I:C abrogated the inhibition, indicating that corticosterone mediates the prolonged effects of ethanol on IL-6, MIP-1 α , and MIP-1 β production in female mice. Aminoglutethimide treatment 13 hours before poly I:C dosing did not affect the inhibition by ethanol (-12hr) except in the case of IL-6 and possibly TNF- α (Table 2). Recall that 2 hr after poly I:C administration is not optimal for TNF- α [2], which may explain the fact that at -12 hr the inhibition by ethanol was not significant. Still, aminoglutethimide administered at -1 hr or -13 hr prior to poly I:C, appears to release the inhibition of TNF- α by ethanol. The poly I:C-induced serum IFN- α and IL-12 (p40) levels were also inhibited by ethanol dosing 12 hours before poly I:C stimulation, however, aminoglutethimide treatment at 1 hour or 13 hours before poly I:C did not affect the inhibition of these cytokines, indicating that corticosterone does not play an important role in this inhibition.

Results in male mice (Table 3) demonstrate the same inhibition of serum cytokines noted in females. Treatment with aminoglutethimide at either time, however, did not abrogate this inhibition, suggesting that the role of corticosterone in the ethanol-mediated inhibition of cytokines and chemokines is limited to females.

DISCUSSION

Acute ethanol exposure has been associated with many immunosuppressive effects, such as decreased neutrophil chemotaxis [24], increased T cell apoptosis [25], decreased NK cell activity (Wu, 94, Gallucci, 94), and reduced proinflammatory cytokine production [26]. Activation of the HPA axis leading to release of glucocorticoids has been shown to play a role in some of these effects [8, 27-30], although the predominant mechanism of cytokine alteration by ethanol does not appear to be either activation of the HPA axis or activation of the SNS [2, 3]. Recently, evidence has indicated that acute ethanol acts, in large part, through direct alterations in cellular membranes as well as through direct effects on receptors and receptor complexes themselves [6, 7]. However, these observations did not address any persistent effects from alcohol consumption that may increase susceptibility to pathogens. These persistent effects of acute ethanol intake on cytokine production are the basis of this study.

Acute ethanol administration 12 hours prior to poly I:C stimulation resulted in significant decreases in serum levels of IFN- α , IL-6, IL-12 (p40), TNF- α , MIP-1 α , and MIP-1 β . Blocking steroid synthesis in females by administering aminoglutethimide 1 hour before poly I:C when ethanol was given 12 hours before poly I:C, partially or completely prevented the ethanol mediated inhibition of production of IL-6, MIP-1 α , MIP-1 β , and TNF- α in female mice. This is consistent with inhibition of corticosterone in this system. However, administration of aminoglutethimide 1 hour before ethanol when ethanol was given 12 hours before poly I:C only prevented the ethanol mediated decrease of IL-6 production. These experiments were designed to allow an assessment of whether the major effect of corticosterone elevation on the relatively persistent action of ethanol given 12 hours before poly I:C occurs early when corticosterone is induced by ethanol or later when it is induced by poly I:C. The results showed that inhibition of corticosterone 1 hour before ethanol was not as effective in preventing the action of ethanol on most cytokines and chemokines as administration 1 hour before poly I:C (11 hours after ethanol). This indicates the initial effects of ethanol on the cells that produce these cytokines and chemokines are not sufficient to inhibit production of most cytokines and chemokines unless the corticosterone associated with poly I:C administration is present. Inhibiting corticosterone production 1hr before poly I:C did not affect poly I:C induced production of most cytokines, as we reported before [1, 3]. Thus, both ethanol exposure and elevated corticosterone from poly I:C exposure are required for the persistent inhibitory effects of ethanol (-12hrs) on most cytokine reduction. Perhaps initial exposure to ethanol at -12 hours changes gene expression, rendering the cells sensitive to subsequent inhibition of cytokine/chemokine production when corticosterone is elevated (by poly I:C). Prior studies have demonstrated small, but significant increases in apoptosis of spleen cells [31] as well as decreases in the number of circulating lymphocytes [12] due to ethanol-induced glucocorticoids, and these factors may play some role in immunosuppression. Because preventing increased corticosterone production induced by ethanol 12 hours before poly I:C only prevented inhibition of IL-6 production, it seems that the persistent changes that cause inhibition of the other cytokines and chemokines are not mediated by corticosterone but by other mediators or mechanisms. Nevertheless, it could involve changes in gene expression leading to corticosterone sensitivity at the time of poly I:C administration.

Male mice demonstrated the same pattern of ethanol-mediated suppression of cytokines and chemokines, but not the prevention of inhibition following aminoglutethimide treatment. It is interesting to note that of the cytokines tested in our lab, IL-6 demonstrated significant differences in serum levels induced by poly I:C in females when ethanol was administered 12 or 24 hours before poly I:C. Interleukin-6 has been shown previously to be regulated by endogenous glucocorticoids [1, 8, 32]. Therefore, it was not surprising that long after

ethanol had been cleared from the body, the remaining effects on IL-6 levels in serum appeared to be mediated by corticosterone in the female mouse experiment. However, the blockade of corticosterone synthesis by aminoglutethimide in male mice did not abrogate the inhibition of IL-6 production by ethanol, indicating that steroids may play a greater role in female IL-6 regulation than in males.

Aminoglutethimide inhibits CYP11A1, blocking the conversion of cholesterol to pregnenolone, thus inhibiting all steroid synthesis, and also inhibits aromatase, blocking production of estrogen from androgens. Aminoglutethimide has been used to treat Cushing's syndrome (often referred to as hypercortisolism) as well as hormone dependent breast cancer in postmenopausal women [33, 34]. Acute ethanol exposure in rodents decreases estrogen concentration and inhibits the hypothalamic-pituitary-gonadal axis in females [35]. In a mouse model similar to the one used in the present study, estradiol substantially increased production of IL-6 and other proinflammatory cytokines by peritoneal macrophages in response to LPS stimulation in ovariectomized mice [36]. Thus, the decrease in estrogen that probably occurs in our mouse model in response to ethanol could contribute to decreased production of IL-6 and other proinflammatory cytokines. However, the additional decrease in estrogen expected in mice treated with aminoglutethimide partially prevented the decrease in proinflammatory cytokines caused by ethanol. Thus, it seems unlikely that effects of ethanol on estrogen concentrations contribute substantially to changes in cytokine production. However, results with human subjects suggest that this interpretation may be too simplistic.

High levels of circulating IL-6 reported in postmenopausal women are associated with decreased production of estrogen by the ovaries [37], and IL-6 gene expression is inhibited by estrogens as well as glucocorticoids [19, 38]. This seems to contrast with the report mentioned above indicating that estrogen added back to ovariectomized mice increases IL-6 production in response to LPS. Although it is generally accepted that estrogen restricts unstimulated levels of IL-6 [39], there have been conflicting results with LPS stimulation of human blood mononuclear cells at different estrogen levels [40, 41]. Estrogen receptors have been detected in macrophages [42] and subsets of T lymphocytes [43]. In fact, there are two different isoforms of estrogen receptor (ER), ER α and ER β , and these isoforms are differentially expressed in tissues [44]. Studies utilizing selective ER α and ER β agonists, antagonists, and knockout mice have demonstrated that the anti-inflammatory effects upon activation of these receptors is variable depending on the tissue or cell type as well as the disease or injury model [45, 46]. The mechanisms of action would likely be tissue or cell type specific, based on the local cytokine milieu as well as the availability of transcription factors, coactivators, or corepressors. Differences in concentrations of estrogen, variable distribution of ER isoforms in tissues, local cytokine levels, and multiple mechanisms of activity for estrogen make generalized statements regarding effects of this hormone on IL-6 production difficult. These factors may explain the observation that decreasing estrogen in post-menopausal women and adding estrogen back to ovariectomized mice both lead to increased concentrations of IL-6. Thus, the role of estrogen (or testosterone) in the differential responses of male and female mice to aminoglutethimide in the present study remains unclear. The similarities we have previously noted in the effects of aminoglutethimide, RU486, and adrenalectomy in female mice [1, 2] suggest that corticosterone is the major factor in the effects of these agents. However, differences in the effects of altered corticosterone responses in aminoglutethimide-treated male and female mice suggest that some interaction with sex hormones occurs. Further research focusing on the hypothalamic-pituitary-gonadal (HPG) axis as it is related to corticosterone concentrations and cytokine production is needed to address this matter.

Interleukin-6 is a pleiotropic cytokine which plays an important role in hematopoiesis, immunity, and induction of the acute phase response [47]. The acute phase is the term for the early response to threatening alterations in homeostasis (e.g., infection or injury), beginning with local clot formation and accumulation of leukocytes at the injury site. Once activated, these leukocytes as well as fibroblasts and endothelial cells are capable of releasing cytokines, namely IL-1, IL-6, and TNF, which aid in establishing the systemic response to pathogens [48]. The release of these cytokines, at least in the case of LPS stimulation has been shown to occur in a specific sequence, with TNF- α secreted first, followed by IL-1 β and IL-6 [49, 50]. Although generally considered an inflammatory cytokine, IL-6 is now believed to play an important role in regulating the transition from the innate immune response to the adaptive immune response. Interleukin-6, IL-1, and TNF are capable of stimulating the HPA axis, resulting in increased glucocorticoid production which plays a crucial role in controlling the cytokine response following immune challenge [51]. In fact, one study demonstrated that with poly I:C (but not with LPS) stimulation, the corticosterone response was dependent upon IL-6 [52]. Interleukin-6 alters chemokine release producing a switch from neutrophil recruitment to monocyte recruitment in an inflammatory response [53, 54]. Two chemokines in this experiment which appear to be modulated by steroids in much the same way as IL-6 are macrophage inflammatory protein-1 alpha and beta (MIP-1 α and MIP-1 β). These chemokines attract monocytes and T lymphocytes, and MIP-1 α expression has been induced by IL-6 in the lung [55], suggesting a possible relationship.

Two observations emerging from these results deserve further consideration. The observation that ethanol exerts inhibitory effects on production of a variety of cytokines and chemokines 12 hr after dosing (long after the ethanol has been cleared) demonstrates persistent effects of ethanol on cells of the innate immune system that perhaps have not been fully appreciated. Because corticosterone only seems to be involved in this inhibition in females for a few cytokines, there must be as yet unknown relatively persistent changes caused by the initial ethanol exposure. The observation that inhibition of corticosterone synthesis (as well as adrenalectomy and a glucocorticoid antagonist in previous studies) does little to increase the production of most cytokines and chemokines induced by TLR stimulation indicates that stress-induced corticosterone increases do not play an important role in regulating production of most cytokines in this model. Interleukin-6 is a notable exception, but the general assumption that stress-induced corticosterone associated with inflammation functions to control the overall inflammatory response by preventing excessive production of a wide range of inflammatory mediators may need to be reconsidered. It is also noteworthy that corticosterone only exhibits anti-inflammatory function at high concentrations, while low concentrations of corticosterone even had a proinflammatory role through a glucocorticoid receptor-dependent mechanism (Hee-Young, 2007 #16963). Although pharmacological doses of glucocorticoids are clearly potent anti-inflammatory agents, it seems that the concentrations of corticosterone achieved during inflammatory responses are not sufficient to significantly regulate most cytokines and chemokines.

The results demonstrated here reinforce the theory that effects of acute ethanol administered shortly before poly I:C on cytokine and chemokine production are not primarily mediated by corticosterone in male or female mice. Additionally, the persistent effects of ethanol administration on most poly I:C-induced cytokines and chemokines are not primarily mediated by increases in corticosterone, but by some other mechanism. In female (but not male) mice, however, corticosteroids do appear to mediate the persistent effects of acute ethanol administration on poly I:C-induced IL-6 levels. The mechanism behind these gender differences is not clear. Further study to determine this mechanism may lead to an understanding of the mediators of gender associated risks in a number of disorders.

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Figure 1. Effects of aminoglutethimide and acute ethanol administration at different timepoints on serum corticosterone levels in poly I:C-treated female mice

Female mice were treated with or without ethanol at 6 g/kg by gavage 15 minutes (poly + EtOH) or 12 hours (poly + EtOH -12hr) before poly I:C at 100 µg/mouse, i.v., and with or without aminoglutethimide at 30 mg/kg, s.c. either 1 or 13 hours before poly I:C. Serum samples were collected 2 hours after treatment with poly I:C. Values shown are means $\pm$ SEM for groups of 5 mice. Results were analyzed by ANOVA followed by Newman-Keuls *post-hoc* test. Lowercase letters above bars indicate the results of that analysis; groups that share any letter in common are not significantly different ($p > 0.05$), and groups that do not share any letter in common are significantly different ($p \leq 0.05$).

Figure 2. Effects of aminoglutethimide and acute ethanol administration at different timepoints on serum corticosterone levels in poly I:C-treated male mice

Male mice were treated with or without ethanol at 6 g/kg by gavage 15 minutes (poly + EtOH) or 12 hours (poly + EtOH -12hr) before poly I:C at 100 µg/mouse, i.v., and with or without aminoglutethimide at 30 mg/kg, s.c. either 1 or 13 hours before poly I:C. Serum samples were collected 2 hours after treatment with poly I:C. Values shown are means $\pm$ SEM for groups of 5 mice. Results were analyzed by ANOVA followed by Newman-Keuls *post-hoc* test. Lowercase letters above bars indicate the results of that analysis; groups that share any letter in common are not significantly different ($p > 0.05$), and groups that do not share any letter in common are significantly different ($p \leq 0.05$).

Figure 3. Effects of aminoglutethimide and acute ethanol administration at different timepoints on serum IL-6 levels in poly I:C-treated female mice

Female mice were treated with or without ethanol at 6 g/kg by gavage 15 minutes (poly + EtOH) or 12 hours (poly + EtOH -12hr) before poly I:C at 100 µg/mouse, i.v., and with or without aminoglutethimide at 30 mg/kg, s.c. either 1 or 13 hours before poly I:C. Serum samples were collected 2 hours after treatment with poly I:C. Values shown are means $\pm$ SEM for groups of 5 mice. Results were analyzed by ANOVA followed by Newman-Keuls *post-hoc* test. Lowercase letters above bars indicate the results of that analysis; groups that share any letter in common are not significantly different ($p > 0.05$), and groups that do not share any letter in common are significantly different ($p \leq 0.05$).

Figure 4. Effects of aminoglutethimide and acute ethanol administration at different timepoints on serum IL-6 levels in poly I:C-treated male mice

Male mice were treated with or without ethanol at 6 g/kg by gavage 15 minutes (poly + EtOH) or 12 hours (poly + EtOH -12hr) before poly I:C at 100 μ g/mouse, i.v., and with or without aminoglutethimide at 30 mg/kg, s.c. either 1 or 13 hours before poly I:C. Serum samples were collected 2 hours after treatment with poly I:C. Values shown are means $\pm$ SEM for groups of 5 mice. Results were analyzed by ANOVA followed by Newman-Keuls *post-hoc* test. Lowercase letters above bars indicate the results of that analysis; groups that share any letter in common are not significantly different ($p > 0.05$), and groups that do not share any letter in common are significantly different ($p \leq 0.05$).

Table 1

Short-term effects aminoglutethimide and acute ethanol administration on serum cytokine and chemokine levels (pg/ml) in poly I:C treated mice with or without ethanol (EtOH) or aminoglutethimide (Am). Am was given 1 hr before EtOH and 1.5 hr before poly I:C.

	Male Naive	Male poly I:C	Male poly I:C + EtOH	Male poly I:C + EtOH + Am	Female Naive	Female poly I:C	Female poly I:C + EtOH	Female poly I:C + EtOH + Am.
IFN- α	324 $\pm$ 52**	5589 $\pm$ 352	298 $\pm$ 34**	92 $\pm$ 33**	155 $\pm$ 18**	6138 $\pm$ 340	794 $\pm$ 213**	1068 $\pm$ 136**
IL-6	32 $\pm$ 31**	53415 $\pm$ 3699	10080 $\pm$ 2829**	16823 $\pm$ 3536**	25 $\pm$ 25**	35906 $\pm$ 3450	10236 $\pm$ 1502**	18863 $\pm$ 2771**
IL-12(p40)	308 $\pm$ 75**	17293 $\pm$ 2101	2252 $\pm$ 392**	2308 $\pm$ 104**	281 $\pm$ 53**	2930 $\pm$ 979	2385 $\pm$ 666**	3986 $\pm$ 582**
TNF- α	0 $\pm$ 0	166 $\pm$ 89	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0**	529 $\pm$ 117	9 $\pm$ 6**	7 $\pm$ 6**
MIP-1 α	0 $\pm$ 0**	1639 $\pm$ 90	0 $\pm$ 0**	0 $\pm$ 0**	0 $\pm$ 0**	1761 $\pm$ 61	295 $\pm$ 137**	1131 $\pm$ 188**
MIP-1 β	0 $\pm$ 0**	5331 $\pm$ 416	328 $\pm$ 67**	434 $\pm$ 140**	0 $\pm$ 0**	4922 $\pm$ 208	729 $\pm$ 122**	2123 $\pm$ 478**

p values are as follows:

* significantly different from poly I:C group ($p < 0.05$);

** significantly different from poly I:C group ($p < 0.01$)

Table 2

Persistent effects of acute ethanol administration on serum cytokine and chemokine levels (pg/ml) in poly I:C treated male mice with or without ethanol (EtOH) or aminoglutethimide (Am).

	Female Naive	Female poly I:C	Female poly I:C + EtOH (-12hr)	Female poly I:C + EtOH (-12hr) + Am (-1hr)	Female poly I:C + EtOH (-12hr) + Am (-13hr)
IFN- α	155 $\pm$ 18**	6138 $\pm$ 340	3287 $\pm$ 306*	3159 $\pm$ 630*	2610 $\pm$ 1483*
IL-6	25 $\pm$ 25**	35906 $\pm$ 3450	17056 $\pm$ 828**	39852 $\pm$ 4565	36988 $\pm$ 12500
IL-12(p40)	281 $\pm$ 53**	12930 $\pm$ 979	5935 $\pm$ 454**	6410 $\pm$ 362**	3639 $\pm$ 2142**
TNF- α	0 $\pm$ 0**	529 $\pm$ 117	199 $\pm$ 57	738 $\pm$ 207	1017 $\pm$ 620
MIP-1 α	0 $\pm$ 0**	1761 $\pm$ 61	948 $\pm$ 182**	1426 $\pm$ 181	455 $\pm$ 398**
MIP-1 β	0 $\pm$ 0**	4922 $\pm$ 208	1921 $\pm$ 237**	3804 $\pm$ 491*	2042 $\pm$ 1152**

p values are as follows:

* significantly different from poly I:C group ($p < 0.05$);

** significantly different from poly I:C group ($p < 0.01$)

Table 3

Persistent effects of acute ethanol administration on serum cytokine and chemokine levels (pg/ml) in poly I:C treated male mice with or without ethanol (EtOH) or aminoglutethimide (Am).

	Male Naive	Male poly I:C	Male poly I:C + EtOH (-12hr)	Male poly I:C + EtOH (-12hr) + Am (-1hr)	Male poly I:C + EtOH (-12hr) + Am (-13hr)
IFN- α	324 $\pm$ 52 **	5589 $\pm$ 352	1018 $\pm$ 433 **	1020 $\pm$ 240 **	2261 $\pm$ 968 **
IL-6	32 $\pm$ 31 **	53415 $\pm$ 3699	23659 $\pm$ 6434 **	36562 $\pm$ 5663 *	30087 $\pm$ 8345 **
IL-12(p40)	308 $\pm$ 75 **	17293 $\pm$ 2101	3941 $\pm$ 1973 **	5238 $\pm$ 648 **	3794 $\pm$ 1532 **
TNF- α	0 $\pm$ 0	166 $\pm$ 89	0 $\pm$ 0	35 $\pm$ 28	81 $\pm$ 43
MIP-1 α	0 $\pm$ 0 **	1639 $\pm$ 90	571 $\pm$ 295 **	782 $\pm$ 122 *	740 $\pm$ 306 *
MIP-1 β	0 $\pm$ 0 **	5331 $\pm$ 416	1669 $\pm$ 741 **	2402 $\pm$ 438 **	1715 $\pm$ 662 **

p values are as follows:

* significantly different from poly I:C group ($p < 0.05$);

** significantly different from poly I:C group ($p < 0.05$)