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Ethanol Inhibits Prolactin- and Tumor Necrosis Factor-α-, But Not Gamma Interferon-Induced Expression of Intercellular Adhesion Molecule-1 in Human Astrocytoma Cells

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Abstract In humans, alcohol consumption has multiple effects on the immune system. Despite an increase in our understanding of the effects of alcohol on the immune system, little is known about the effect of alcohol on the neuroimmune response. In the central nervous system (CNS), astrocytes and microglial function as immune effector cells. In response to infection of injury, astrocytes increase in number and size, express several proinflammatory cytokines, MHC class I and II antigens, and several adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1). Interactions between ICAM-1 and its counter-receptors play an important role in the regulation of neuroimmune response. In this study, cultured human astrocytoma cells were used to examine the effect of ethanol on ICAM-1 expression. Western blot analyses show that quiescent astrocytes express, at least, four immunoreactive ICAM-1 proteins with apparent molecular weights 55, 67, 82, and 90 kDa. Incubation of human astrocytoma cells with tumor necrosis factor-α (TNF-α) or prolactin (PRL) resulted in marked increases in all four immunoreactive ICAM-1 proteins. In the presence of ethanol, however, PRL- and TNF-α-induced increases in all four immunoreactive ICAM-1 proteins were markedly inhibited. ICAM-1 is a cell surface transmembrane glycoprotein. Using a cell surface specific ICAM-1 adhesion assay we found that in human astrocytoma cells TNF-α, interferonγ (IFN-γ) and PRL increased cell surface ICAM-1 expression. Consistent with our Western blot analyses, ethanol significantly inhibited TNF-α- and PRL-induced cell surface ICAM-1 expression. By contrast, IFN-γ-induced ICAM-1 expression was not inhibited by exposure of the cells to ethanol. Expression of ICAM-1 is regulated predominantly at the transcriptional level. In the present report, we show that TNF-α increased ICAM-1 mRNA levels in human astrocytoma cells and that ethanol markedly blocked TNF-α-induced increases in ICAM-1 mRNA levels. Further, we found that PRL-induced ICAM-1 expression was, at least in part, due to a PRL-induced increase in TNF-α syntheses and secretion. Our results clearly indicate that ethanol has a pronounced effect on ICAM-1 expression in human astrocytoma cells, thus suggesting that ETOH exposure may impair the immune response in the CNS by blocking leukocytes adhesion and migration into the CNS in response to injury or infection. J. Cell. Biochem. 77:455–464, 2000.

Key words: ethanol; prolactin; astrocytoma; intercellular adhesion molecule-1; tumor necrosis factor-α

The immunosuppressive effects of chronic alcohol use have been well documented. Acute or chronic ethanol intake affects the functions of T and B lymphocytes, natural killer (NK) cells, and neutrophil and monocyte activities [Szabo et al., 1995; Verma et al., 1993; Szabo, 1993]. Similarly, fetal alcohol exposure results in immunodeficiency in humans and animals [Gottesfeld and Abel, 1991]. Despite the multiple effects of ethanol in the central nervous system (CNS), and compelling evidence indicating that alcohol is a immunosuppressive drug, little is known about

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the cellular and molecular effects of ethanol on the neuroimmune system.

In the CNS, astrocytes function as immunocompetent cells. In response to injury or infection astrocytes divide, increase in number and size, express an array of cytokines, adhesion molecules, MHC class I and II antigens, and can present antigen to T-cell clones in a MHC-restricted response [Benveniste, 1992; Hickey et al., 1985]. This process termed astrogliosis, is the most frequent cellular reaction to CNS injury or infection, and is found in many neurological disorders, including multiple sclerosis (MS), acquired immune deficiency syndrome dementia complex, Alzheimer's disease, and the animal model for MS, experimental allergic encephalomyelitis (EAE) [Hickey et al., 1985; Benveniste, 1992; Martin et al., 1992].

Adhesion molecules play a role in many biological processes, such as embryonal development, hematopoiesis and are critical for normal immune function. Adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) are involved in the regulation and recruitment of immunocompetent cells, mediate antigen presentation and effector functions of T cells, B cells, and NK cells by increasing intercellular contact [Springer, 1990; Dougherty et al., 1988; van de Stople and van der Saag, 1996]. ICAM-1 is a cell surface glycoprotein belonging to the immunoglobulin (Ig) supergene family and serves as a counter-receptor for leukocyte β-2-integrins, e.g., LAF-1 (CD11a/CD18) and Mac-1 (CD11b/CD18), and sialophorin (CD43) [Diamond et al., 1990; Sanchez-Madrid et al., 1983; Staunton et al., 1988]. Interactions between ICAM-1 and these counter-receptors play an important role in T-cell-mediated defense mechanisms and in the generation of an inflammatory response [Springer, 1990].

ICAM-1 is expressed on numerous hematopoietic and nonhematopoietic cells, such as lymphocytes, endothelial cells and in the CNS [Shibayama et al., 1996; Moynagh et al., 1994; Satoh et al., 1991]. In the CNS, ICAM-1 can be expressed by several cell types including neurons, oligodendrocytes, microglia, and astrocytes [Ballestas and Benveniste, 1995]. Regulation of ICAM-1 expression is cell type specific and depends on the cytokine/hormone receptors, signal transduction pathways and transcription factors available in a given cell type. In astrocytes, ICAM-1 mRNA and protein are constitutively expressed, and ICAM-1 expression is enhanced by proinflammatory cytokines, such as TNF-α, IFN-γ and IL-1 [Shrikant et al., 1994, 1995; Ballestas and Benveniste, 1995; Frohman et al., 1989; Hurwitz et al., 1992]. In the CNS, PRL is a novel cytokine stimulating astrocyte mitogenesis and cytokine expression [DeVito et al., 1992, 1993, DeVito et al., 1995b]. We have shown that ethanol inhibits prolactin-induced mitogenesis and TNF-α expression in cultured rat astrocytes [DeVito et al., 1997]. In this report, we examined the effect of alcohol on growth factor and cytokine-induced ICAM-1 expression in human astrocytoma cells. We report that ethanol markedly inhibits PRL- and TNF-α-, but not IFN-γ-induced ICAM-1 expression. Similarly, ethanol blocked TNF-α-induced increases in ICAM-1 mRNA levels. Our results indicate that ethanol can inhibit cytokine and hormone induced ICAM-1 expression which could result in an inappropriate neuroimmune response.

MATERIALS AND METHODS

Materials

Recombinant human TNF-α and IFN were obtained from the Genzyme Corporation (Cambridge, MA). Other materials were purchased from the following sources: D,L-dithiothreitol (DTT), N-oleoylethanolamine, RPMI, sodium dodecyl sulfate (SDS), glycerol, β-mercaptoethanol, and bromophenol blue from Sigma Chemical Co. (St. Louis, MO); calf serum from Gibco-BRL (Grand Island, NY). All other chemicals and reagents were obtained from commercial sources and were of reagent or molecular biology grade.

Cell Culture

Human astrocytoma cells were obtained from American Type Culture Collection (Rockville, MD). Cells were seeded at 2×10^5 cell/cm^2 in RMPI medium 1640 containing 1% fetal bovine serum (FBS) and cultured for 7 days at 37°C, humidified incubation under an atmosphere of 5% CO_2, 95% air. Before use, cells were dispersed by trypsin, counted in a hemocytometer and cell viability determined by exclusion of Trypan blue.

Western Blot Analysis of ICAM-1

Cells were homogenized in 25 mM Tris-HCl pH 7.4, including 0.25 M sucrose, 1 mM phe-

nylmethylsulfonyl fluoride (PMSF) and aprotinin 100 kIU, and centrifuged (1,000*g*) for 15 min at 4°C. The supernatant was centrifuged for 30 min at 100,000*g* at 4C and the resulting microsomal fraction was dissolved in sample buffer (0.65 M Tris, 4% sodium dodecyl sulfate (SDS), 20% glycerol, 10% β-mercaptoethanol, and 0.01% bromophenol blue) and proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) on 0.75-mm-thick slab gels, using a 4% polyacrylamide stacking gel and a 15% resolving gel. Electrophoresis was carried out at 20 mA for approximately 2 h using a Hoefer sturdier electrophoresis apparatus (Hoefer Scientific Instruments, San Francisco, CA). Proteins were electrophoretically transferred onto nitrocellulose at 100 V for 2 h at 4°C. ICAM-1 immunoreactive proteins were detected by enhanced chemiluminescence (ECL), using a human anti-ICAM-1 antibody (Santa Cruz) as previously described [DeVito et al., 1995a].

TNF-α Bioassay

TNF-α activity was determined in a biologic assay using WEHI 164 mouse fibrosarcoma cells as described by Chung and Benveniste [Chung and Benveniste, 1990]. The absolute concentration of TNF-α activity was determined by extrapolation from the standard curve, which was generated using known amounts of recombinant human TNF-α. Briefly, cells growing in log-phase were harvested and resuspended at a concentration of 4×10^5 cells/ml RPMI 1640 media. A total of 100 μl of this suspension was added per well, 100 μl of either sample or TNF-α standards were added and incubated at 37°C in triplicate. After incubation, cytotoxicity was assessed using the MTT cytotoxicity assay. Ten ul of MTT (5 mg/ml) was added to each well, and incubated for 4 h at 37°C, and crystals were solubilized by the addition of acid-isopropanol (100 μl of 0.04 M HCl in isopropanol) and mixed in. After 15 min at room temperature, the plates were read on a Labsystems Multiskan MS plate reader at a test wavelength of 595 nm and a reference wavelength of 650 nm. TNF-α concentrations were extrapolated from the linear portion of the standard curve.

ICAM-1 Cell Adhesion Assay

Astrocytoma cells were plated into 96-well microtiter plates (1×10^4 cells/0.2 ml) in RPMI 1640 complete medium and allowed to adhere for 48 h. Cells were pretreated with ETOH for 18 h and then stimulated with PRL, IFN-γ, or TNF-α in the presence or absence of ethanol as describe in the figure legends. Stimulation was terminated by removal of medium followed by 2 washes with 200 μl RPMI containing 2.5% (v/v) FBS. Cells were incubated with anti-human ICAM-1 (0.15 μg/100 μl) in RPMI containing 2.5% (v/v) FBS for 45 min at room temperature. Cells were washed 3 times with phosphate-buffered saline (PBS) containing 2.5% FBS (v/v) and treated with 0.025% (v/v) glutaraldehyde for 5 min at room temperature. Cells were washed twice with PBS containing 2.5% FBS (v/v) for 5 min and once with media for 45 min. Cells were incubated with anti-mouse IgG AP conjugate for 45 min at room temperature. Cells were then washed twice with PBS containing 2.5% (v/v) FBS and once with PBS alone. Cells were incubated with 3 mM NPP containing 0.05 M $NaCO_3$ and 0.05 mM $MgCl_2$. Color development was terminated by the addition of 0.5 N NaOH and measured spectrophotometrically at 405 nm in a Labsystems Multiskan MS plate reader.

Isolation of Total Cellular RNA And Northern Blot Analysis

Total cellular RNA was isolated by the acid guanidium-thiocyanate-chloroform extraction method [Chomczynski and Sacchi, 1987]. Equal amounts of total RNA were fractionated by electrophoresis and blotted on Duralose-ultraviolet (UV) filters (Stratagene, La Jolla, CA) as previously described [Tang et al., 1995]. The filters were hybridized to a ^{32}P-labeled human ICAM-1 probe overnight at 42° or 55°C. After several washes, filters were exposed to Fuji RX film (Fuji Photo Film Co., Tokyo, Japan). Blots were stripped and hybridized to a ^{32}P-labeled human GAPDH probe. The relative amounts of ICAM-1 mRNA were normalized to GAPDH mRNA content.

RESULTS

To determine the molecular identity of ICAM-1 in human astrocytoma cells Western blot analyses were performed using a specific ICAM-1 antibody. In quiescent human astrocytoma cells, Western blot analysis detected low levels of four ICAM-1 immunoreactive proteins with apparent molecular weights 55, 67, 82,

and 90 kDa (Fig. 1). The 55-kDa immunoreactive protein is consistent with the predicted molecular weight of the nonglycosylated ICAM-1 core protein. The 90-kDa immunoreactive protein is consistent with the reported size of the mature cell surface ICAM-1 protein. To examine the effect of ethanol on TNF-α- and PRL-induced ICAM-1 proteins human astrocytoma cells were preincubate with ethanol (50 mM) for 18 h and then stimulated with TNF-α or PRL for 18 h in the presence, or absence of ethanol. Incubation of human astrocytoma cells with TNF-α (50 ng/ml) or PRL (10 nM) for 18 h resulted in marked increases in all four immunoreactive ICAM-1 proteins (Fig. 1, lanes 2 and 3). As shown in Figure 1, incubation of quiescent cells with ethanol (50 mM) for 36 h did not markedly affect the levels of the four immunoreactive ICAM-1 proteins (Fig. 1, lane 4). In the presence of ethanol, however, PRL- and TNF-α-induced increases in all four immunoreactive ICAM-1 proteins were markedly inhibited (Fig. 1, lanes 5 and 6).

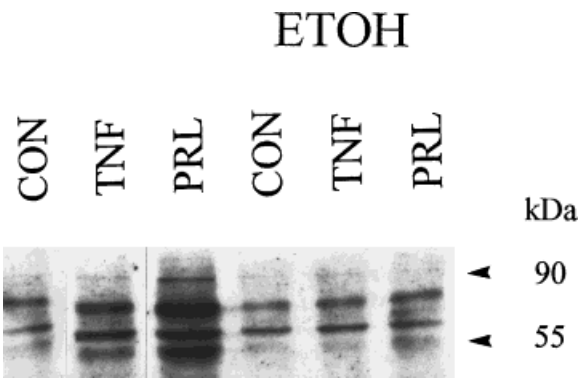

Fig. 1. Western blot analysis of extracts from human astrocytoma cells, using an ICAM-1-specific antibody. Cells were incubated in control media or in media containing ethanol (50 mM) for 18 h before stimulation. Cells were then stimulated with PRL (10 nM) or tumor necrosis factor-α (TNF-α) (100 ng/ml) for 18 h in the absence (**lanes 2,3**), or presence of ETOH (**lanes 5,6**). Whole cell extracts were prepared from control and ETOH-treated cells, separated SDS-PAGE, transferred to nitrocellulose membranes, and probed with a ICAM-1 specific antibody.

ICAM-1 is a cell surface transmembrane glycoprotein. To examine the effect of ethanol on cell surface expression of ICAM-1 we used a specific ICAM-1 adhesion assay. In unstimulated human astrocytoma cells, cell surface ICAM-1 was easily detected by the ICAM-1 adhesion assay (Fig. 2). In the absence of ethanol, incubation of human astrocytoma cells TNF-α for 18 h resulted in a dose-dependent increase in cell surface ICAM-1 levels, with 100 ng/ml TNF-α increasing ICAM-1 expression by fourfold (Fig. 2). In human astrocytoma cells exposed to ethanol (50 mM) before and during TNF-α stimulation, completely blocked TNF-induced increases in cell surface ICAM-1 levels. As found with TNF-, incubation of human astrocytoma cells with PRL (10 and 100 nM) resulted in a marked increase in cell surface ICAM-1 expression (Fig. 3). In the presence of ethanol (50 mM), however, PRL-induced increases in cell surface ICAM-1 expression were completely blocked (Fig. 3). In rat pups blood ethanol concentrations in the range of 50 mM results in microencephaly. Therefore, we determine whether lower ethanol concentrations inhibits TNF-induced ICAM-1 expression. As illustrated in Figure 4,

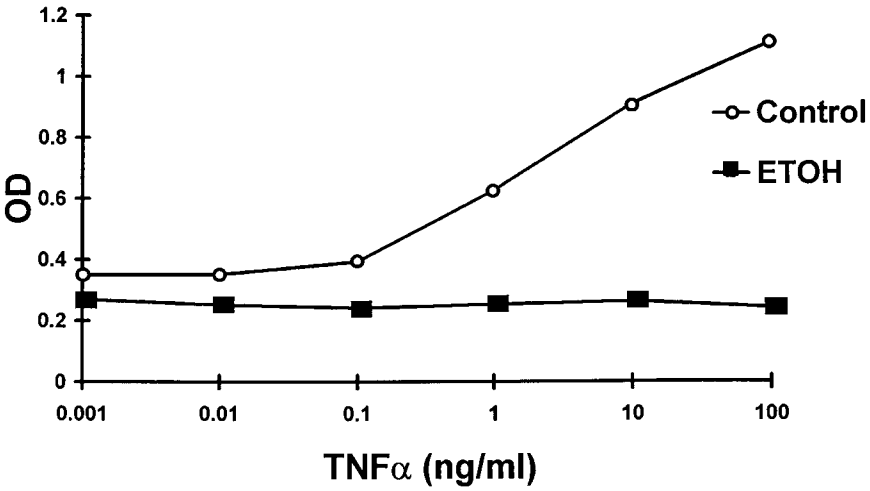

Fig. 2. Effect of ethanol on tumor necrosis factor-α (TNF-α)-induced ICAM-1 expression in human astrocytoma cells. Cells were incubated in control media or in media containing ethanol (50 mM) for 18 h before stimulation. Cells were then stimulated with increasing concentrations of TNF-α in the presence and in the absence of ethanol (50 mM) for 18 h.

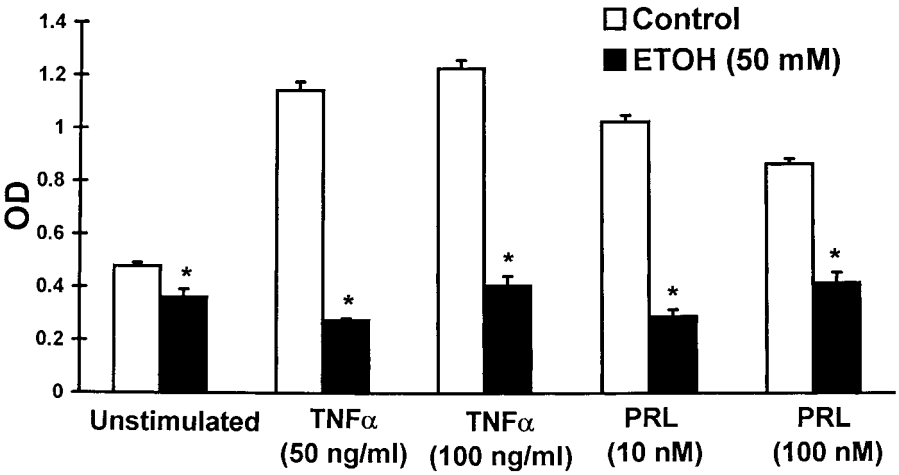

Fig. 3. Effect of ethanol on tumor necrosis factor-α (TNF-α)- and prolactin (PRL)-induced expression of ICAM-1 in human astrocytoma cells. Cells were incubated in control media or media containing ethanol (50 mM) for 18 h before stimulation. Cells were then stimulated with TNF-α or PRL in the presence and in the absence of ethanol (50 mM) for 18 h. Each value represents the mean ± SEM, n = 5. *$P < 0.05$), as compared with controls.

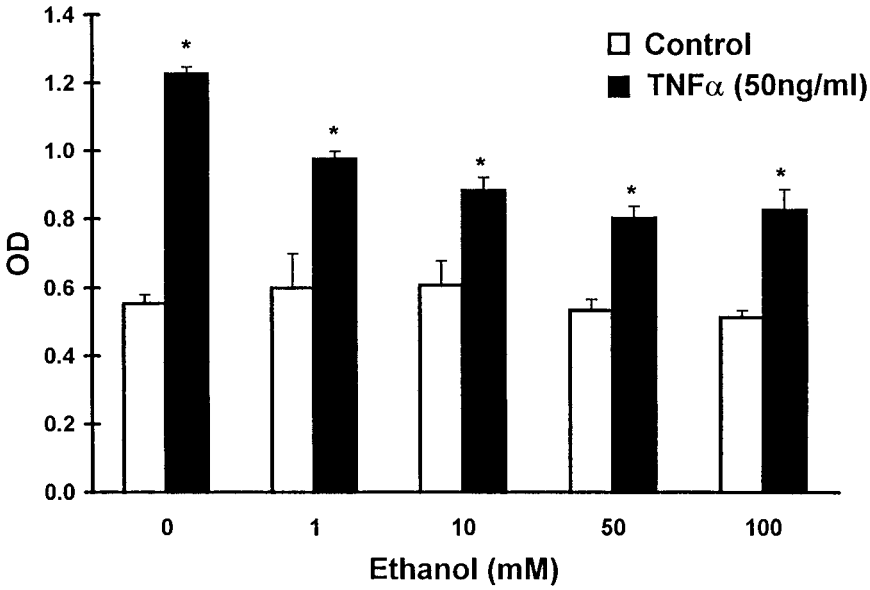

Fig. 4. Effect of ethanol on tumor necrosis factor-α (TNF-α)-induced expression of ICAM-1 in human astrocytoma cells. Cells were incubated in control media to confluence. Cells were then incubated for 18 h in control media or in media containing increasing concentrations of ethanol for 18 h. Cells were then stimulated with TNF-α in the absence (open bars) or presence (closed bars) of ethanol for 18 h. Each value represents the mean ± SEM, n = 5. *$P < 0.05$), as compared with TNF-α stimulated controls in the absence of ethanol.

incubation of human astrocytoma cells with ethanol concentrations ranging from 1 to 100 mM revealed that low concentrations of ethanol (e.g., 1 mM), inhibited TNF-induced cell surface ICAM-1 expression (Fig. 4). Maximal inhibition of ICAM-1 expression was found in human astrocytes incubated with approximately 50 mM. Using the MTT cytotoxic assay we found that ethanol concentrations of >100 mM were cytotoxic under the assay conditions employed in these studies (data not presented).

To determine whether ethanol inhibits other known activators of ICAM-1 expression we examined the effect of ethanol on IFN-γ-induced ICAM-1 expression in human astrocytoma cells. In human astrocytoma cells grown in the absence of ethanol, IFN-γ increased cell surface ICAM-1 expression (Fig. 5). By contrast to the inhibitory effect of ethanol on TNF-α-induced ICAM-1 expression, however, ethanol did not inhibit IFN-γ-induced ICAM-1 expression.

TNF-α regulates the expression of ICAM-1 predominantly at the transcriptional level. In quiescent human astrocytoma cells, Northern blot analyses revealed low or undetectable levels of ICAM-1 mRNA (Fig. 6). In the absence of ethanol, TNF-α (50 ng/ml) resulted in a time-dependent increase in ICAM-1 mRNA levels reaching peak levels at 4 h and returned to low or undetectable levels 24 h after stimulation. By contrast, in human astrocytoma cells preincubated with ethanol (25 mM) and stimulated with TNF-α in the presence of ethanol, there were

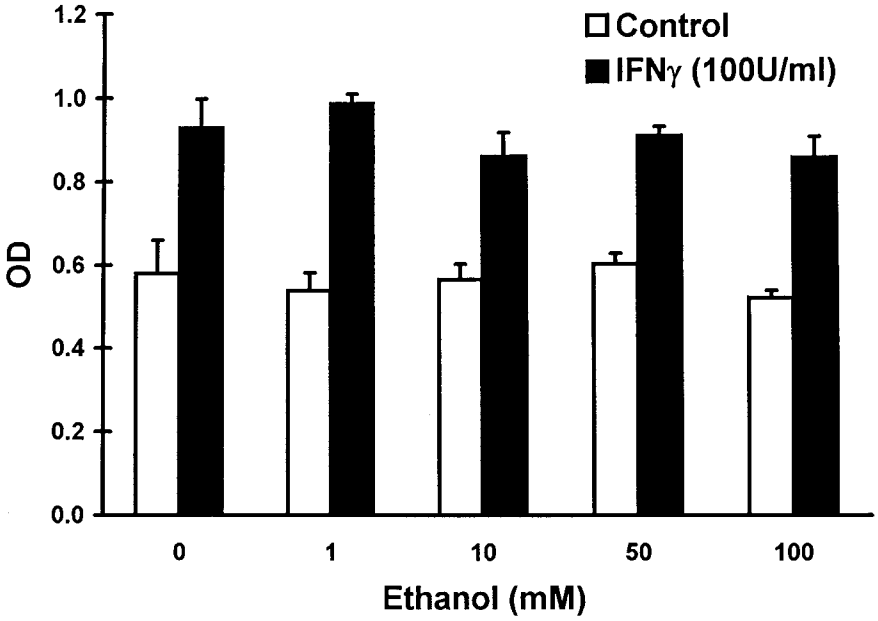

Fig. 5. Effect of ethanol on interferon-γ (IFN-γ)-induced expression of ICAM-1 in human astrocytoma cells. Cells were incubated in control media to confluence. Cells were then incubated for 18 h in control media or in media containing increasing concentrations of ethanol for 18 h. Cells were then stimulated with tumor necrosis factor-α (TNF-α) in the absence (open bars) or in the presence (closed bars) of ethanol for 18 h. Each value represents the mean ± SEM, n = 5. $*P < 0.05$), as compared with TNF-α-stimulated controls in the absence of ethanol.

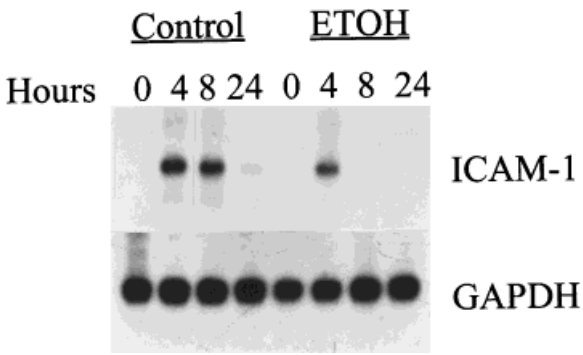

Fig. 6. Representative Northern blot analysis of the effect of ethanol (50 mM) on tumor necrosis factor-α (TNF-α)-induced increases in ICAM-1 mRNA levels in human astrocytoma cells. Cells were incubated in control media or media containing ethanol (50 mM) for 18 h before stimulation. Cells were then stimulated with TNF-α in the absence or in the presence of ethanol (50 mM) for 4–24 h.

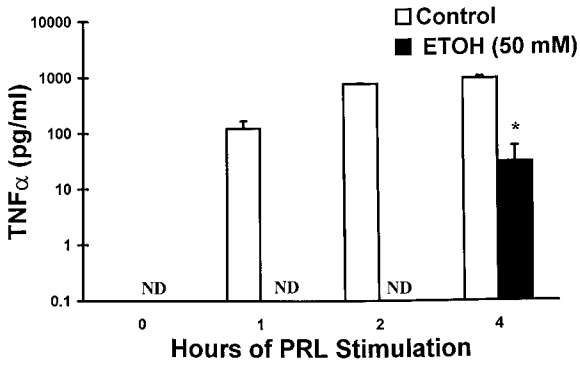

Fig. 7. Effect of ethanol of prolactin-induced tumor necrosis factor-α (TNF-α) secretion in human astrocytoma cells. Cells were incubated in control media or in media containing ethanol (50 mM) for 18 h before stimulation. Cells were then stimulated with prolactin (10 nM) for 4 h in the presence and in the absence of ethanol. $*P < 0.05$), as compared with controls. Each value represents the mean ± SEM, n = 3. ND-TNF-α concentrations were not detectable.

marked decrease in the relative amount and duration of TNF-α-induced ICAM-1 mRNA levels.

We have shown that in cultured rat astrocytes PRL increases TNF-α content [DeVito et al., 1997]. Here we show that in human astrocytoma cells, PRL induced a dose- and time-dependent increase in TNF-α secretion (Figs. 7, 8). In the presence of ETOH, however, PRL-induced TNF-α secretion was markedly inhibited. Further time course analysis of PRL- and TNF-α-induced ICAM-1 expression indicated that TNF-α-induced ICAM-1 expression preceded the PRL-induced increase in ICAM-1 expression (data not presented). This finding suggested that the PRL-induced expression of ICAM-1 may be due in part to a PRL-induced increase in TNF-α expression in human astrocytes. To determine whether the PRL-induced increase in TNF-α expression played a role in the PRL-induced ICAM-1 expression, human astrocytoma cells were stimulated with PRL in the presence of a TNF-α neutralizing antibody. As shown in Figure 9, stimulation of human astrocytoma cells in

the presence of increasing concentrations of a TNF-α-neutralizing antibody blocked PRL-induced increases in the cell surface expression of ICAM-1.

DISCUSSION

The immunosuppressive effects of chronic alcohol use have been well documented [Szabo et al., 1995; Verma et al., 1993; Szabo, 1993]. Similarly, fetal alcohol exposure results in immunodeficiency in humans and animals [Gottesfeld and Abel, 1991]. While compelling evidence indicates that alcohol is a immunosuppressive drug, the effect of alcohol on the neuroimmune system remains an unexplored area. In this study, we examined the effect of ethanol on PRL-, IFN-γ, and TNF-α- induced expression of ICAM-1 in human astrocytoma cells. In quiescent human astrocytoma cells, Western blot analyses detected four immunoreactive proteins with apparent molecular weights of 55, 67, 82, and 90 kDa. This finding is consistent with the molecular cloning and characterization of ICAM-1, which show that ICAM-1 is a single-chain glycoprotein with a polypeptide core with a apparent molecular weight of 55 kDa, containing a short cytoplasmic tail and five extracellular Ig-like domains [van de Stople and van der Saag, 1996]. The molecular weight of the mature glycosylated form of ICAM-1 is tissue, and species specific and ranges from 80 to 114 kDa [van de Stople and van der Saag, 1996]. In this study, the 90-kDa immunoreactive ICAM-1 protein identified in human astrocytoma cells is consistent with the size of the mature membrane-bound ICAM-1 glycoprotein found in human and rat astrocytes [Xiao et al., 1996; Shrikant et al., 1994, 1995]. The ICAM-1 immunoreactive proteins with apparent molecular weights of 67 and 82 kDa most likely represent proteins at different stages of glycosylation. In primary astrocyte cultures, TNF-α increases the production of the mature ICAM-1 protein [Frohman et al., 1989; Shrikant et al., 1994, 1995]. Here we show that in human astrocytoma cells that PRL and TNF-α increase in the relative abundance of all four immunoreactive ICAM-1 proteins, including the mature 90-kDa protein. In quiescent human astrocytoma cells, ethanol

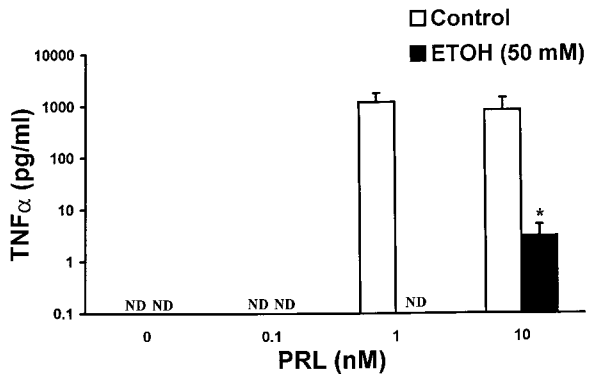

Fig. 8. Effect of ethanol of prolactin-induced tumor necrosis factor-α (TNF-α) secretion in human astrocytoma cells. Cells were incubated in control media or media containing ethanol (50 mM) for 18 h before stimulation. Cells were then stimulated with increasing concentrations of prolactin in the presence and absence of ethanol (50 mM) for 4 h. $*P < 0.05$), as compared with controls (0 mM ethanol). Each value represents the mean ± SEM, n = 3. ND-TNF-α concentrations were not detectable

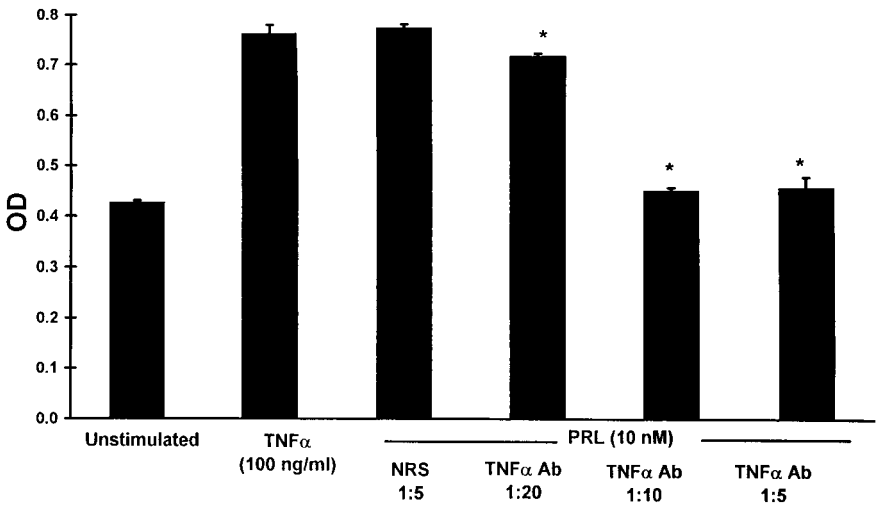

Fig. 9. Effect of a tumor necrosis factor-α (TNF-α)neutralizing antibody on prolactin-induced ICAM-1 expression in human astrocytoma cells. Cells were incubated in control media or media containing ethanol (50 mM) for 18 h before stimulation. Cells were then stimulated with prolactin (10 nM) for 18 h in the control media or media containing normal rabbit serum or increasing concentration of a TNF-α-specific antibody. $*P < 0.05$), as compared with controls. Each value represents the mean ± SEM, n = 3.

had little effect on the relative abundance of these proteins. In TNF-α- and PRL-stimulated cells, however, ethanol blocked the PRL- and TNF-α-induced increases in the ICAM-1 immunoreactive proteins. Together, these results suggest that ethanol may inhibit the synthesis and posttranscriptional modification of ICAM-1.

ICAM-1 is an inducible cell surface glycoprotein that promotes leukocyte adhesion and migration in immune and inflammatory responses. Accordingly, the effect of ethanol on cell surface expression of ICAM-1 was examined using a cell surface ICAM-1-specific enzyme-linked immunosorbent assay (ELISA). In quiescent cells, cell surface ICAM-1 expression was easily detected. Stimulation of astrocytoma cells with PRL, INF-γ and TNF-α resulted in marked increases in cell surface ICAM-1 expression. Consistent with our Western blot analyses, we found that in quiescent cells ethanol did not decrease cell surface expression of ICAM-1. In the presence of ethanol, however, TNF-α- and PRL-induced increases in cell surface ICAM-1 expression were markedly decreased. By contrast, the IFN-γ-induced increase in cell surface ICAM-1 expression was not blocked by ethanol exposure. The failure of ethanol to decrease basal and IFN-γ-stimulated ICAM-1 cell surface expression indicates that ethanol does on inhibit PRL- and TNF-α-induced cell surface ICAM-1 expression by altering the biophysical properties of the cell membrane, which could result in the proteolytic release and metabolism of ICAM-1. Further, analysis of cell viability by the MTT cytotoxic assay indicated that treatment of human astrocytoma cells with 50 mM ethanol for 36 h did not decrease cell viability (data not presented). Alternatively, our Western blot analyses suggest that the ethanol-induced decrease in cell surface ICAM-1 expression may be at the transcriptional and/or post-transcriptional level. Further, the failure of ethanol to inhibit IFN-γ-induced ICAM-1 expression indicates that the inhibitory effect of ethanol on ICAM-1 expression is not the result of a general decrease in protein synthesis.

In primary rat astrocytes, TNF-α increases ICAM-1 mRNA levels through the transcriptional activation of the ICAM-1 gene [Shrikant et al., 1994; Ballestas and Benveniste, 1995; Hurwitz et al., 1992]. To determine whether ethanol inhibits ICAM-1 gene expression in human astrocytoma cells, Northern blot analyses were performed on TNF-α-stimulated human astrocytoma cells. We found that TNF-α increased ICAM-1 mRNA levels in a time-dependent manner. When astrocytoma cells were pre-exposed to ethanol, however, the TNF-α-induced increase in ICAM-1 mRNA was markedly inhibited. Our studies indicate that ethanol inhibits TNF-α-induced ICAM-1 gene expression, resulting in decreases in ICAM-1 production and cell surface expression.

TNF-α exerts its pleiotropic activities through binding to two distinct membrane-anchored receptors with molecular masses of 55 kDa and 75 kDa [Smith et al., 1994]. The cellular events involved in the regulation of TNF-α-induced ICAM-1 expression after ligand binding are unclear. In other cell systems, induction of many TNF-α-responsive genes is mediated through the activation of the nuclear transcription factor system, NFκB. In the ICAM-1 promoter the κB enhancer represents the most important transcriptional-regulatory element and conveys responsiveness to TNF-α, TPA, and lipopolysaccharide. Thus, further studies are required to determine which cellular mechanisms are involved in mediating TNF-α-induced ICAM-1 gene expression in human astrocytoma and at what level(s) ethanol inhibits the transcriptional regulation of the ICAM-1 gene.

Most of the studies described in this report used an ethanol concentration of 50 mM. This concentration was used because it results in maximal inhibition of PRL- and TNF-α-induced ICAM-1 expression, without decreasing cell viability. This concentration, while slightly greater then those associated with impaired mental capabilities and coordination (10 mM) or resulting in ataxia (20 mM), in humans is within the physiological relevant range. That is, exposure to 50 mM ethanol is similar to, or less than, the blood alcohol levels achieved in animal models of binge-like drinking [Thomas et al., 1996; Maier et al., 1997; Goodlett et al., 1997]. Further, studies in drinking women have reported blood alcohol concentrations above 50 mM [Urso et al., 1981; Church and Gerkin, 1988; Hammond et al., 1973; Wells and Barhill, 1996], including a report of one woman with a blood alcohol level of approximately 330 mM (1 1/2%) It is important to note, however, that in this study we observed inhibitory effects of ethanol on TNF-α-induced ICAM-1 expression in cells exposed to

1 mM ethanol (less than 0.01%). This finding suggests the possibility that moderate alcohol exposure may have a negative impact on the neuroimmune response.

Originally considered a reproductive hormone, it is now clear that prolactin is a multifunctional hormone with effects on humoral and cellular immune responses. In vivo, hypoprolactinemia induced by hypophysectomy, or by administration of bromocriptine or CB-154, impairs humoral, cell-mediated, and autoimmune responses [Lunkin, 1960; Prentice et al., 1976; Cross et al., 1984; Nagy and Berczi, 1978; Gala, 1991]. We have shown that, in the CNS, PRL is a novel growth factor and is involved in the regulation of astrocyte mitogenesis and cytokine expression, including TNF-α and IL-1 [Vick et al., 1987; DeVito et al., 1993, 1995a,b]. Further, using an in vivo model of CNS immune activation (i.e., wounding) we found that a local increase in PRL synthesis at a CNS wound site is involved in the regulation of the brains' response to injury or trauma [DeVito et al., 1995b]. Recently, we have shown that exposure of cultured rat astrocytes to low concentrations of ethanol markedly inhibits PRL-induced astrocyte proliferation and TNF-α expression [DeVito et al., 1997]. Further, PRL-induced mitogenesis and TNF-α content were markedly decreased in primary astrocyte cultures prepared from rats exposed to alcohol prenatally [DeVito et al., 1997]. Here we show that in human astrocytoma cells, PRL markedly increased TNF-α secretion, and that the PRL-induced increase in TNF-α secretion was markedly inhibited by preexposure to ethanol. This finding suggests that the PRL-induced increase in ICAM-1, may in part, be due to PRL-induced increases in TNF-α synthesis and release. To test this hypothesis, immunoneutralization studies were performed using a TNF-α neutralizing antiserum. We found that stimulating human astrocytoma cells with PRL in the presence of a TNF-α neutralizing antiserum blocked the PRL-induced increase of cell surface ICAM-1 expression. This finding indicates that PRL-induced ICAM-1 expression, and ethanol-induced inhibition of PRL-induced ICAM-1 expression, are secondary events involving TNF-α synthesis and secretion.

In summary, interactions between lymphoid cells, astrocytes and other glial cells at the site of inflammation in the CNS involve an initial step of intercellular contact. Interactions between integrins and cell surface ICAM-1 molecules plays an important role in the generation of inflammatory responses, and a co-stimulatory role in the activation of T-cells. Impairment of immunological responses, such as transendothelial migration of neutrophils into the site of inflammation, mixed lymphocyte reactions, and contact hypersensitivity in ICAM-1 knock out mice confirms that ICAM-1 plays an important role as a mediator of inflammatory responses [van de Stople and van der Saag, 1996]. The present study demonstrates that, in human astrocytoma cells, ethanol inhibits TNF-α-induced ICAM-1 expression, one of the key regulators of ICAM-1. The cellular mechanisms involved in mediating the inhibitory effect of ethanol on TNF-α-induced ICAM-1 expression, however, remain to be determined. Furthermore, studies are required to determine whether ethanol inhibits ICAM-1 expression in vivo and are currently in progress.

REFERENCES

Ballestas ME, Benveniste EN. 1995. Interleukin 1-β and tumor necrosis factor-α mediate regulation of ICAM-1 gene expression in astrocytes requires protein kinase C activity. Glia 14:267–278.

Benveniste EN. 1992. Inflammatory cytokines within the central nervous system: sources, function, and mechanism of action. Am J Physiol 263:C1–C16.

Chomczynski P, Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium-thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159.

Chung IY, Benveniste EN. 1990. Tumor necrosis factor-alpha production by astrocytes: induction by lipopolysaccharide, interferon-gamma, and interleukin-1. J Immunol 144:2999–3007.

Church MW, Gerkin KP. 1988. Hearing disorders in children with fetal alcohol syndrome: findings from case reports. Pediatrics 82:1071–1076.

Cross RJ, Markesbery WR, Brooks WH, Roszman TL. 1984. Hypothalamic-immune interactions. Neuromodulation of natural killer activity by lesions of the anterior hypothalamus. Immunology 51:399–405.

DeVito WJ, Okulicz WC, Stone S, Avakian C. 1992. Prolactin-stimulated mitogenesis of cultured astrocytes. Endocrinology 130:2549–2556.

DeVito WJ, Avakian C, Stone S, Okulicz WC. 1993. Prolactin-stimulated mitogenesis of cultured astrocytes is mediated by a protein kinase C-dependent mechanism. J Neurochem 60:835–842.

DeVito WJ, Avakian C, Stone S, Okulicz WC, Tang K-T, Shamgochian M. 1995a. Prolactin induced expression of interleukin-1 alpha, tumor necrosis factor-alpha and transforming growth factor-alpha in cultured astrocytes. J Cell Biochem 57:290–298.

DeVito WJ, Stone S, Shamgochian M. 1995b. Prolactin induced expression of glial acidic protein and tumor necrosis factor-alpha at a wound site in the rat brain. Mol Cell Endocrinol 108:125–130.

DeVito WJ, Stone S, Mori K. 1997. Low concentrations of ethanol inhibits prolactin-induced mitogenesis and cytokine expression in cultured astrocytes. Endocrinology 138:922–928.

Diamond MS, Staunton DE, de Fougerolles AR, Stacker SA, Garcia-Aguilar J, Hibbs ML, Springer TA. 1990. (CD54): a counter-receptor for Mac-1 (CD11b/CD18). J Cell Biol 111:3129–3139.

Dougherty GJ, Murdoch S, Hogg N. 1988. The function of human intercellular adhesion molecule-1 (ICAM-1) in the generation of an immune response. Eur J Immunol 18:35–39.

Frohman EM, Frohman TC, Dustin L, Vayuvegula B, Choi B, Gupta A, van den Noort S, Gupta S. 1989. The induction of intracellular adhesion molecule 1 (ICAM-1) expression on human fetal astrocytes by interferon-γ, tumor necrosis factor-α, lymphotoxin, and interleukin-1: relevance to intercerebral antigen presentation. J Neuroimmunol 23:117–124.

Gala RR. 1991. Prolactin and growth hormone in the regulation of the immune system. Proc Soc Exp Biol Med 198:513–527.

Goodlett CR, Peterson SD, Lundahl KR, Pearlman AD. 1997. Binge-like alcohol exposure of neonatal rats via intragastric intubation induces both Purkinje cell loss and cortical astrogliosis. Alcohol Clin Exp Res 21:1010–1017.

Gottesfeld Z, Abel EL. 1991. Maternal and paternal alcohol use: effects on the immune system of the offspring. Life Sci 48:1–8.

Hammond KB, Rumack BH, Rodgerson DO. 1973. Blood ethanol. A report of unusually high levels in a living patient. JAMA 226:63–64.

Hickey WF, Osborn JP, Kirby WM. 1985. Expression of Ia molecules by astrocytes during acute experimental allergic encephalomyelitis in the Lewis rat. Cell Immunol 91:528–535.

Hurwitz AA, Lyman WD, Guida MP, Calderon TM, Berman JW. 1992. Tumor necrosis factor α induces adhesion molecule expression on human fetal astrocytes. J Exp Med 176:1631–1636.

Lunkin PM. 1960. Action of hypophysectomy on antibody formation in the rat. Acta Pathol Microbiol Scand 48(suppl):351–355.

Maier SE, Chen WJ, Miller JA, West JR. 1997. Fetal alcohol exposure and temporal vulnerability regional differences in alcohol-induced microencephaly as a function of the timing of binge-like alcohol exposure during rat brain development. Alcohol Clin Exp Res 21:1418–1428.

Martin R, McFarland HF, McFarlin DE. 1992. Immunological aspects of demyelinating diseases. Annu Rev Immunol 10:153–187.

Moynagh PN, Williams DC, O'Neill LAJ. 1994. Activation of NF-κB and induction of vascular cell adhesion molecule-1 and intracellular adhesion molecule-1 expression in human glial cells by IL-1. J Immunol 153: 2681–2690.

Nagy E, Berczi I. 1978. Immunodeficiency in hypophysectomized rats. Acta Endocrinol 89:530–537.

Prentice ED, Lipscomb H, Metcalf WK, Sharp JG. 1976. Effects of hypophysectomy on DNBC-induced contact sensitivity in rats. Scand J Immunol 5:955–961.

Sanchez-Madrid F, Nagy JA, Robbins E, Simon P, Springer TA. 1983. A human leukocyte differentiation antigen family with distinct α-subunits and a common β-subunit. J Exp Med 158:1785–1803.

Satoh J, Kim SU, Kastrrukoff LF, Takei F. 1991. Expression and induction of intercellular adhesion molecules (ICAMs) and major histocompatibility complex (MHA) antigens on cultured murine oligodendrocytes and astrocytes. J Neurosci Res 29:1–12.

Shibayama M, Kuchiwaki H, Inao S, Yoshida K, Ito M. 1996. Intercellular adhesion molecule-1 expression on glia after brain injury: participation of interleukin-1β. J Neurotrauma 13:801–808.

Shrikant P, Chung IY, Ballestas M, Benveniste EN. 1994. Regulation of intercellular adhesion molecule-1 gene expression by tumor necrosis factor-α, interleukin-β, and interferon-γ in astrocytes. J Neuroimmunol 51:209

Shrikant P, Weber E, Jilling T, Benveniste EN. 1995. Intercellular adhesion molecule-1 gene expression by glial cells. J Immunol 155:1489–1501.

Smith CA, Farrah T, Goodwin RG. 1994. The TNF-receptor superfamily of cellular and viral proteins: activation, costimulation and death. Cell 76:959–962.

Springer TA. 1990. Adhesion receptors of the immune system. Nature 346:425–434.

Staunton DE, Marlin SD, Stratowa C, Dustin ML, Springer TA. 1988. Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell 52:925–933.

Szabo G. 1993. Monocyte-mediated immunosuppression after acute ethanol exposure (abst). In: Watson RR, editor. Alcohol, drugs of abuse and immunomodulation. Tarrytown, NY: Pergamon Press. p 121–133.

Szabo G, Mandrekar P, Catalano D. 1995. Inhibition of superantigen-induced T cell proliferation and monocyte IL-1β, TNF-α, and IL-6 production by acute ethanol treatment. J Leukocyte Biol 58:342–350.

Tang K-T, Braverman LE, DeVito WJ. 1995. Tumor necrosis factor-α and interferon-γ modulate gene expression of type I 5′-deiodinase, thyroid peroxidase and thyroglobulin in FRTL-5 thyroid cells. Endocrinology 136:881–888.

Thomas JD, Wasserman EA, West JR, Goodlett CR. 1996. Behavioral defects induced by bingelike exposure to alcohol in neonatal rats: importance of developmental timing and number of episodes. Dev Psychobiol 29:433–452.

Urso T, Gavaler JS, Van Thiel DH. 1981. Blood ethanol levels in sober alcohol users seen in an emergency room. Life Sci 28:1053–1056.

van de Stople A, van der Saag PT. 1996. Intercellular adhesion molecule-1. J Mol Med 74:13–33.

Verma BK, Fogarasi M, Szabo G. 1993. Down-regulation of TNF-α activity by acute ethanol treatment in human peripheral blood monocytes. J Clin Immunol 13:8–22.

Vick RS, Wong LY, Witorsch RJ. 1987. Biological, immunological and biochemical characterization of cleaved prolactin generated by lactating mammary gland. Biochim Biophys Acta 931:196–204.

Wells DJ, Barhill MTJ. 1996. Unusually high ethanol levels in two emergency medicine patients. J Anal Toxicol 20:272

Xiao B-G, Zhang GX, Link M. 1996. Transforming growth factor-beta 1 (TGF-β1) mediated inhibition of glial cell proliferation and down-regulation of intercellular adhesion molecule-1 (ICAM-1) are interrupted by interferon-gamma (IFNΓ). Clin Exp Immunol 103:475–481.