Alcohol Causes Both Tolerance and Sensitization of Rat Kupffer Cells via Mechanisms Dependent on Endotoxin

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Background & Aims: Ethanol causes both tolerance and sensitization of Kupffer cells. This study was designed to evaluate temporal effects of ethanol in an attempt to understand this paradox. **Methods:** Rats were given ethanol (4 g/kg body wt) intragastrically, and Kupffer cells were isolated 0–48 hours later. After addition of lipopolysaccharide (LPS), intracellular calcium concentration ($[Ca^{2+}]_i$) was measured using a microspectrofluorometer with the fluorescent indicator fura-2, and tumor necrosis factor α (TNF- α) was measured by enzyme-linked immunosorbent assay. CD14 was evaluated by Western and Northern analysis. **Results:** Two hours after ethanol administration, the LPS-induced increase in $[Ca^{2+}]_i$ and TNF- α release by Kupffer cells was diminished by 50%, and these parameters were reciprocally enhanced twofold at 24 hours. Sterilization of the gut with antibiotics blocked all effects of ethanol on $[Ca^{2+}]_i$ and TNF- α release completely. Twenty-four hours after ethanol, CD14 in Kupffer cells was elevated about fivefold. **Conclusions:** Kupffer cells isolated from rats early after ethanol exhibited tolerance to LPS, whereas sensitization was observed later. It is likely that both of these phenomena are caused by gut-derived endotoxin and that sensitization in Kupffer cells is caused by increases in CD14.

Intercellular calcium ($[Ca^{2+}]_i$) most likely plays an important role in Kupffer cell activation.^{1,2} For example, a transient increase of $[Ca^{2+}]_i$ is required for lipopolysaccharide (LPS)-induced expression of tumor necrosis factor α (TNF- α) by a macrophage cell line.³ Further, Kupffer cells contain voltage-dependent calcium channels, which cause transmembrane Ca^{2+} influx.⁴ Short-term ethanol treatment in vivo reduced the probability that calcium channels in Kupffer cells would be open, whereas long-term ethanol treatment in vivo made them easier to open.^{5,6} Moreover, Kupffer cells are involved in alcohol-induced liver injury. Inactivation of

Kupffer cells with gadolinium chloride and intestinal sterilization with antibiotics (polymyxin B and neomycin) prevented early alcohol-induced liver injury in the enteral feeding model of Tsukamoto and French.^{7,8} However, the effect of ethanol on isolated Kupffer cells remains unclear. Although short-term administration of alcohol enhances endotoxin hepatotoxicity,⁹ a majority of studies report that short-term ethanol inactivates Kupffer cells.^{10–13} It is therefore paradoxical that Kupffer cells are involved in alcoholic liver injury based on in vivo data with gadolinium chloride and antibiotics, yet ethanol blunts activation of isolated Kupffer cells. Accordingly, the purpose of this study was to attempt to understand this paradox by studying the temporal effect of ethanol in vivo on the response of subsequently isolated Kupffer cells. Preliminary accounts of this work have appeared elsewhere.¹⁴

Materials and Methods

Animals and Treatments

Female Sprague-Dawley rats weighing between 200 and 250 g were used. All animals were given humane care in compliance with institutional guidelines. Rats were given ethanol (4 g/kg body wt, orally) before experiments.^{15,16} Some rats were treated for 4 days with polymyxin B and neomycin¹⁷ to prevent growth of intestinal bacteria, the primary source of endotoxin in the gastrointestinal tract. Based on the results of preliminary experiments, 150 mg · kg⁻¹ · day⁻¹ of polymyxin B and 450 mg · kg⁻¹ · day⁻¹ of neomycin were given orally. Under these conditions, gut sterilization was achieved.⁸

Analytical Methods

Rats were forced to breathe into a closed heated chamber (37°C) for 20 seconds, and 1 mL of breath was

Abbreviations used in this paper: $[Ca^{2+}]_i$, intracellular calcium; DMEM, Dulbecco's modified Eagle medium; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; LBP, LPS-binding protein; LPS, lipopolysaccharide; SDS, sodium dodecyl sulfate; SSC, standard saline citrate; TNF- α , tumor necrosis factor α .

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collected using a gas-tight syringe to measure ethanol. Ethanol was analyzed by gas chromatography.^{15,16} Blood was collected to measure endotoxin from the portal vein in pyrogen-free heparinized syringes and centrifuged, and plasma was stored at -20°C in pyrogen-free glass test tubes until measurement using the limulus amebocyte lysate assay (Whitaker Bioproducts Inc., Walkersville, MD). Levels of endotoxin in plasma from normal rats were below limits of detection. Serum was stored at -20°C in microtubes, and aspartate transaminase (AST) and alanine aminotransferase (ALT) levels were measured by standard enzymatic procedures.¹⁸ Livers were formalin-fixed, embedded in paraffin, and stained with H&E to assess inflammation and necrosis.

Kupffer Cell Preparation, Culture, and Measurement of $[\text{Ca}^{2+}]_i$

Kupffer cells were isolated by collagenase digestion and differential centrifugation using Percoll (Pharmacia, Uppsala, Sweden) as described elsewhere with slight modifications.^{19,20} Cells were seeded onto 25-mm glass coverslips and cultured in Dulbecco's modified Eagle medium (DMEM; GIBCO Laboratories Life Technologies Inc., Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/mL of penicillin G and 100 $\mu\text{g}/\text{mL}$ of streptomycin sulfate) at 37°C with 5% CO_2 . Nonadherent cells were removed after 1 hour by replacing buffer, and cells were cultured for 24–48 hours before experiments.

$[\text{Ca}^{2+}]_i$ was measured fluorometrically using the fluorescent calcium indicator dye fura-2 and a microspectrofluorometer (PTI, South Brunswick, NJ) interfaced with an inverted microscope (Diaphot, Nikon, Japan) as described in detail previously.²⁰

TNF- α Detection

Kupffer cells were seeded onto 24-well plates and cultured in DMEM supplemented with 10% FBS and antibiotics at 37°C in the presence of 5% CO_2 . Cells were incubated with fresh media containing LPS (0–1000 ng/mL supplemented with 5% rat serum) for an additional 4 hours. Samples of media were collected and kept at -80°C until assay. TNF- α in the culture media was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Genzyme Corp., Cambridge, MA), and data were corrected for dilution.

Western Blotting for CD14

Total protein extracts of cultured Kupffer cells were obtained by homogenizing samples in a buffer containing 10 mmol/L HEPES (pH 7.6), 25% glycerol, 420 mmol/L NaCl, 1.5 mmol/L MgCl_2 , 0.2 mmol/L ethylenediaminetetraacetic acid, 0.5 mmol/L dithiothreitol, 40 $\mu\text{g}/\text{mL}$ bestatin, 20 mmol/L β -glycerophosphate, 10 mmol/L 4-nitrophenylphosphate, 0.5 mmol/L pepabloc, 0.7 $\mu\text{g}/\text{mL}$ pepstatin A, 2 $\mu\text{g}/\text{mL}$ aprotinin, 50 $\mu\text{mol}/\text{L}$ Na_3VO_4 , and 0.5 $\mu\text{g}/\text{mL}$ leupeptin. Protein concentration was determined using the Bradford assay kit (Bio-Rad Laboratories, Hercules, CA). Extracted protein

was separated by 10% sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. Membranes were blocked by Tris-buffered saline–Tween 20 containing 5% skim milk, probed with mouse anti-rat ED9 monoclonal antibody (Serotec, Oxford, England), followed by horseradish peroxidase–conjugated secondary antibody as appropriate. Membranes were incubated with a chemiluminescence substrate (ECL reagent; Amersham Life Science, Buckinghamshire, England) and exposed to X-OMAT films (Eastman Kodak Co., Rochester, NY).

RNA Preparation and Northern Blotting

Total liver RNA was prepared by guanidinium/CsCl centrifugation as described elsewhere.²¹ The integrity and concentration of RNA was determined by measuring absorbance at 260 nm followed by electrophoresis on agarose gels.

For Northern blotting, 10 μg of total RNA was separated on 1% agarose gels containing formaldehyde followed by capillary transfer to nylon membranes. Membranes were prehybridized in a buffer containing 50% formamide, $5\times$ standard saline citrate (SSC), 100 $\mu\text{g}/\text{mL}$ salmon sperm DNA, and $1\times$ Denhardt's solution, hybridized in the same buffer with 10×10^6 cpm of random prime labeled complementary DNA (cDNA) for CD14 or LPS-binding protein (LBP) overnight, washed with $2\times$ SSC and 0.1% SDS at 50°C for 30 minutes and $0.1\times$ SSC and 0.1% SDS at 55°C for 30 minutes, and subjected to autoradiography. Subsequently, membranes were stripped and reprobbed using cDNA for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase.

Statistical Analysis

All results were expressed as mean $\pm$ SEM. Statistical differences between means were determined using analysis of variance (ANOVA) or ANOVA on ranks as appropriate. $P < 0.05$ was selected before the study to reflect significance.

Results

Blood Ethanol and Endotoxin Levels

After oral administration of ethanol (4 g/kg) to untreated, normal rats, blood ethanol levels increased gradually and reached a value of 274 ± 34 mg/dL after 90 minutes (Figure 1A). Similar results were obtained in rats treated with antibiotics. Subsequently, levels declined toward basal values over the next 6 hours. Plasma endotoxin levels were increased by ethanol treatment to values of 77 ± 6 pg/mL at 90 minutes and declined subsequently to the baseline (Figure 1B). Levels were about fourfold higher than values from rats treated with antibiotics.

Effect of Ethanol and LPS, Liver Histology, and Serum Transaminases

Liver specimens were collected for histology 24 hours after administration of LPS (5 mg/kg). Histology

Figure 1. Blood ethanol and endotoxin levels. (A) Rats were given ethanol as described in Materials and Methods, and ethanol was analyzed in breath by gas chromatography. (B) Portal endotoxin was determined by the limulus amoebocyte lysate pyrogen test. Results are mean $\pm$ SEM of 4 (A) individual rats or (B) samples. * $P < 0.05$ vs. 90 minutes control by two-way ANOVA.

was normal in control rats (Figure 2A), whereas LPS caused focal necrosis and neutrophil infiltration in the liver as expected (Figure 2B). Twenty-four hours after ethanol treatment, necrosis and neutrophil infiltration due to LPS were increased dramatically (Figure 2C). These histological changes were blunted by treatment with antibiotics (Figure 2D).

Two and 24 hours after ethanol treatment, LPS (5 mg/kg) was injected via the tail vein, and blood samples were collected 24 hours later for serum AST and ALT measurements (Figure 3). Mean values for AST and ALT in the control group were 71 ± 3 and 64 ± 7 IU/L, respectively. Values were not altered 2 hours after ethanol in control rats or animals treated with antibiotics; however, 24 hours after ethanol, LPS increased transaminases dramatically to 779 ± 125 and 760 ± 134 IU/L,

respectively. This increase was also blunted significantly by antibiotics.

Effect of Ethanol Treatment on LPS-Induced Increases in $[Ca^{2+}]_i$ in Kupffer Cells

Addition of LPS (10 μ g/mL) to Kupffer cells from untreated control rats caused a transient elevation in $[Ca^{2+}]_i$ followed by a gradual return to the baseline over 3 minutes. The maximal $[Ca^{2+}]_i$ level achieved was 303 ± 14 nmol/L (Figure 4A); however, values only reached 134 ± 6 nmol/L in Kupffer cells from rats treated with ethanol for 2 hours (Figure 4B). On the other hand, values reached 367 ± 48 nmol/L in cells isolated from rats treated 24 hours previously with ethanol (Figure 4C). After addition of a lower dose of LPS (100 ng/mL), $[Ca^{2+}]_i$ levels in cells from normal rats increased to 84 ± 6 nmol/L; however, values were over twofold higher (201 ± 15 nmol/L) in cells from rats treated with ethanol 24 hours earlier (Figure 4D and F). The time course of changes in the $[Ca^{2+}]_i$ response to LPS is summarized in Figure 5. Treatment of rats with antibiotics prevented both the early decline and later elevation in $[Ca^{2+}]_i$ caused by LPS after ethanol treatment (Figure 6). However, the response of Kupffer cells isolated from control rats to LPS was not affected by treatment with antibiotics (data not shown).

Effect of Ethanol Treatment In Vivo on LPS-Induced Production of TNF- α by Isolated Kupffer Cells

Treatment of rats with antibiotics prevented both the decrease in TNF- α production caused by exposure to ethanol for 2 hours as well as the stimulation observed after 24 hours (Figure 7). LPS-induced TNF- α production by isolated Kupffer cells was measured after ethanol treatment (Figure 8). In the absence of added LPS, TNF- α production was minimal and not different between the groups studied. However, after exposure to 10–1000 ng/mL LPS, TNF- α production increased about threefold over basal levels in cells from control rats, but did not change in cells from rats treated 2 hours earlier with ethanol. TNF- α production increased about eightfold over basal levels in cells from rats exposed to ethanol 24 hours earlier (Figure 8).

Effect of Ethanol and Antibiotics on CD14 Expression in Kupffer Cells and Production of LBP in the Liver

To determine whether the LPS/LBP receptor (CD14) was altered in Kupffer cells by ethanol treatment

Figure 2. Photomicrographs (H&E) of liver tissue from rats treated as described in Materials and Methods. (A) No treatment; (B) 24 hours after LPS (5 mg/kg intravenously); (C) 24 hours ethanol exposure and 24 hours LPS; and (D) antibiotics for 4 days, ethanol for 24 hours, and LPS for 24 hours. Original magnification 100 $\times$. Typical photomicrographs.

in vivo, Western blotting was performed using ED-9 antibody, which recognizes rat CD14 (Figure 9A). As expected, Kupffer cells from control rats expressed the 58-kilodalton CD14; however, the band was about fivefold more intense in Kupffer cells isolated from rats treated 24 hours earlier with ethanol. This increase was blunted by treatment with antibiotics. On the other hand, CD14 was not changed in Kupffer cells isolated from rats treated 2 hours earlier with ethanol.

Ethanol treatment also increased CD14 messenger RNA (mRNA) twofold 24 hours after ethanol treatment (Figure 9B).

Because LBP is necessary for CD14 to recognize LPS, mRNA for LBP in the liver after ethanol treatment was measured by Northern blotting. LBP mRNA was increased about twofold in livers from rats treated with ethanol for 6 hours followed by a gradual decline (Figure 9C). This increase was also blunted by antibiotics.

Figure 3. Effect of short-term ethanol treatment on LPS-induced increases in serum (A) AST and (B) ALT levels. Rats were treated with ethanol (EtOH) as described in Materials and Methods, and blood samples were collected 24 hours after LPS (5 mg/kg, *Escherichia coli* serotype O111: B4; Sigma Chemical Co., St. Louis, MO). Some rats were given antibiotics for 4 days before experiments (150 mg · kg⁻¹ · day⁻¹ of polymyxin B and 450 mg · kg⁻¹ · day⁻¹ of neomycin). A, antibiotics. Results are mean ± SEM for 4 rats per group. **P* < 0.05 vs. control. #*P* < 0.05 vs. ethanol 24 hours + LPS, 5 mg/kg. ***P* < 0.05 vs. control. ##*P* < 0.05 vs. ethanol 24 hours + LPS, 5 mg/kg, by ANOVA.

Discussion

Kupffer Cells Are Involved in Alcohol-Induced Liver Injury

Activation of Kupffer cells is a prominent event in the initiation of alcoholic liver disease.^{22,23} Kupffer cells are activated by endotoxin leading to rapid increases in intracellular calcium³ followed by release of inflammatory mediators (e.g., cytokines and lipid metabolites), as well as reactive oxygen intermediates.^{9,24,25} TNF- α is produced predominantly by the monocyte-macrophage lineage, and the major cell type of this lineage is the hepatic Kupffer cell.²⁶ TNF- α is likely a critical factor in

Figure 4. Effect of short-term ethanol treatment on LPS-induced increase in [Ca²⁺]_i in isolated Kupffer cells. [Ca²⁺]_i in isolated Kupffer cells was measured fluorometrically using fura-2 as described in Materials and Methods. Changes in [Ca²⁺]_i after addition of (A–C) 10 μ g/mL or (D–F) 100 ng/mL LPS, supplemented with 5% rat serum, are plotted. LPS was added to (A and D) Kupffer cells from control rats, (B and E) Kupffer cells from rats treated with ethanol for 2 hours before isolation, or (C and F) Kupffer cells from rats treated with ethanol 24 hours earlier. Data are representative traces of experiments repeated four times.

the progression of alcoholic liver disease because it stimulates generation of toxic superoxide anion from mitochondrial complex III in parenchymal cells,²⁷ expression of factors for neutrophil chemotaxis (interleukin 8/cytokine-induced neutrophil chemoattractant, macrophage inflammatory protein, MIP-2), and expression of intracellular adhesion molecule 1, leading to microcirculatory disturbances.²⁸ This scenario is supported by the fact that early injury in the Tsukamoto–French model is diminished by anti-TNF- α antibody.²⁹ However, the effect of ethanol on Kupffer cell sensitivity remains

Figure 5. Effect of ethanol on time course of LPS-induced changes in $[Ca^{2+}]_i$ in Kupffer cells. Experimental design is as in Figure 4. Mean $\pm$ SEM of peak $[Ca^{2+}]_i$ levels from four individual experiments were plotted. * $P < 0.05$ vs. control (10 μ g/mL LPS). ** $P < 0.05$ vs. control (100 ng/mL LPS) by ANOVA on ranks using Dunn's post hoc test.

unclear. For example, Fukui et al. reported that long-term alcohol-fed rats had higher plasma TNF- α levels after endotoxin than controls.^{30,31} Moreover, short-term ethanol enhances endotoxin hepatotoxicity⁹ (Figures 2 and 3). On the other hand, many studies show that short-term administration of ethanol inactivates Kupffer cells, which presents a paradox.^{10–13} To try to understand this paradox, here temporal changes in $[Ca^{2+}]_i$, TNF- α , and CD14 were monitored in Kupffer cells isolated from livers of rats given ethanol for various times. The LPS-stimulated increase in intracellular calcium was blunted in Kupffer cells isolated from rats treated for 2 hours (Figure 4). In contrast, the opposite result was obtained in Kupffer cells isolated from rats treated with ethanol 24 hours earlier (Figure 4). TNF- α production and histological changes after LPS followed a similar

Figure 6. Effect of antibiotics on LPS-induced increases in $[Ca^{2+}]_i$ in isolated Kupffer cells from ethanol-treated rats. Experimental design is as in Figure 4. Rats were treated with antibiotics for 4 days (150 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of polymyxin B and 450 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of neomycin) before experiments. A, antibiotics. Results are mean $\pm$ SEM of 4 rats per group. * $P < 0.05$ vs. control. # $P < 0.05$ vs. ethanol 2 hours. ** $P < 0.05$ vs. control. ## $P < 0.05$ vs. ethanol 24 hours by ANOVA.

pattern. Thus, it is concluded that both tolerance and sensitization to LPS can occur after a single dose of ethanol depending on timing. This most likely explains previous conflicting results.

Mechanism of Tolerance to LPS by Ethanol

Kupffer cells isolated from rats exposed to ethanol for 2 hours were less sensitive to LPS reflected by diminished $[Ca^{2+}]_i$ and TNF- α production. This confirms work of others who showed that short-term alcohol administration decreased endotoxin-induced increases in serum TNF- α .^{10–12} Importantly, this effect was blocked by antibiotics (Figures 6 and 7), supporting the hypothesis that tolerance due to alcohol involves gut-derived endotoxin (Figure 10).¹² CD14 was not decreased significantly 2 hours after ethanol treatment when the response

Figure 7. Influence of antibiotics on ethanol-mediated LPS-induced TNF- α production in isolated Kupfer cells from ethanol-treated rats. Some rats were treated with antibiotics before experiments for 4 days ($150 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of polymyxin B and $450 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of neomycin). Zero, 2, and 24 hours after oral administration of ethanol (4 g/kg body wt, orally), Kupfer cells were isolated and cultured in 24-well culture plates. After 24 hours, LPS (final concentration, $1 \mu\text{g/mL}$ in 5% rat serum) was added and incubation was continued for 4 hours. TNF- α was measured by ELISA as described in Materials and Methods. A, antibiotics. Results are mean $\pm$ SEM; $n = 4$. * $P < 0.05$ vs. control. # $P < 0.05$ vs. 2 hours of ethanol. ** $P < 0.05$ vs. control. ## $P < 0.05$ vs. ethanol 24 hours by ANOVA.

of the Kupfer cell to endotoxin was clearly blunted. However, Hijioka et al.⁵ showed that short-term ethanol treatment rapidly inactivated voltage-dependent calcium channels in Kupfer cells. Thus, inactivation of voltage-dependent calcium channels in Kupfer cells may be involved in mechanisms of rapid tolerance to ethanol.

Mechanism of Sensitization to LPS by Ethanol

Shibayama et al. showed that short-term administration of ethanol enhanced endotoxin hepatotoxicity.⁹ In the present study, Kupfer cells became sensitized to LPS

Figure 8. Effect of ethanol on dose-dependence of LPS-induced TNF- α production by isolated Kupfer cells. Kupfer cells were isolated from rats treated with ethanol for 2 or 24 hours and cultured on 24-well plates with DMEM + 10% FBS for 24 hours (see Materials and Methods). Culture media was exchanged at 24 hours with new media containing LPS (0–1000 ng/mL, supplemented with 5% rat serum) and incubated for 4 hours. TNF- α in the culture media was measured by ELISA. Data represent mean $\pm$ SEM of preparations from 4 rats. * $P < 0.05$ vs. control and ethanol 2 hours. # $P < 0.05$ vs. 2 hours of ethanol by two-way ANOVA on ranks.

in cells isolated 24 hours after ethanol as reflected by increased $[\text{Ca}^{2+}]_i$, TNF- α production, and large increases in CD14 (Figures 5 and 8–10). These effects were all blocked by antibiotics (Figures 6, 7, and 9), indicating that sensitization of Kupfer cells by ethanol is also mediated by gut-derived endotoxin. Moreover, it was shown that Kupfer cells from rats treated with ethanol 24 hours earlier expressed much higher levels of CD14 than controls or rats given antibiotics. This most likely explains why Kupfer cells ex vivo “remember” that they were treated with ethanol in vivo. Because gut sterilization removes portal endotoxin, Kupfer cells are not sensitized (Figures 6 and 7) and liver damage after LPS injection is blunted in vivo (Figures 2 and 3).

TNF- α increases in alcoholics consistent with the hypothesis that Kupfer cells of patients with alcoholic liver disease are activated. Further, Kupfer cells contain Ca^{2+} channels, which are easier to open after prolonged exposure to ethanol.⁶ Reports that the calcium channel blocker nimodipine decreased alcohol-induced liver injury in the Tsukamoto–French enteral alcohol model support the hypothesis that Kupfer cell calcium channels play an important role in mechanisms of alcoholic liver disease.³² Recently, Su et al. showed that CD14 was up-regulated in Kupfer cells from rats exposed to long-term alcohol using the Tsukamoto–French enteral

ethanol delivery model.³³ Moreover, LBP, which forms a complex with LPS in serum, enhances the effect of LPS on macrophages via CD14 receptors on the plasma membrane.³⁴ Here, LBP mRNA was increased in livers from rats treated with ethanol for 6 hours followed by a gradual decline. This increase was also blunted by antibiotics (Figure 9C). The main purpose of these experiments, however, was to understand the sensitivity of isolated Kupffer cells to LPS. Therefore, all experiments used serum from untreated rats (i.e., LBP was constant). Accordingly, sensitization of isolated Kupffer

Figure 10. Working hypothesis depicting the mechanism by which alcohol causes sensitization of Kupffer cells. Ethanol increases gut-derived endotoxin in the circulation. Endotoxin is removed from the circulation primarily by Kupffer cells, which causes both early tolerance and later sensitization in Kupffer cells. Changes in CD14 are responsible for the later sensitization to LPS observed after treatment with ethanol.

cells to LPS was dependent exclusively on increases in CD14 under these conditions.

In summary, Kupffer cells isolated from rats early after ethanol exhibited tolerance to LPS, and sensitization was

Figure 9. Effect of ethanol on CD14 and LBP expression in Kupffer cells. (A) Protein extracts from cultured Kupffer cells before and 2 and 24 hours after ethanol treatment were analyzed by Western blotting using mouse anti-rat ED-9 antibody. Specific bands for CD14 (55 kilodaltons) are shown. Lane 1, Kupffer cells from control rats; lane 2, Kupffer cells from rats treated with ethanol for 2 hours before isolation; lane 3, Kupffer cells from rats treated with ethanol for 24 hours before isolation; lane 4, Kupffer cells from rats treated with antibiotics for 4 days and ethanol for 24 hours before isolation. (B) Total RNA (20 µg) from livers before and 2, 6, 12, and 24 hours after ethanol treatment (4 g/kg intragastrically) was analyzed by Northern blotting for CD14. Lane 1, livers from control rats; lanes 2–5, livers from rats treated with ethanol for 2, 6, 12, and 24 hours before sampling, respectively. (C) Total RNA from livers before and 6 hours after ethanol treatment was analyzed by Northern blotting for LBP. Lane 1, livers from control rats; lane 2, livers from rats treated with antibiotics for 4 days; lane 3, livers from rats treated with ethanol for 6 hours before sampling; lane 4, livers from rats treated with antibiotics for 4 days and ethanol for 6 hours before sampling. Data shown are representative of three individual experiments.

observed later (Figure 10). It is likely that both of these phenomena were caused by gut-derived endotoxin and sensitization-involved increases in CD14.

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