Stabilization of Tumor Necrosis Factor \( \alpha \) mRNA by Chronic Ethanol

ROLE OF A + U-RICH ELEMENTS AND p38 MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING PATHWAY*

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Increased expression of tumor necrosis factor \( \alpha \) (TNF\( \alpha \)) in response to chronic ethanol has been implicated in the pathogenesis of alcoholic liver disease. However, the molecular mechanisms by which ethanol increases the levels of TNF\( \alpha \) are not well characterized. Utilizing Kupffer cells isolated from rats fed an ethanol containing diet and a murine macrophage cell line, RAW264.7, exposed to ethanol in culture, we have demonstrated that exposure to chronic ethanol results in an enhanced expression of lipopolysaccharide (LPS)-induced TNF\( \alpha \). While chronic ethanol had no effect on the rate of LPS-induced TNF\( \alpha \) transcription as measured by nuclear run-on experiments, TNF\( \alpha \) mRNA half-life was increased by chronic ethanol. Chronic ethanol also potentiated the activation of LPS-induced p38 mitogen-activated protein (MAP) kinase in Kupffer cells, as well as in RAW264.7 cells. Specific inhibition of p38 MAP kinase activation by SB203580 in Kupffer cells or by overexpression of dominant negative p38 MAP kinase in RAW264.7 cells blocked ethanol-mediated TNF\( \alpha \) mRNA stabilization. Furthermore, using chimeric reporter constructs, we have shown that A + U-rich elements in the 3′-untranslated region of TNF\( \alpha \) mRNA are not sufficient to impart ethanol-mediated stabilization on TNF\( \alpha \) mRNA.

The inflammatory response is a key component of host defense. Macrophages participate in this tightly regulated process, at least in part, via the secretion of proinflammatory cytokines, such as tumor necrosis factor \( \alpha \) (TNF\( \alpha \)\(^1\)) and interleukin-1 (IL-1), in response to various stimuli encountered in the tissue microenvironment. TNF\( \alpha \) is one of the principal mediators of the inflammatory response in mammals, transducing differential signals that regulate cellular activation and proliferation, cytotoxicity, and apoptosis (1, 2). In addition to its role in acute septic shock, TNF\( \alpha \) has been implicated in the pathogenesis of a wide variety of inflammatory diseases (3–5) as well as in the progression of alcoholic liver disease (6–9). Data from a number of animal studies, in combination with clinical studies, indicate that long-term alcohol consumption results in the development of fatty liver and steatohepatitis with progression to more severe liver damage including fibrosis, cirrhosis, and hepatocellular carcinoma (9). At least some of these alcohol-induced liver abnormalities have been linked to the overexpression of TNF\( \alpha \). Indeed, enhanced levels of TNF\( \alpha \) have been observed in the liver of ethanol-fed animals, as well as in the circulation of patients with alcoholic liver disease (10, 11). However, the molecular mechanisms leading to this overexpression in response to alcohol are poorly understood.

Considering the pleiotropic actions of TNF\( \alpha \), it is not surprising that its biosynthesis is under the control of multiple and complex regulatory mechanisms. TNF\( \alpha \) expression is regulated at transcriptional, post-transcriptional, as well as translational levels (1, 2, 12). Modulation of mRNA stability is an important mechanism of TNF\( \alpha \) biosynthesis (12, 13). Stabilization of mRNAs contributes to the strong and rapid induction of genes in the inflammatory process. Although the mechanisms involved in post-transcriptional gene regulation are complex, the mRNA itself contains sequence-specific information that determines its stability (14–16). TNF\( \alpha \) mRNA, like other short-lived mRNAs, contains A + U-rich elements (ARE) in its 3′-untranslated region (UTR) which function as destabilizing elements as demonstrated in TNF\( \alpha \)-ARE knockout mouse in vivo (17), as well as in various in vitro systems (13, 18). AREs have also been implicated in stimulus-induced stabilization of otherwise unstable mRNAs (19, 20). Sequences in the 5′-UTRs or coding regions, acting either in concert with AREs in the 3′-UTR or independently, also contribute to mRNA stabilization of certain genes (19, 21). However, the signaling pathways involved in the control of mRNA stabilization are not well defined.

Proinflammatory cytokines and external stressors act through receptor-dependent signaling cascades that diverge into multiple pathways, including the NF\( \kappa \)B pathway (22) and each of the three mitogen-activated protein (MAP) kinases: extracellular-regulated kinase (ERK), stress-activated protein kinase/c-jun N-terminal kinase (SAPK/JNK), and p38 MAP kinase (23, 24). While each of these signaling pathways contributes to the activation of gene transcription (22, 25), their role in controlling gene expression at the level of mRNA stability is not well understood. Although some studies have shown the involvement of ERK1/2 and SAPK/JNK pathways in the mRNA stability (21, 26–28), the majority of studies suggest that mRNAs encoding for proinflammatory genes including, cyclooxygenase-2 (COX-2), IL-6, IL-8, and TNF\( \alpha \), are stabilized upon activation of the p38 MAP kinase pathway (20, 29, 30). This p38 MAP kinase-mediated stabilization is dependent on ARE sequences in the 3′-UTRs of respective genes (20, 29, 31). Thus, while AREs confer instability on mRNAs, they also allow mRNA stabilization following activation of p38 MAPK pathway (30).
Despite the suggested role of TNFα in the pathogenesis of alcoholic liver disease, the mechanisms by which chronic alcohol exposure increases TNFα expression are not well understood. Ethanol modulates the activity of several important signaling molecules (32) including ERK1/2, p38 MAPK (33), JNK (33, 34), and the transcription factors NfκB (35) and Egr-1.2 However, it is not known whether ethanol-induced changes in these pathways contribute to abnormal TNFα transcription and/or translation.

To investigate the mechanism for ethanol-mediated increases in TNFα, we have studied the effect of chronic ethanol in the regulation of LPS-induced TNFα expression, both in Kupffer cells isolated from ethanol-fed rats, as well as in a mouse macrophage like cell line, RAW264.7, exposed to chronic ethanol in culture. We demonstrate, for the first time, that ethanol stabilizes LPS-induced TNFα mRNA. We further demonstrate that activation of p38 MAP kinase pathway is required for the ethanol-mediated TNFα mRNA stabilization, but that the AREs in the TNFα-3′-UTR are not sufficient to mediate ethanol-induced stabilization of TNFα mRNA.

MATERIALS AND METHODS

Reagents—Cell culture reagents were from Life Technologies, Inc. (Grand Island, NY). Antibodies were from the following sources: mouse phospho-p38 MAPK, Promega; Mouse phospho-ERK1/2, Promega; and ERK1/2, Upstate Biotechnology, Lake Placid, NY; anti-rabbit IgG-peroxidase was purchased from Roche Molecular Biochemicals (Indianapolis, IN). PD98059 and SB230580 were from Calbiochem (La Jolla, CA). LPS from Escherichia coli serotype 026:B6 and actinomycin D were purchased from Sigma. Ribonuclease Protection Assay (RPA) kit and reagents were purchased from BD Pharmingen (San Diego, CA) and Ambion (Austin, TX). Sequagel and related buffers were from National Diagnostics (Atlanta, GA). PerkinElmer Life Sciences (Boston, MA) was the source for [α-32P]UTP. Transfase transcription reagent and pSP-Luc+ vector used as DNA template for Luciferase RPA probe were purchased from Promega.

Constructs—Kinase-dead dominant negative constructs for p38, ERK1, and ERK2 MAP kinases were a kind gift from Dr. R. L. Eckert and have been described before (36). Chimeric luciferase-TNF3′ UTR constructs (98-18 luciferase-TNF3′ UTR and 98-19 luciferase-TNF-ARE mutant) were kindly provided by Dr. V. Kruys.

Animals and Chronic Ethanol Feeding Protocol—Adult male Wistar rats weighing 150 g were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). Lieber DeCarli ethanol diet was purchased from Dyets (Bethlehem, PA). Rats were acclimated for 3 days before arrival and provided with free access to Purina rat chow and water. All rats were then allowed free access to liquid diet (37) without ethanol for 2 days and then randomly assigned to the ethanol-fed or pair-fed groups. The ethanol-fed group was allowed free access to liquid diet with 17% of calories as ethanol for the remainder of the feeding period. Culture dishes were wrapped in parafilm to minimize the evaporation during culture. Culture dishes with untreated cells were also wrapped in parafilm. For transfections, RAW264.7 cells were grown in 100-mm dishes to 60% confluency and were transiently transfected with appropriate control and expression vectors using transfast transfection reagent (Promega), following the manufacturer’s instructions. Transfected cells were subcultured, treated, or not with 25 mM ethanol for 48 h; stimulated with LPS (100 ng/ml) and treated or not with actinomycin D according to experimental requirements, before the isolation of protein or RNA.

Nuclear Run-on—Nuclear run-on experiments to measure nascent RNA transcripts were essentially performed as described elsewhere (39). Briefly, Kupffer cells from ethanol-or pair-fed rats and RAW264.7 cells pretreated or not with 25 mM ethanol for 48 h, were stimulated with 0 or 100 ng/ml LPS. Following stimulation, nuclei were isolated from 2 × 107 cells/experimental group and were incubated with 2 × reaction buffer (10 mM Tris-Cl, 5 mM MgCl2, 0.3 mM KCl, 10 mM each of ATP, GTP, CTP, 1 mM dithiothreitol) in the presence of [α-32P]UTP at 30 °C for 30 min and were further incubated with RNase-free DNase 1 (1 mg/ml) for 5 min at 30 °C and with Proteinase K (20 mg/ml) for an additional 30 min at 42 °C. Labeled RNA was harvested and hybridized with mouse TNFα and GAPDH cDNAs. Autoradiogram was mounted on nitrocellulose filters for 24 h at 80 °C. Membranes were thoroughly washed and processed for autoradiography.

Ribonuclease Protection Assay—Kupffer cells from ethanol- and pair-fed rats were stimulated with 100 ng/ml LPS for 1 h. Total RNA was isolated by Trizol method (Life Technologies, Inc.). For mRNA stability experiments, cells were further cultured in the presence or absence of actinomycin D (5 μg/ml) for the indicated times following LPS treatment before RNA was isolated. For some experiments Kupffer cells were pretreated with either 20 μM SB203580 or 50 μM PD98059 before LPS stimulation and RNA isolation. RAW264.7 cells were cultured in the presence or absence of 25 mM ethanol for 48 h prior to the LPS stimulation and actinomycin D treatment. Rat or mouse cytokine multiprobe DNA templates (Pharmingen) were used to synthesize in vitro transcribed antisense riboprobes and RPAs were carried out following the manufacturer’s instructions. Samples were run on 5% sequencing gels, dried, and autoradiographed.

Western Blot Analysis—Kupffer cells isolated from ethanol- and pair-fed rats were stimulated with 0 or 100 ng/ml LPS for the indicated times, washed twice with cold PBS and lysed directly in 2 × Laemmli buffer. Lysates were passed through a 21-gauge needle to shear DNA, boiled for 10 min, and centrifuged briefly. Samples were separated by 10% SDS-PAGE, transferred to PVDF membranes, and probed with specific phospho-anti-MAP kinase antibodies. The membrane was then stripped and reprobed with the corresponding total anti-MAP kinase antibody as a loading control. Bound antibody was detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ). Immunoreactive protein quantity was assessed by scanning densitometry.

Statistical Analysis—Because of the limited number of Kupffer cells available from each animal, data from several feeding trials are presented in this paper; each trial consisted of 6 rats per feeding group. Values reported are mean ± S.E. Data were analyzed by Student’s t test or general linear models procedure (SAS, Carey, IN), blocking for trial effects if data from more than one trial was used.

RESULTS

Chronic Ethanol Consumption Increases LPS-induced TNFα mRNA Levels in Kupffer Cells—We have previously reported that exposure of RAW264.7 cells to chronic ethanol (25 mM for 48 h) increases the level of LPS-induced TNFα mRNA expression.2 To investigate whether chronic ethanol would have a similar effect in vivo, TNFα mRNA expression was analyzed in Kupffer cells isolated from pair-fed and ethanol-fed rats. As shown in Fig. 1, chronic ethanol consumption increased LPS-induced TNFα mRNA accumulation in Kupffer cells isolated

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from ethanol-fed rats compared with pair-fed rats. Interestingly, ethanol feeding did not affect LPS-induced expression of other proinflammatory cytokines, including IL-4, IL-5, and IL-6. LPS-induced expression of IL-1β was, however, reduced in response to chronic ethanol feeding. Taken together these results suggest that chronic ethanol specifically enhances LPS-induced TNFα mRNA expression.

**Chronic Ethanol Does Not Affect LPS-induced Transcription of TNFα Message**—To investigate whether increased expression of LPS-induced TNFα in response to chronic ethanol reflects increased transcription, nuclear run-on experiments were carried out in Kupffer cells and RAW264.7 cells. LPS stimulated de novo TNFα transcription was comparable between Kupffer cells from ethanol-fed and pair-fed rats (Fig. 2A). Similarly, prior exposure of RAW264.7 cells to 25 mM ethanol for 48 h had no effect on the rate of LPS-induced TNFα transcription compared with control cells (Fig. 2B). These results suggest that chronic ethanol does not increase LPS-induced TNFα expression at the level of gene transcription.

**LPS-induced TNFα mRNA Is Stabilized by Chronic Ethanol**—There is ample evidence regarding regulation of TNFα expression at the level of mRNA stability. To elucidate whether ethanol-mediated increases in the level of TNFα mRNA might reflect an increased half-life of the transcripts, mRNA stability experiments were performed in Kupffer cells and RAW264.7 cells. Kupffer cells from ethanol- and pair-fed rats were stimulated with 0 or 100 ng/ml LPS for 60 min. Cells were further incubated in the presence or absence of actinomycin D for 1–2 h. RNA was harvested and analyzed for TNFα mRNA expression (Fig. 3A). Chronic ethanol consumption stabilized LPS-induced TNFα mRNA in Kupffer cells isolated from ethanol-fed rats (t1/2 > 100 min), compared with those isolated from pair-fed rats (t1/2 < 40 min). In contrast, the half-life of IL-1β mRNA was not affected by chronic ethanol in the same experiment (data not shown). A similar effect of chronic ethanol was observed on the TNFα mRNA stability in RAW 264.7 cells (Fig. 3B). In control cells, LPS-induced TNFα mRNA decayed with an approximate half-life of 36 min. However, treatment of cells with 25 mM ethanol for 48 h not only increased the accumulation of TNFα mRNA, but also substantially stabilized the TNFα transcript (t1/2 > 100 min). These data clearly demonstrate that exposure to chronic ethanol both in vivo and in vitro results in the marked stabilization of LPS-induced TNFα mRNA.

**Ethanol-mediated Stabilization of LPS-induced TNFα mRNA Is Gene Specific**—mRNA stability of labile genes can be regulated by ARE sequences in their 3’-UTRs. TNFα, like other short-lived cytokine genes, has multiple AREs in its 3’-UTR. To investigate whether exposure to chronic ethanol stabilized other short-lived, ARE-containing cytokine mRNAs, we studied the effect of chronic ethanol on IL-12 p40, IL-1β, IL-6, and IL-2 mRNA stability. The mRNA decay rate for LPS-induced IL-12 p40, IL-1β, IL-6 (Fig. 4), and IL-2 (Fig. 3B) was similar in RAW264.7 cells cultured with or without ethanol. These results show that chronic ethanol had no effect on the mRNA stability of the other ARE-containing cytokine mRNAs, suggesting that ethanol specifically targets the stabilization of TNFα mRNA.

**Chronic Ethanol Potentiates LPS-induced Activation of p38 MAP Kinase**—Short- and long-term ethanol exposure modulates agonist-mediated activation of p38, ERK1/2 as well as SAPK/JNK MAP kinases in some cell types (33, 34). We have previously shown that chronic ethanol potentiates the LPS-induced activation of ERK1/2 MAP kinases in RAW264.7 cells.2 Since p38 MAP kinase has been implicated in stabilization of cytokine mRNA, we investigated the effect of chronic ethanol on the activation of p38 MAP kinase. Ethanol potentiated and prolonged the LPS-induced phosphorylation of p38 MAP kinase in Kupffer cells isolated from rats fed ethanol compared with pair-fed (Fig. 5A). A similar increase and prolongation in LPS-induced phosphorylation of p38 MAP kinase was observed in vivo.
RAW264.7 cells cultured in presence of 25 mM ethanol for 48 h compared to control cells (Fig. 5B).

Inhibition of p38 MAP Kinase Specifically Eliminates Ethanol-mediated Stabilization of TNFα mRNA—Activation of p38 and ERK1/2 MAP kinases has been linked to mRNA stabilization of otherwise short-lived cytokine and other immediate early response genes (20). Therefore, we tested whether ethanol-induced potentiation of p38 or ERK1/2 MAP kinases was involved in the stabilization of TNFα mRNA observed after chronic ethanol exposure. Kupffer cells isolated from ethanol- and pair-fed rats were pretreated with either 20 μM SB203580 or 50 μM PD98059 followed by stimulation with LPS. After stimulation with LPS, Kupffer cells were further treated or not with actinomycin D. Total RNA from each treatment group was analyzed for TNFα mRNA levels (Fig. 6). As observed earlier, TNFα mRNA was substantially stabilized in Kupffer cells isolated from ethanol-fed rats. Inhibition of p38 activation completely abrogated ethanol-mediated stabilization of TNFα mRNA. In contrast, inhibition of ERK1/2 activation by PD98059 had no effect on ethanol-mediated stabilization of TNFα mRNA.

To further substantiate the role of MAP kinases in ethanol-mediated stabilization of TNFα mRNA, we tested the efficacy of p38 and ERK1/2 dominant negative constructs to block the ethanol-mediated TNFα mRNA stability. RAW264.7 cells were transfected either with dominant negative p38 or dominant negative ERK1/2 and mRNA stability experiments were carried out as described earlier (Fig. 7). Efficacy of transfections was determined by Western blots. Overexpression of either p38 or ERK1/2 dominant negative constructs completely blocked the phosphorylation of p38 and ERK1/2, respectively (data not shown). Inhibition of either p38 or ERK1/2 activation reduced LPS-induced TNFα mRNA expression by ~50% compared with cells transfected with vector alone, likely a result of reduced transcription. Exposure to chronic ethanol stabilized TNFα mRNA in RAW264.7 cells transfected with vector alone. However, this stabilization of TNFα mRNA was completely blocked in cells transfected with dominant negative p38 MAP kinase. Transfection of dominant negative ERK1/2 MAP kinase had no discernible effect on the ethanol-mediated TNFα mRNA stabilization. These results demonstrate specificity of p38 MAP kinase activation in ethanol-mediated stabilization of TNFα mRNA.

ARE Sequences in TNFα-3’-UTR Are Not Sufficient to Impart Ethanol-mediated Stabilization on the TNFα mRNA—Since ARE sequences have previously been shown to...
be an important determinant of TNFα mRNA stability, we next determined the role of TNFα-ARE sequences in ethanol-mediated stabilization of the TNFα transcript. RAW264.7 cells were transiently transfected either with a reporter construct containing full-length 3’-UTR of TNFα linked to luciferase reporter gene (pGL3-TNF3’UTR) or a similar chimera in which the 70-nucleotide ARE-containing sequence from TNFα 3’-UTR was deleted (pGL3-TNF AUmut). A vector containing lucifer-
an increased TNFα production (40–42). Indeed, ethanol-induced liver injury was blunted by treating ethanol-fed rats with antibiotics, further supporting the association of endotoxins with the ethanol-induced liver injury (43). However, little is known about the mechanism by which chronic ethanol up-regulates LPS-induced expression of proinflammatory mediators including TNFα. Our data show that ethanol specifically enhanced TNFα mRNA level in rat Kupffer cells, but did not increase the accumulation of other proinflammatory cytokines (Fig. 1). Interestingly, chronic ethanol decreased the LPS-induced mRNA accumulation of IL-1β. IL-1β expression is regulated by many of the mechanisms as TNFα, involving modulation of gene transcription, mRNA stability and mRNA translation (44, 45). Thus, the differential effect of chronic ethanol on LPS-induced mRNA expression of TNFα and IL-1β in Kupffer cells suggests that chronic ethanol must impact on distinct molecular mechanisms and signaling pathways to differentially regulate the expression of TNFα compared with IL-1β.

Cell type and stimulus specific transcriptional regulation of TNFα has been reported in a number of studies (1, 2, 46). We have recently demonstrated that exposure of RAW264.7 macrophages to chronic ethanol modulates the LPS-induced DNA binding activity of at least two transcription factors to the TNFα promoter. Chronic ethanol increases Egr-1 binding activity, but decreases the binding activity of NFκB (2). However, in the present study, we found no discernible changes in the rate of de novo transcribed TNFα in response to chronic ethanol (Fig. 2). These results indicate that despite multiple effects on DNA binding activity of key transcription factors, there is no net effect on the rate of TNFα transcription in response to chronic ethanol and that post-transcriptional mechanism(s) must contribute to ethanol-mediated up-regulation of TNFα mRNA.

Chronic ethanol stabilized the LPS-induced TNFα mRNA both in a mouse macrophage cell line RAW264.7 and in Kupffer cells isolated from rats fed ethanol for 4 weeks (Fig. 3). Utilizing a wide variety of cell types, several studies have shown stimulus-specific stabilization of otherwise labile mRNAs that contributes to a rapid increase in their abundance. Phorbol ester treatment increases the half-life of granulocyte macrophage-colony stimulating factor mRNA (16, 47). Calcium ionophore transiently stabilizes IL-3 mRNA in a murine mast cell line (48). In T cells, IL-2 mRNA is stabilized by co-stimulatory signal through CD-28, upon stimulation of T cell receptor (49). More recently a number of studies have shown the stimulus-induced stabilization of COX-2, IL-6, IL-8, GROα, MCP-1, and TNFα mRNAs in macrophage/monocytes, as well as in other cell types (19, 20, 28, 29, 31, 50). However, this is the first study to demonstrate ethanol-mediated modulation of TNFα stability in any cell type. Whether this phenomenon is a more generalized effect of ethanol or is restricted to macrophages remains to be elucidated.

LPS is known to activate all members of the MAP kinase family. To gain insight into the role of MAP kinase signaling pathways in the ethanol-mediated stabilization of TNFα transcript, we studied the effect of chronic ethanol on the LPS-induced phosphorylation of p38 and ERK1/2 MAP kinases. Chronic ethanol exposure increased LPS stimulated phosphorylation of p38 MAP kinase (Fig. 5). This observation is consistent with a number of other studies reporting the effect of ethanol on MAP kinase activation. Ethanol exposure, both long- and short-term, potentiates agonist-induced activation of p38, ERK1/2, as well as SAPK/JNK MAP kinases in hepatocytes (33, 34). Long-term ethanol exposure increases NGF-stimulated ERK1/2 in PC12 cells (41). In NIH 3T3 fibroblasts, ethanol po-

![Fig. 8. Ethanol-induced stabilization of TNFα mRNA is not mediated by AU rich elements. RAW264.7 cells were transiently transfected either with control luciferase vector or with pGL3-TNF3′UTR or pGL3-TNF AUmut vectors. Transfected cells were subcultured in the presence or absence of 25 mM ethanol for 48 h, stimulated with 100 ng/ml LPS, and further treated or not with actinomycin D for 2 h. RNA was harvested and was analyzed for luciferase and β-actin mRNA levels. Autoradiograph is representative of three similar experiments.

FIG. 8. Ethanol-induced stabilization of TNFα mRNA is not mediated by AU rich elements. RAW264.7 cells were transiently transfected either with control luciferase vector or with pGL3-TNF3′UTR or pGL3-TNF AUmut vectors. Transfected cells were subcultured in the presence or absence of 25 mM ethanol for 48 h, stimulated with 100 ng/ml LPS, and further treated or not with actinomycin D for 2 h. RNA was harvested and was analyzed for luciferase and β-actin mRNA levels. Autoradiograph is representative of three similar experiments.

Discussion

Although enhanced expression of TNFα has been implicated in the pathogenesis of alcoholic liver disease, the molecular mechanisms regulating TNFα overexpression are not well understood. In the present study, utilizing an ethanol-fed rat model, as well as mouse macrophage cells exposed to ethanol in culture, we have demonstrated that chronic ethanol specifically increases the LPS-induced TNFα mRNA stability. Furthermore, stabilization of TNFα mRNA by ethanol was dependent on activation of p38 MAP kinase, but independent of the ARE sequences in the 3′-UTR of the TNFα mRNA. Chronic ethanol specifically enhanced the accumulation of TNFα; this specific increase in TNFα mRNA level was observed both in mouse RAW264.7 macrophage cells after in vitro exposure to ethanol and in Kupffer cells isolated from rats fed ethanol in vivo. Furthermore, in both types of macrophages, chronic ethanol increased the half-life of LPS-induced TNFα mRNA, without affecting the rate of TNFα transcription. Increased stability of TNFα mRNA was directly associated with an increase in LPS-induced activation of p38 MAP kinase; inhibition of p38 MAP kinase activation by two different approaches blocked the ethanol-mediated stabilization of TNFα mRNA, while inhibition of ERK1/2 activation did not affect TNFα mRNA stability. Moreover, AREs in the 3′-UTR of TNFα mRNA destabilized luciferase reporter mRNA but were not sufficient to render ethanol-mediated stabilization on the transgene.

There is now general agreement that long-term alcohol consumption leads to an increased expression of TNFα in liver. It has been suggested that chronic ethanol consumption compromises the barrier function of gastrointestinal mucosa, overexposing liver cells to endotoxins, such as LPS, and resulting in...
tentatively sphingosine-1 phosphate-stimulated ERK1/2 activation, but not p38 MAP kinase (51). More recently we have shown that in RAW264.7 mouse macrophage cells, chronic ethanol increases LPS-stimulated phosphorylation of ERK1/2 MAP kinases.2 The mechanism by which ethanol potentiates LPS-stimulated activation of p38 remains an unresolved question. Since chronic ethanol increases both LPS-stimulated ERK1/2 and p38, we are currently investigating whether ethanol acts at a common upstream signaling intermediate linking LPS-receptor activation to both these members of the MAP kinase family. Alternatively, ethanol could also enhance ERK1/2 and p38 activity by decreasing MAP kinase phosphatase-1 activity, which can inactivate both phosphorylated ERK1/2 and p38.

There is growing body of evidence indicating the participation of MAP kinases in stimulus-induced stabilization of various short-lived transcripts. Studies on the signaling pathways involved in mRNA stability have shown p38, ERK1, and SAPK/JNK MAP kinases, as well as CAMP-dependent protein kinase A, are important contributors to this post-transcriptional regulatory mechanism (21, 28, 52). The emerging picture from these studies suggests that stabilization of mRNAs can be achieved by different signaling events; effectors of multiple MAP kinases are involved in mRNA stability. One potentially important target of ethanol-mediated mRNA stability is the protein kinase C (PKC) pathway. Ethanol is known to regulate PKC activity in many cell types; the δ and ε isoforms are particularly sensitive to ethanol (32). PKC-mediated mRNA stabilization has been reported in a number of studies (58–60); PKCδ has been specifically implicated in regulation of iNOS mRNA stability (61). While PKC is involved in many LPS-mediated responses in macrophages (62), we are currently investigating whether a specific PKC isoform is involved in TNFα mRNA stability and if that isoform is targeted by chronic ethanol exposure.

Considering the association between ethanol-mediated up-regulation in TNFα expression and alcoholic liver disease, it is critical to understand the mechanism by which ethanol increases TNFα biosynthesis. Our data provide direct evidence for a novel mechanism for ethanol-mediated up-regulation of TNFα biosynthesis. While other studies have demonstrated that ethanol increases TNFα mRNA expression, this is the first study to demonstrate that chronic ethanol, signaling selectively through the p38 MAP kinase pathway, enhances the LPS-induced TNFα expression by stabilizing the TNFα transcript. Moreover, our data demonstrate that ethanol specifically enhances TNFα mRNA stability. Therefore, this response is likely to contribute to the critical role that TNFα plays in the progression of inflammation during alcoholic liver disease.

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Stabilization of Tumor Necrosis Factor α mRNA by Chronic Ethanol: ROLE OF A + U-RICH ELEMENTS AND p38 MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING PATHWAY

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