Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 332 (2005) 37–42
BBRC
www.elsevier.com/locate/ybbrc

Ethanol alters cellular activation and CD14 partitioning in lipid rafts

Qun Dai, Jun Zhang, Stephen B. Pruett *

Department of Cellular Biology and Anatomy, Louisiana State University Health Science Center, 1501 Kings Highway, Shreveport, LA 71130, USA

Received 15 April 2005
Available online 27 April 2005

Abstract

Alcohol consumption interferes with innate immunity. In vivo EtOH administration suppresses cytokine responses induced through Toll-like receptor 4 (TLR4) and inhibits TLR4 signaling. Actually, EtOH exhibits a generalized suppressive effect on signaling and cytokine responses induced by through most TLRs. However, the underlying mechanism remains unknown. RAW264.7 cells were treated with LPS or co-treated with EtOH or with lipid raft-disrupting drugs. TNF-α production, IRAK-1 activation, and CD14 partition were evaluated. EtOH or nystatin, a lipid raft-disrupting drug, suppressed LPS-induced production of TNF-α. The suppressive effect of EtOH on LPS-induced TNF-α production was additive with that of methyl-β-cyclodextrin (MCD), another lipid raft-disrupting drug. EtOH interfered with IRAK-1 activation, an early TLR4 intracellular signaling event. Cell fractionation analyses show that acute EtOH altered LPS-related partition of CD14, a critical component of the LPS receptor complex. These results suggest a novel mechanism of EtOH action that involves interference with lipid raft clustering induced by LPS. This membrane action of EtOH might be one of the mechanisms by which EtOH acts as a generalized suppressor for TLR signaling.

Keywords: Ethanol; Toll-like receptors; Lipid rafts; RAW264.7 cells; CD14

Alcohol consumption interferes with innate immunity. Acute EtOH has been found to cause suppression of pro-inflammatory cytokines and inhibition of neutrophil chemotaxis and activation [1,2]. Rodents receiving acute alcohol administration become more susceptible to a wide range of pathogens [3–6]. Mammalian Toll-like receptors (TLRs) are key innate immune receptors responsible for recognizing pathogen-associated molecular patterns, which represent conserved molecular features of a given microbial class. Bacterial LPS is the natural ligand for TLR4. TLRs activate signaling pathways that are critical for triggering innate immune responses to microbial challenge [7,8]. Recent results from a few laboratories (including ours) indicate that in vivo EtOH administration suppresses cytokine responses induced through TLR4 [9–11] and inhibits TLR4 signaling [12–14]. Actually, EtOH exhibits a generalized suppressive effect on signaling and cytokine responses induced through most TLRs [9]. However, the underlying mechanism remains unknown.

The suppression of innate immune responses by alcohol in rodent models is no doubt a systemic manifestation. There is evidence that the suppressive effect of EtOH does not require cellular metabolism of EtOH to acetaldehyde via alcohol dehydrogenase [15,16]. An interaction of EtOH or acetaldehyde with circulating corticosterone is not involved in EtOH-mediated attenuation of LPS-stimulated iNOS or TNF-α production in the lung [15,16]. So, it is reasonable to focus on the direct effect of EtOH on cells in vitro at physiological concentrations, rather than investigate potential indirect mechanisms.

Since the 1970s there have been many study results suggesting a membrane site of action for alcohol and related drugs [17]. Acute EtOH increases membrane fluidity [18–20] and influences the function of membrane proteins in vitro [21–23]. Recently, a model of innate

* Corresponding author. Fax: +1 318 675 5889.
E-mail address: spruet@lsuhsc.edu (S.B. Pruett).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.04.088

immune recognition of bacterial LPS has been proposed, in which the ligation of CD14 by LPS and the recruitment of multiple signaling molecules to lipid rafts are the basis for cellular activation by LPS [24,25]. The key question is whether EtOH suppresses TLR4 signaling by subtle alterations of lipid rafts that affect partitioning of important proteins (such as CD14) among these membrane regions.

In this study, we demonstrate that acute EtOH alters LPS-related partition of CD14 (a key component of the TLR4 receptor complex) in lipid raft fractions. This interferes with an early TLR4 intracellular signaling event and suppresses LPS-induced TNF-α production by the RAW264.7 murine macrophage cell line. This effect is additive with the suppressive effect of methyl-β-cyclodextrin (MCD), a lipid raft-disrupting drug, suggesting a common mechanism of action.

Materials and methods

Materials. Cell culture media and supplements were purchased from Gibco. Lipopolysaccharide from *Escherichia coli* (*E. coli*) serotype 0128:B12, protease inhibitor cocktail, HRP-conjugated cholera toxin B subunit, methyl-β-cyclodextrin, and nystatin suspension were purchased from Sigma. A mouse TNF ELISA kit was purchased from BD Pharmingen. Rabbit Polyclonal anti-IRAK-1 (H-273), anti-CD14 (M-305), and horseradish peroxidase-conjugated goat anti-rabbit IgG were obtained from Santa Cruz Biotechnology.

Cell culture and measurement of TNF-α. The RAW264.7 macrophage-like cell line was obtained from the American Type Culture Collection and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and penicillin–streptomycin at 37 °C and 5% CO_2. For experiments, RAW264.7 cells were seeded at 3×10^5/ml in 24-well plates. After incubation for 16–24 h, cells were treated with 100 ng/ml LPS (serotype 0128:B12, Sigma) with or without EtOH at various concentrations from 0.05 to 0.4%(wt/vol). TNF-α levels in cell culture medium were measured with the Mouse TNF ELISA kit 3 h after treatments, according to the manufacturer's protocol.

Raft fractionation by sucrose density gradient centrifugation. Raft fractions were prepared by sucrose density gradient centrifugation and defined according to Ganglioside GM1 distribution essentially as described previously [26]. RAW264.7 cells were grown to subconfluency in a 150 mm dish and treated with LPS (100 ng/ml) or LPS plus EtOH (0.4% wt/vol) in complete medium for 15 min. All the subsequent steps were performed on ice. The monolayer was rinsed with phosphate-buffered saline (PBS) and scraped with a rubber policeman in 4 ml PBS. The cells were collected by centrifugation and lysed in 2 ml TNE/Triton X-100 buffer (20 mM Tris–Cl [pH 7.4], 1 mM EDTA, 140 mM NaCl, and 0.5% TX-100) with protease inhibitor (Sigma) for 30 min. The lysates were homogenized by passage 10 times through a 25G 5/8 inch needle. After centrifugation at 800*g* for 10 min at 4 °C, equal amounts of post-nuclear supernatants from different treatment groups were brought to 40%(wt/vol) with sucrose by mixing with an equal volume of 80% sucrose in TNE without TX-100. A discontinuous sucrose density gradient was made in ultracentrifuge tubes by sequential layering of 3 ml of 60% sucrose, 3.6 ml of the cell lysates adjusted to 40% sucrose, 2 ml of 30% sucrose, 1.2 ml of 20% sucrose, and 1 ml of 10% sucrose all in TNE buffer. The gradients were centrifuged at 39,000 rpm at 4 °C for 18–21 h in an SW41 rotor (Beckman). Fractions of 1 ml each were collected from the bottom of the tubes. The GM1 distribution pattern was detected with CTxB-HRP by dot blot using a 2 μl sample from each fraction. Total proteins in each fraction were concentrated by TCA precipitation for detecting CD14 by Western blot.

TCA precipitation. Trichloroacetic acid (TCA) solution was added to each fraction to reach a final concentration of TCA equal to 10%. The samples were vortexed briefly and incubated for over 30 min on ice. After the proteins were precipitated, the samples were microcentrifuged for 15 min at 12,000*g* at 4 °C. Then the pellets were washed with 1 ml of ice-cold acetone (kept in the freezer at −20 °C) and air-dried. In the first experiment, the same volume of cell lysates from each group was subjected to fractionation. In the second experiment, cell lysates containing equal amounts of protein were subjected to fractionation. After fractionation and TCA precipitation, air-dried samples were resuspended in equal volumes of sample loading buffer. An equal volume from each sample was used for SDS–PAGE (see next section). Because the two methods of sample preparation described here yielded very similar results, the data from these two experiments were pooled for analysis (see Results).

Western blots. A whole cell lysate was used for IRAK-1 analysis and concentrated protein in sucrose gradient fractions was used for CD14 analysis. Protein concentrations were determined using the microplate version of the BCA protein assay (Pierce). Equal amounts of protein (10–20 μg for detecting IRAK-1) were separated by 7.5% SDS–PAGE gel and blotted onto a PVDF membrane (Bio-Rad). Nonspecific binding sites were blocked with 5% non-fat milk in wash buffer (20 mM Tris–HCl, pH 7.6, 0.05% Tween 20, and 150 mM NaCl). The membrane was incubated with the primary antibody in 5% non-fat milk containing wash buffer overnight at 4 °C, followed by incubation with the secondary antibody conjugated to the HRP at room temperature for 1 h. The membrane was washed again and proteins were visualized by using the ECL chemiluminescence detection kit (Amersham). A BioRad Versa Doc 3000 imaging system was used to obtain the digital images. The volume analysis (mean intensity × area) of bands was determined using Quantity 1.1 software.

Statistical analysis. Differences between the means of groups were analyzed by one-way ANOVA followed by LSD post hoc test. Most of the analyses were performed with SPSS 11.0 software. Gaussian distribution analysis was done by Prism 4.0 software.

Results

EtOH has similar effects on LPS-induced production of TNF-α as two known lipid raft-disrupting drugs

RAW264.7 cells, initially derived from Balb/c mice infected with Abelson leukemia virus, are extremely sensitive to LPS [27]. Many of the responses of activated macrophages to LPS have been observed in cultured RAW264.7 cells [28]. As shown in Fig. 1A, LPS induced a dramatic increase of TNF-α production by RAW264.7 cells 2 or 4 h after treatment. Either EtOH or nystatin, a lipid raft-disrupting drug, can suppress induction of TNF-α production, and this is more prominent at 4 h after treatment. The results shown in Fig. 1B indicate that another lipid raft disrupting drug, methyl-β-cyclodextrin (MCD), also suppressed LPS-induced TNF-α production. When EtOH and MC were both included in cultures, the effects were concentration-dependent and approximately additive (Fig. 1C) until near maximal levels of suppression were attained at the higher concentrations of both compounds. Additive effects of

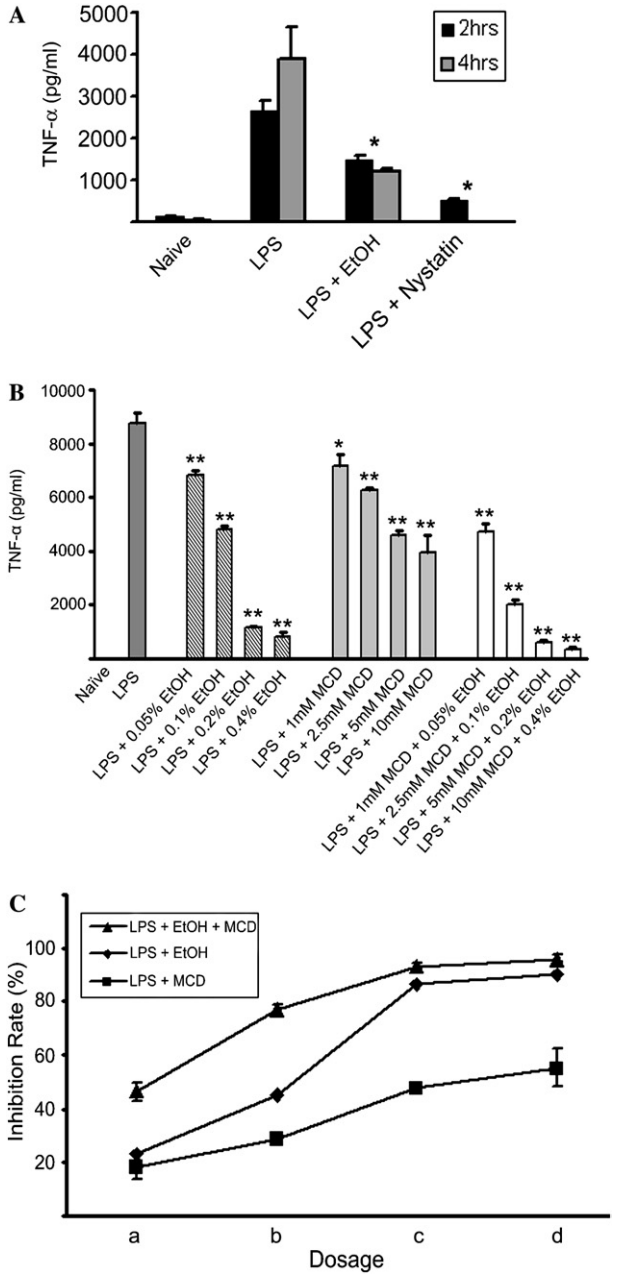

Fig. 1. Suppressive effect of nystatin, methyl-β-cyclodextrin (MCD), EtOH, and combinations of EtOH and MCD on LPS-induced production of TNF-α by RAW264.7 cells. (A) After 16–24 h of incubation of RAW264.7 cells, EtOH (0.4% wt/vol), nystatin (60 μg/ml), and LPS (100 ng/ml) were added directly to the culture medium at time 0. Cell culture medium taken at 2 and 4 h was analyzed using an ELISA kit for TNF-α. Results shown are means ± SEM ($n = 4$ wells/group). Values significantly different from the LPS group are indicated by ** ($P < 0.01$). (B) EtOH and MCD were added directly to the culture medium at time 0 and the cell culture medium was taken at 3 h for TNF-α measurement using an ELISA kit for TNF-α. Values significantly different from the LPS group are indicated by * ($P < 0.05$) or ** ($P < 0.01$). (C) Inhibitory rate (%) of LPS-induced TNF-α production by EtOH and/or MCD, based on results of (B). Concentrations of EtOH: (a) 0.05%(wt/vol); (b) 0.1%; (c) 0.2%; and (d) 0.4%. Concentrations of MCD: (a) 1 mM; (b) 2.5 mM; (c) 5 mM; and (d) 10 mM. Concentrations of EtOH plus MCD: (a) 0.05%(wt/vol) + 1 mM; (b) 0.1% + 2.5 mM; (c) 0.2% + 5 mM; and (d) 0.4% + 10 mM.

this type are consistent with a common mechanism of action for ethanol and MCD in this system.

Acute EtOH inhibits LPS-induced IRAK-1 activation

To determine if there is a correlation between altered signaling with suppressed TNF-α production in RAW264.7 cells, IRAK-1 degradation was evaluated. IRAK-1 is normally phosphorylated just after ligand-induced interaction of the adapter molecules with the TLRs [29], and this contributes to subsequent signaling events. The hyper-phosphorylation of IRAK-1 triggers its ubiquitination and degradation, which is an excellent indicator of activation of early events in TLR4 signaling [29,30]. Data shown in Fig. 2 demonstrate that IRAK-1 is constitutively expressed in untreated RAW264.7 cells and acute EtOH at 0.4%(wt/vol) partially prevents the LPS-induced IRAK-1 degradation. Alteration of degradation of IRAK-1 suggests that EtOH affects an early step in TLR signaling. We have previously reported similar results for acute EtOH in vivo in mice [31].

Acute EtOH alters the LPS-induced distribution pattern of CD14-containing fractions

Because the ligation of CD14 by LPS and the recruitment of multiple signaling molecules within the lipid rafts are the basis for cellular activation by LPS [24,25], the distribution of CD14 in lipid raft fractions was investigated. CD14 is a 55-kDa glycosyl phosphatidylinositol-linked protein [32], which resides constitutively within rafts [33–35]. As seen from Fig. 3A, lipid rafts are mainly in fractions 9 and 10 and, to a lesser

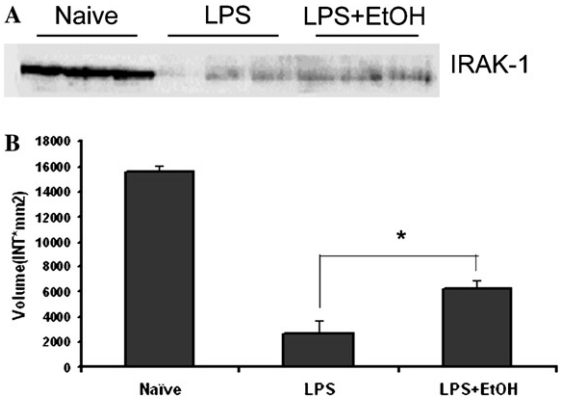

Fig. 2. Acute EtOH inhibits LPS-induced IRAK-1 activation. RAW264.7 cells were treated with LPS (100 ng/ml) with or without EtOH (0.4% wt/vol) for 30 min and whole cell lysates were prepared for IRAK-1 analysis using Western blot. (A) Western blot. (B) The bands were compared quantitatively by the Quantity One software package (Bio-Rad). The volume of each band (Y axis) refers to the mean value of the intensity times the area. Differences between the group means were analyzed by one-way ANOVA followed by LSD post hoc test using SPSS 11.0 software. Statistically significant differences are indicated by * ($P < 0.05$).

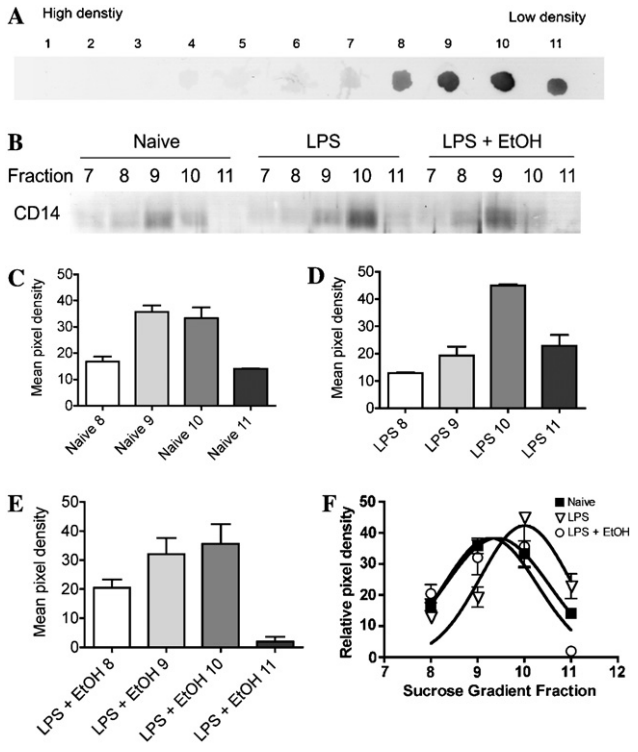

Fig. 3. Acute EtOH alters the LPS-induced distribution pattern of lipid raft fractions containing CD14. GM1 ganglioside distribution in gradient fractions was detected with CTxB-HRP (Sigma) by dot blot using a 2 μl sample from each fraction (A). RAW264.7 cells were treated with LPS (100 ng/ml) with or without EtOH (0.4% wt/vol) for 15 min. Equal amounts of protein samples from different treatment groups were subjected to discontinuous sucrose density gradient centrifugation. Raft fractions of the lysates defined according to the GM1 distribution pattern (as seen in A) were concentrated by TCA precipitation and the same proportion of protein sample in each fraction was taken for detection of CD14 using Western blot (B). (C–E) Mean ± SEM for densitometry analysis of the CD14 band in each fraction of two independent experiments done using the Quantity One software package (Bio-Rad). (F) Gaussian analysis of the distribution pattern of fractions of each treatment done by Prism 4.0 software. The 95% confidence intervals for these distributions demonstrate that the distribution for the LPS group is significantly different from that for the naïve group, but the distribution for the LSP plus EtOH group is not significantly different from naïve.

extent, fractions 8 and 11, according to the dot blot signal intensity of the lipid raft marker, ganglioside GM1. Subconfluent RAW264.7 cells were left untreated (Naïve group), treated with LPS (100 ng/ml) (LPS group), or treated with EtOH (0.4% wt/vol) along with LPS (LPS + EtOH) for 15 min. The cold lysate was subjected to discontinuous sucrose density gradient centrifugation. GM1 distribution patterns were the same among groups, which indicates that EtOH does not affect the buoyancy of lipid rafts in general (data not shown). Raft fractions defined according to the GM1 distribution patterns were concentrated by TCA precipitation for detection of CD14 (Fig. 3B). Without any treatment, CD14 appears mostly in fraction 9. LPS induces the peak CD14 signal shift to fraction 10 while EtOH prevents this shift. Gaussian distribution analysis showed that the distribution patterns of CD14 in fractions of Naïve group and LPS plus EtOH group are overlapping while the LPS treatment alone causes an obvious shift of the distribution pattern of CD14 in fractions. Apparently, EtOH blunted LPS-induced redistribution of CD14 from less buoyant lipid raft fractions to more buoyant lipid raft fractions, which is correlated with the suppressive effects of EtOH on LPS-induced IRAK-1 activation and TNF-α production.

Discussion

Alcohol consumption has long been recognized as a risk factor for infections. Previous study results in our laboratory and other laboratories have clearly demonstrated that acute EtOH (EtOH) interferes with the innate immune response. One important cellular mechanism responsible for the impairment of innate immunity by acute alcohol treatment is suppression of TNF-α production by mononuclear phagocytes [36–41]. The suppressive effect of EtOH found from this in vitro study is similar to the suppressive effect of acute EtOH administration on cytokine responses induced by TLR ligands in vivo [10,31,42].

The effects reported here occurred at relevant EtOH concentrations. The experiment on the distribution of CD14 in lipid rafts involved the highest concentration of EtOH (0.4%), primarily because of the limited sensitivity of the methodology, which can only detect relatively large differences in density, due to the 10% interval in the discontinuous sucrose gradient. This concentration of EtOH is clearly high with regard to human blood levels, but it is not rare [43]. The other results suggest that the effects noted are dose-responsive, with significant changes at much lower EtOH concentrations. The use of RAW264.7 cells allowed consistent and reproducible populations, which are necessary for lipid raft studies. These cells have been very widely used as a model system to understand a wide variety of macrophage functions at the molecular level. In addition, EtOH has been reported to suppress LPS-induced cytokine production by a wide variety of macrophage types in vivo and in vitro, and by RAW264.7 and other macrophage-like cell lines [12–14,16,38,40]. This suggests that this particular effect of EtOH is not dependent on the type of macrophage and that it occurs similarly in normal macrophages and in macrophage-like tumor cells.

It is generally accepted that rafts can be both positive and negative regulators, probably by means of controlling physical recruitment or sequestration of proteins involved in activating and suppressing signaling [44]. There is ligand-specific receptor clustering in lipid rafts

upon LPS stimulation of monocytes. Our study found that acute EtOH actually alters the LPS-induced lipid raft partition pattern of CD14 in RAW264.7 cells as analyzed by Gaussian distribution. CD14 of naïve group and LPS plus EtOH group have similar Gaussian distribution pattern while that of LPS group is substantially different (as indicated by the 95% confidence intervals). It is becoming apparent that lipid rafts are heterogeneous both in terms of their protein and their lipid content [35,45]. It is probable that LPS-induced receptor clusters are heterogeneous in nature due to the recruitment of co-receptors as well as the interacting lipids surrounding those molecules. The different buoyant density reflects the heterogeneous nature of CD14 containing fractions. From this point of view, EtOH may interfere with receptor clustering, which causes the alteration of CD14 partitioning in the process of LPS recognition. Alternatively, EtOH may cause subtle changes in the lipid portion of rafts, causing changes in clustering or sequestration of proteins within rafts.

Early in the 1970s, McCreery found a close correlation between membrane solubility and intoxicating potency for a variety of alcohols and other intoxicant-anesthetics, indicating a membrane site of action for alcohol and related drugs [17]. Later studies demonstrated that acute EtOH increases membrane fluidity [18–20]. In this study, we provide the first biochemical evidence that acute EtOH alters the LPS-induced distribution pattern of CD14 in lipid raft fractions. However, the results also indicate that rafts are not grossly altered by EtOH, and the nature of the subtle changes that affect CD14 distribution is not clear.

If this effect of EtOH contributes to the inhibition of signaling and TNF-α production, it would be expected that other lipid raft disrupting drugs would have similar effects. In this study, we used two commonly used lipid raft-disrupting drug, nystatin, a polyene antibiotic [46], and MCD. They are sterol binding reagents and have their effect by sequestering and depleting membrane cholesterol, which is crucial for formation and maintenance of Triton X-100-resistant membrane domains [47]. Both agents suppress LPS-induced TNF-α production. EtOH and MCD have linear concentration-dependent inhibitory effects on LPS-induced TNF-α, and these effects are approximately additive. This suggests similar modes of action. These results do not definitively establish that EtOH-induced alteration of distribution of CD14 into lipid rafts is responsible for disrupted signaling and cytokine production, but the results are consistent with this possibility. The finding of similar effects of two structurally distinct drugs known to act by disrupting lipid rafts and of additive effects of EtOH and one of these drugs strengthens this possibility. The idea that EtOH acts at a very early stage of LPS signaling is also supported by our observation that IRAK-1 degradation is decreased (Fig. 2).

The results of the present study do not necessary preclude other mechanisms of action that have been proposed by others. For example, Zhao et al. [16] demonstrated that EtOH suppresses LPS-induced TNF secretion by inhibiting interactions between TNF and its converting enzyme. The regulation of inflammatory cytokine production is very complex. TNF-α induced by LPS is controlled at transcriptional, post-transcriptional, and post-translational levels [48]. Alteration of lipid rafts could conceivably affect most of these levels of regulation by altering cell surface-mediated events. However, it is also possible that additional distinct mechanisms exist that are not related to alterations in lipid rafts. Further studies are needed to address this issue.

Acknowledgments

This work was supported by Grants AA009505 and ES09158 from the National Institutes of Health.

References

[1] P.J. Spagnuolo, R.R. MacGregor, Acute thanol effect on chemotaxis and other components of host defense, J. Lab. Clin. Med. 86 (1975) 24–31.
[2] S. Nelson, G.J. Bagby, B.G. Bainton, W.R. Summer, The effects of acute and chronic alcoholism on tumor necrosis factor and the inflammatory response, J. Infect. Dis. 160 (1989) 422–429.
[3] G.J. Bagby, P. Zhang, D.A. Stoltz, S. Nelson, Suppression of the granulocyte colony-stimulating factor response to *Escherichia coli* challenge by alcohol intoxication, Alcohol Clin. Exp. Res. 22 (1998) 1740–1745.
[4] S.S. Greenberg, X. Zhao, L. Hua, J.F. Wang, S. Nelson, J. Ouyang, Ethanol inhibits lung clearance of *Pseudomonas aeruginosa* by a neutrophil and nitric oxide-dependent mechanism, in vivo, Alcohol Clin. Exp. Res. 23 (1999) 735–744.
[5] J. Nishida, W. Ekataksin, D. McDonnell, R. Urbaschek, B. Urbaschek, R.S. McCuskey, Ethanol exacerbates hepatic microvascular dysfunction, endotoxemia, and lethality in septic mice, Shock 1 (1994) 413–418.
[6] C.S. Pavia, C.M. Harris, M. Kavanagh, Impaired bactericidal activity and host resistance to *Listeria monocytogenes* and *Borrelia burgdorferi* in rats administered an acute oral regimen of ethanol, Clin. Diagn. Lab. Immunol. 9 (2002) 282–286.
[7] B. Abel, N. Thieblemont, V.J. Quesniaux, N. Brown, J. Mpagi, K. Miyake, F. Bihl, B. Ryffel, Toll-like receptor 4 expression is required to control chronic *Mycobacterium tuberculosis* infection in mice, J. Immunol. 169 (2002) 3155–3162.
[8] R.M. Wooten, M.A. Ying, R.A. Yoder, J.P. Brown, J.H. Weis, J.F. Zachary, C.J. Kirschning, J.J. Weis, Toll-like receptor 2 plays a pivotal role in host defense and inflammatory response to *Borrelia burgdorferi*, Vector Borne Zoonotic Dis. 2 (2002) 275–278.
[9] S.B. Pruett, Q. Zheng, R. Fan, K. Matthews, C. Schwab, Acute exposure to ethanol affects Toll-like receptor signaling and subsequent responses: an overview of recent studies, Alcohol 33 (2004) 235–239.
[10] S.B. Pruett, Q. Zheng, R. Fan, K. Matthews, C. Schwab, Ethanol suppresses cytokine responses induced through Toll-like receptors

as well as innate resistance to *Escherichia coli* in a mouse model for binge drinking, Alcohol 33 (2004) 147–155.
[11] J.K. Kolls, J. Xie, D. Lei, S. Greenberg, W.R. Summer, S. Nelson, Differential effects of in vivo ethanol on LPS-induced TNF and nitric oxide production in the lung, Am. J. Physiol. 268 (1995) L991–L998.
[12] S. Yamashina, M.D. Wheeler, I. Rusyn, K. Ikejima, N. Sato, R.G. Thurman, Tolerance and sensitization to endotoxin in Kupffer cells caused by acute ethanol involve interleukin-1 receptor-associated kinase, Biochem. Biophys. Res. Commun. 277 (2000) 686–690.
[13] J. Goral, M.A. Choudhry, E.J. Kovacs, Acute ethanol exposure inhibits macrophage IL-6 production: role of p38 and ERK1/2 MAPK, J. Leukoc. Biol. 75 (2004) 553–559.
[14] P. Mandrekar, A. Dolganiuc, G. Bellerose, K. Kodys, L. Romics, R. Nizamani, G. Szabo, Acute alcohol inhibits the induction of nuclear regulatory factor kappa B activation through CD14/toll-like receptor 4, interleukin-1, and tumor necrosis factor receptors: a common mechanism independent of inhibitory kappa B alpha degradation? Alcohol. Clin. Exp. Res. 26 (2002) 1609–1614.
[15] S.S. Greenberg, J. Xie, J. Ouyang, X. Zhao, Ethanol metabolism is not required for inhibition of LPS-stimulated transcription of inducible nitric oxide synthase, Alcohol 17 (1999) 203–213.
[16] X.J. Zhao, L. Marrero, K. Song, P. Oliver, S.Y. Chin, H. Simon, J.R. Schurr, Z. Zhang, D. Thoppil, S. Lee, S. Nelson, J.K. Kolls, Acute alcohol inhibits TNF-alpha processing in human monocytes by inhibiting TNF/TNF-alpha-converting enzyme interactions in the cell membrane, J. Immunol. 170 (2003) 2923–2931.
[17] M.J. McCreery, W.A. Hunt, Physico-chemical correlates of alcohol intoxication, Neuropharmacology 17 (1978) 451–461.
[18] D.B. Goldstein, Ethanol-induced adaptation in biological membranes, Ann. N.Y. Acad. Sci. 492 (1987) 103–111.
[19] R.A. Harris, R. Burnett, S. McQuilkin, A. McClard, F.R. Simon, Effects of ethanol on membrane order: fluorescence studies, Ann. N.Y. Acad. Sci. 492 (1987) 125–135.
[20] W.G. Wood, C. Gorka, F. Schroeder, Acute and chronic effects of ethanol on transbilayer membrane domains, J. Neurochem. 52 (1989) 1925–1930.
[21] E. Rubin, H. Rottenberg, Ethanol-induced injury and adaptation in biological membranes, Fed. Proc. 41 (1982) 2465–2471.
[22] K.W. Miller, L.L. Firestone, S.A. Forman, General anesthetic and specific effects of ethanol on acetylcholine receptors, Ann. N.Y. Acad. Sci. 492 (1987) 71–87.
[23] M. Lasner, L.G. Roth, C.H. Chen, Structure–functional effects of a series of alcohols on acetylcholinesterase-associated membrane vesicles: elucidation of factors contributing to the alcohol action, Arch. Biochem. Biophys. 317 (1995) 391–396.
[24] M. Triantafilou, K. Brandenburg, T. Gutsmann, U. Seydel, K. Triantafilou, Innate recognition of bacteria: engagement of multiple receptors, Crit. Rev. Immunol. 22 (2002) 251–268.
[25] G. Schmitz, E. Orso, CD14 signalling in lipid rafts: new ligands and co-receptors, Curr. Opin. Lipidol. 13 (2002) 513–521.
[26] H. Kono, T. Suzuki, K. Yamamoto, M. Okada, T. Yamamoto, Z. Honda, Spatial raft coalescence represents an initial step in Fc gamma R signaling, J. Immunol. 169 (2002) 193–203.
[27] W.C. Raschke, S. Baird, P. Ralph, I. Nakoinz, Functional macrophage cell lines transformed by Abelson leukemia virus, Cell 15 (1978) 261–267.
[28] R.A. Zoeller, P.D. Wightman, M.S. Anderson, C.R. Raetz, Accumulation of lysophosphatidylinositol in RAW 264.7 macrophage tumor cells stimulated by lipid A precursors, J. Biol. Chem. 262 (1987) 17212–17220.
[29] J. Hu, R. Jacinto, C. McCall, L. Li, Regulation of IL-1 receptor-associated kinases by lipopolysaccharide, J. Immunol. 168 (2002) 3910–3914.
[30] J.L. Swantek, M.F. Tsen, M.H. Cobb, J.A. Thomas, IL-1 receptor-associated kinase modulates host responsiveness to endotoxin, J. Immunol. 164 (2000) 4301–4306.
[31] S.B. Pruett, C. Schwab, Q. Zheng, R. Fan, Suppression of innate immunity by acute ethanol administration: a global perspective and a new mechanism beginning with inhibition of signaling through TLR3, J. Immunol. 173 (2004) 2715–2724.
[32] D.T. Golenbock, Y. Liu, F.H. Millham, M.W. Freeman, R.A. Zoeller, Surface expression of human CD14 in Chinese hamster ovary fibroblasts imparts macrophage-like responsiveness to bacterial endotoxin, J. Biol. Chem. 268 (1993) 22055–22059.
[33] D.A. Brown, J.K. Rose, Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface, Cell 68 (1992) 533–544.
[34] D. Brown, Structure and function of membrane rafts, Int. J. Med. Microbiol. 291 (2002) 433–437.
[35] H.A. Lucero, P.W. Robbins, Lipid rafts-protein association and the regulation of protein activity, Arch. Biochem. Biophys. 426 (2004) 208–224.
[36] P. Mandrekar, D. Catalano, G. Szabo, Inhibition of lipopolysaccharide-mediated NFkappaB activation by ethanol in human monocytes, Int. Immunol. 11 (1999) 1781–1790.
[37] G. Szabo, P. Mandrekar, L. Girouard, D. Catalano, Regulation of human monocyte functions by acute ethanol treatment: decreased tumor necrosis factor-alpha, interleukin-1 beta and elevated interleukin-10, and transforming growth factor-beta production, Alcohol. Clin. Exp. Res. 20 (1996) 900–907.
[38] D.A. Stoltz, S. Nelson, J.K. Kolls, P. Zhang, R.P. Bohm Jr., M. Murphey-Corb, G.J. Bagby, In vitro ethanol suppresses alveolar macrophage TNF-alpha during simian immunodeficiency virus infection, Am. J. Respir. Crit. Care Med. 161 (2000) 135–140.
[39] Z. Zhang, J. Cork, P. Ye, D. Lei, P.O. Schwarzenberger, W.R. Summer, J.E. Shellito, S. Nelson, J.K. Kolls, Inhibition of TNF-alpha processing and TACE-mediated ectodomain shedding by ethanol, J. Leukoc. Biol. 67 (2000) 856–862.
[40] B.K. Verma, M. Fogarasi, G. Szabo, Down-regulation of tumor necrosis factor alpha activity by acute ethanol treatment in human peripheral blood monocytes, J. Clin. Immunol. 13 (1993) 8–22.
[41] M.P. Nair, N.M. Kumar, Z.A. Kronfol, J.F. Greden, J.S. Lwebuga-Mukasa, S.A. Schwartz, Alcohol inhibits lipopolysaccharide-induced tumor necrosis factor alpha gene expression by peripheral blood mononuclear cells as measured by reverse transcriptase PCR in situ hybridization, Clin. Diagn. Lab. Immunol. 3 (1996) 392–398.
[42] S.B. Pruett, R. Fan, Q. Zheng, Acute ethanol administration profoundly alters poly I:C-induced cytokine expression in mice by a mechanism that is not dependent on corticosterone, Life Sci. 72 (2003) 1825–1839.
[43] T. Urso, J.S. Gavaler, D.H. Van Thiel, Blood ethanol levels in sober alcohol users seen in an emergency room, Life Sci. 28 (1981) 1053–1056.
[44] L.J. Pike, Lipid rafts: bringing order to chaos, J. Lipid Res. 44 (2003) 655–667.
[45] L.J. Pike, Lipid rafts: heterogeneity on the high seas, Biochem. J. 378 (2004) 281–292.
[46] T. Ikehara, H. Yamaguchi, K. Hosokawa, T. Yonezu, H. Miyamoto, Effects of nystatin on intracellular contents and membrane transport of alkali cations, and cell volume in HeLa cells, J. Membr. Biol. 90 (1986) 231–240.
[47] D.P. Cerneus, E. Ueffing, G. Posthuma, G.J. Strous, A. van der Ende, Detergent insolubility of alkaline phosphatase during biosynthetic transport and endocytosis. Role of cholesterol, J. Biol. Chem. 268 (1993) 3150–3155.
[48] K.A. Papadakis, S.R. Targan, Tumor necrosis factor: biology and therapeutic inhibitors, Gastroenterology 119 (2000) 1148–1157.