Activation of Nuclear Factor Kappa B and Cytokine Imbalance in Experimental Alcoholic Liver Disease in the Rat

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Inflammatory stimuli and lipid peroxidation activate nuclear factor kappa B (NF-κB) and upregulate proinflammatory cytokines and chemokines. The present study evaluated the relationship between pathological liver injury, endotoxemia, lipid peroxidation, and NF-κB activation and imbalance between pro- and anti-inflammatory cytokines. Rats (5 per group) were fed ethanol and a diet containing saturated fat, palm oil, corn oil, or fish oil by intragastric infusion. Dextrose isocalorically replaced ethanol in control rats. Pathological analysis was performed and measurements of endotoxin were taken, lipid peroxidation, NF-κB, and messenger RNA (mRNA) levels of proinflammatory cytokines (tumor necrosis factor-α [TNFα], interleukin-1 β [IL-1β], interferon-γ, [IFN-γ], and IL-12), C-C chemokines (regulated upon activation, normal T cell expressed and secreted [RANTES], monocyte chemotactic protein [MCP]-1, macrophage inflammatory protein [MIP]-1α), C-X-C chemokines (cytokine induced neutrophil chemoattractant (CINC), MIP-2, IP-10, and epithelial neutrophil activating protein [ENA]-78), and anti-inflammatory cytokines (IL-10, IL-4, and IL-13). Activation of NF-κB and increased expression of proinflammatory cytokines C-C and C-X-C chemokines was seen in the rats exhibiting necroinflammatory injury (fish oil–ethanol [FE] and corn oil–ethanol[CE]). These groups also had the highest levels of endotoxin and lipid peroxidation. Levels of IL-10 and IL-4 mRNA were lower in the group exhibiting inflammatory liver injury. Thus, activation of NF-κB occurs in the presence of proinflammatory stimuli and results in increased expression of proinflammatory cytokines and chemokines. The Kupffer cell is probably the major cell type showing activation of NF-κB although the contribution of endothelial cells and hepatocytes cannot be excluded. Downregulation of anti-inflammatory cytokines may additionally exacerbate liver injury. (Hepatology 1999;30:934-943.)

Alcoholic liver injury is a complex process involving several injury mechanisms and multiple cellular targets.[1] Several lines of investigation indicate that endotoxin and oxidative stress are important pathogenic mechanisms in alcohol-induced liver injury.[1-5] It is well known that endotoxin is hepatotoxic and concentrations of endotoxin increase in experimental and human alcoholic liver disease.[2,3,6,7] Lipid peroxides are also potentially hepatotoxic and increased levels are seen after alcohol administration.[8,9] A concerted effect of endotoxin and lipid peroxides in promoting liver injury probably occurs.

One pathway by which endotoxemia and oxidative stress can cause liver injury is via nuclear factor kappa B (NF-κB).[10-12] NF-κB is a ubiquitous transcription factor that is implicated in the activation of many genes including those involved in alcoholic liver injury. In unstimulated cells, NF-κB is a heterodimeric complex that is sequestered in the cytoplasm by its interaction with the IκB family of inhibitors. When these cells are stimulated, IκB is phosphorylated with subsequent release of NF-κB; NF-κB then translocates into the nucleus where it binds to specific sequences in the promoter region of target genes.[10-12] Among the genes activated by NF-κB are several proinflammatory and cytotoxic cytokines.

Our hypothesis is that activation of NF-κB and upregulation of cytokine production occurs in alcoholic liver injury in association with endotoxemia and lipid peroxidation, and that this activation is associated with pathological liver injury. Regulation of necrosis and inflammation by cytokines involves an intricate balance between pro- and anti-inflammatory cytokines. Among the proinflammatory cytokines that are likely to play an important role in alcoholic liver injury are tumor necrosis factor-α (TNFα), interleukin 1 (IL-1), interferon-gamma (IFN-γ), and IL-12.[13,14] There is also increasing support for a role for chemokines in alcoholic liver injury.[15,16] Chemokines are a group of cytokines that exhibit strong chemoattractant activities and are involved in inflammatory cell migration.[17,18]

To evaluate the relationship between pathological liver injury and activation of NF-κB and cytokine disregulation, we used the rat intragastric feeding model for alcoholic liver disease.[19,20] In this model, we and other investigators have

Abbreviations: NF-κB, nuclear factor kappa B; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon; MCTE, medium-chain triglycerides plus ethanol; PE, palm oil plus ethanol; CE, corn oil plus ethanol; FD, fish oil plus dextrose; FE, fish oil plus ethanol; ALT, alanine transaminanse; EMSA, electrophoretic mobility shift assay; CINC, cytokine induced neutrophil chemoattractant; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; RANTES, regulated upon activation, normal T cell expressed and secreted; PCR, polymerase chain reaction; mRNA, messenger RNA; RT, reverse transcription; IP, inducible protein; ENA, epithelial neutrophil activating protein.

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Received February 5, 1999; accepted July 8, 1999.

K.J. was supported by grants from the Academy of Finland, Finnish Cultural Foundation, and Yrjö Jahnsson Foundation.

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0270-9139/99/3004-0017$3.00/0

shown that an inverse relationship exists between the saturation of lipids in the diet and the severity of alcohol-induced liver injury.[21-24] Rats fed ethanol and diets enriched in saturated fatty acids are protected from liver injury, whereas diets enriched in polyunsaturated fatty acids promote alcoholic liver injury. Thus, the rat intragastric feeding model, in which ethanol is fed with different dietary fats, is useful to elucidate the pathogenesis of alcoholic liver disease. In the current study, we used this model of liver injury to study the relationship between liver pathology and NF-κB and pro- and anti-inflammatory cytokines.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing between 275 and 300 g were fed a liquid diet by continuous infusion through permanently implanted gastric tubes as described previously.[19,20] The rats were given their total nutrient intake by intragastric infusion. The percentage of calories derived from fat was 35% of total calories. Vitamins and minerals were given as described previously.[19,20] The amount of ethanol was modified to maintain high levels of blood ethanol (150 to 300 mg/dL) throughout the day. The amount of ethanol was initially 10 g/kg/d, and was increased up to 16 g/kg/d as tolerance developed. Each ethanol-fed rat had at least 2 measurements of blood alcohol taken.

Eight groups of rats (5 rats per group) fed different dietary fats with ethanol or dextrose were studied. These groups were fed medium-chain triglycerides plus dextrose or medium-chain triglycerides plus ethanol (MCTE), palm oil plus dextrose or palm oil plus ethanol (PE), corn oil plus dextrose or corn oil plus ethanol (CE), and fish oil plus dextrose (FD) or fish oil plus ethanol (FE). All control rats were pair-fed the same diet as the ethanol-fed rats except that ethanol was isocalorically replaced by dextrose. All diets were prepared fresh daily. The fish oil diet was stored in air-tight containers, filled with nitrogen, and kept in a cold room at 4°C. Lipid peroxidation was measured to exclude the possibility of auto-oxidation. All animals were killed after 1 month of treatment with the experimental diets. When the animals were killed, a sample of liver was obtained for histopathological analysis, and the remainder of the liver was rapidly excised, washed with ice-cold 1.15% (wt/vol) KC1, and cut into small pieces that were transferred to plastic vials and placed in liquid nitrogen. The vials were stored at −80°C. All animals received humane care in compliance with the National Institutes of Health criteria for care of laboratory animals.

Histopathological Analysis. A small sample of the liver was obtained and formalin-fixed when the rats were killed. Hematoxylin-eosin stain was used for light microscopy. The severity of liver pathology was assessed as follows: steatosis (the percentage of liver cells containing fat) was scored 1+ with less than 25% of the cells containing fat; 2+ with 26% to 50% of cells containing fat; 3+ with 51% to 75% of the cells containing fat; and 4+ with greater than 75% of the cells containing fat. Necrosis was quantified as the number of necrotic foci per square millimeter, and inflammation was scored as the number of inflammatory cells per square millimeter. At least 3 different sections were examined per sample of liver. The pathologist evaluating the sections was unaware of the treatment groups when assessing the histology.

Measurements of Alcohol and Alanine Transaminase in Blood. Blood was collected from the tail vein and ethanol concentration was measured using an alcohol dehydrogenase kit from Sigma Chemical Company (St. Louis, MO). Alanine transaminase (ALT) activity was measured using an automated analyzer (Hitachi 747, Boehringer Mannheim, Indianapolis, IN).

Measurement of Plasma Endotoxin. Blood samples were collected in endotoxin-free vials (Sigma) and centrifuged at 400*g* for 15 minutes at 4°C. Samples were then diluted 1:10 in pyrogen-free water and heated to 75°C for 30 minutes to remove endotoxin inhibitors from plasma. The Limulus Amoebocyte Lysate test (Kinetic-QLC; Whittaker Bioproducts, Walkersville, MD) was used for measurement of endotoxin. Samples were incubated at 37°C for 10 minutes with Limulus amebocyte lysate. The substrate solution was added subsequently and the incubation continued for 20 minutes. The reaction was stopped with 25% acetic acid, and the samples were read spectrophotometrically at 410 nm.

Measurement of conjugated dienes and thiobarbituric acid reactive substances were performed. Conjugated dienes and thiobarbituric acid reactive substances in liver were measured according to previous published methods.[25]

Preparation of Cytosolic and Nuclear Extracts. The livers were fractionated as described previously[26] with some modification. One gram of the liver tissue was homogenized using a Dounce homogenizer (Lab Glass, Vineland, NJ) in 5-mL buffer (0.32 mol/L sucrose, 50 mmol/L Tris-HC1 [pH 7.5], 25 mmol/L KC1, 5 mmol/L $MgC1_2$, 0.5 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 10 μg/mL tosyllysylchloromethyl ketone [TLCK], 10 μg/mL tosylphenylalonylchloromethyl ketone [TPCK]) and centrifuged at 600*g* at 4°C for 10 minutes. Two thirds of the supernatant was removed and centrifuged at 7,000*g* for 10 minutes, and the obtained supernatant was stored at −80°C as a cytosolic extract. The pellet obtained from the first centrifugation was resuspended in 2.5 mL of 2 mol/L sucrose-Tris HCl, KLl, and $MgCl_2$ (TKM) buffer and homogenized. The homogenate was centrifuged at 40,000*g* at 4°C for 2 hours. The supernatant was carefully removed and the pellet containing the nuclear extract was resuspended in 40 μ of buffer A (10 mmol/L HEPES/KOH [pH 7.9], 2 mmol/L $MgC1_2$, 0.1 mmol/L ethylenediaminetetraacetic acid, 10 mmol/L KC1, 1 mmol/L dithiothreitol , 0.5 mmol/L phenylmethylsulfonyl fluoride) and left on ice for 10 minutes, mixed, and centrifuged at 15,000*g* at 4°C for 15 seconds. The pellet was then resuspended in 1.0 mL of buffer B (10 mmol/L HEPES/KOH [pH 7.9], 50 mmol/L KCI, 300 mmol/L NaC1, 0.1 mmol/L ethylenediaminetetraacetic acid, 10% (vol/vol) glycerol, 1 mmol/L dithiothreitol, 0.5 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 10 μg/mL TPCK) and put in ice for 20 minutes. After centrifugation at 15,000*g* at 4°C for 5 minutes, the supernatant was stored at −80°C as a nuclear extract. Before the experiments, the protein concentration of the cytosolic and nuclear extracts was determined by the BCA method (Pierce, Rockford, IL) in one of the aliquots.

Electrophoretic Mobility Shift Assay. The electrophoretic mobility shift assay (EMSA) method used is that described previously[27] with slight modifications. Briefly, equal amounts of nuclear extracts (12 μg of protein) were incubated for 1 hour at room temperature with ^{32}P-labeled NF-κB oligonucleotide probe (Promega, Madison, WI) in binding buffer (10 mmol/L HEPES [pH 7.9], 50 mmol/L KC1, 0.2 mmol/L ethylenediaminetetraacetic acid, 2.5 mmol/L dithiothreitol, 10% glycerol, 0.05% NP-40), final volume 20 μL.

TABLE 1. Pathological Changes and Plasma ALT Levels in the Different Experimental Groups

Treatment Group	Fatty Liver	Necrosis (foci/mm²)	Inflammation (cells/mm²)	ALT (μ/L)
MCTD	0.0	0.0	0.0	13 ± 2
MCTE	0.3 ± 0.4	0.03 ± 0.01	0.0	15 ± 3
PD	0.0	0.03 ± 0.01	0.0	16 ± 4
PE	2.6 ± 0.5*	0.06 ± 0.03	0.07 ± 0.03*	30 ± 6*
CD	0.0	0.0	0.08 ± 0.04	15 ± 2
CE	3.6 ± 0.5*	0.6 ± 0.2*,†	4.1 ± 1.3*,†	53 ± 12*,†
FD	0.0	0.0	0	16 ± 4
FE	4.0 ± 0.0*	1.3 ± 0.3*,†,‡	24.3 ± 7.1*,†,‡	72 ± 11*,†,§

Abbreviations: MCTD, medium-chain triglycerides plus dextrose; PD, palm oil plus dextrose; CD, corn oil plus dextrose.

*$P < .01$ vs. dextrose-fed controls and MCTE.

†$P < .01$ vs. MCTE and PE.

‡$P < .01$ vs. CE.

§$P < .05$ vs. CE.

TABLE 2. Lipid Peroxidation (Conjugated Dienes, A_{232}, and TBARS) and Plasma Endotoxin Levels in the Different Experimental Groups

Treatment Group	A_{232}	TBARS (nmol/mg protein)	Plasma Endotoxin (pg/mL)
MCTD	0.08 ± 0.02	0.09 ± 0.03	6 ± 2
MCTE	0.12 ± 0.05	0.27 ± 0.06*	20 ± 7*
PD	0.10 ± 0.04	0.18 ± 0.04	7 ± 3
PE	0.27 ± 0.07†	0.54 ± 0.09†‖	21 ± 6*
CD	0.16 ± 0.07	0.16 ± 0.05	5 ± 3
CE	0.57 ± 0.09‡	0.75 ± 0.09†‡	58 ± 12†‡
FD	0.40 ± 0.08	0.63 ± 0.10	8 ± 4
FE	0.80 ± 0.18‡	1.61 ± 0.31†‡§	72 ± 18†‡

Abbreviations: TBARS, thiobarbituric acid reactive substances; MCTD, medium-chain triglycerides plus dextrose; PD, palm oil plus dextrose; CD, corn oil plus dextrose.

†$P < .01$ vs. dextrose-fed controls and MCTE.

‡$P < .01$ vs. MCTE and PE.

*$P < .01$ vs. MCTD.

§$P < .01$ vs. all other groups.

‖$P < .01$ vs. PD.

DNA-protein complexes were separated in 7% nondenaturing polyacrylamide gels at a constant voltage of 100 V at room temperature. The gel was then autoradiographed on Fuji radiograph film at −80°C with intensifying screens. NF-κB oligonucleotide was labeled using (γ^{32}P) adenosine triphosphate (3,000 Ci/mmol/L; NEN Life Science Products, Boston, MA) and polynucleotide kinase (Promega). The specificity of binding was determined by prior addition of 100-fold excess of unlabeled competitor consensus oligonucleotide. Supershift experiments were performed on 5% nondenaturing gels using antiserum directed against the $_p$50 subunit of NF-κB (Santa Cruz Biotechnology, Santa Cruz, CA). Densitometric analysis of NF-κB activation was performed using laser scanning densitometry using a Molecular Dynamics Densitometer and Image Quant Software (Molecular Dynamics, Sunnyvale, CA).

Western Blot Analysis. Western blot analysis was conducted using 50 μg of cytosolic protein. Samples were electrophoresed on 10% sodium dodecyl sulfate–polyacrylamide gel at a constant current of 20 mA for 1 hour and proteins were then electroblotted onto polyvinyldiene diflouride (PVDF) membranes (Sigma). The blots were stained with Ponceau S to confirm the uniformity of protein loading in each lane. Membranes were then blocked overnight with 5% nonfat milk and Tween–phosphate buffered saline (137 mmol/L NaC1, 2.7 mmol/L KC1, 8.1 mmol/L Na_2HPO_4, 1.5 mmol/L KH_2PO_4, 0.1% Tween 20) and incubated with the primary antibody against IκB-α and p65 (Santa Cruz Biotechnology) at a dilution of 1:500 in 1% nonfat milk Tween–phosphate buffered saline. Membranes were incubated with a secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G) at a dilution of 1:10,000 in Tween–phosphate buffered saline for 1 hour. After 4 washes with Tween–phosphate buffered saline, antibody-reactive bands were visualized by the use of an enhanced chemiluminescence assay using reagents from NEN Life Science Products.

RNA Extraction and Analysis of Messenger RNAs by Reverse Transcription Polymerase Chain Reaction. To examine the expression of various cytokines and chemokines in liver tissue, total RNA was isolated according to the guanidinium isothiocyanate method.[28] The integrity of RNA was assessed by agarose gel electrophoresis and ethidium bromide staining. We reverse-transcribed 0.5 to 1 μg of total RNA by adding 30 μL of a master mix with reverse transcriptase buffer (0.6 mmol/L $MgC1_2$, 15 mmol/L KC1, and 10 mmol/L Tris-HC1 [pH 8.3]), 40 pmol of downstream primer, 0.5 mmol/L deoxynucleoside triphosphate mixture, 1 U/μL ribonuclease inhibitor, and 13.3 U/μL Moloney murine leukemia virus reverse transcriptase (GIBCO-BRL, Grand Island, NY) (final concentrations indicated). Samples were incubated, first for 60 minutes at 42°C and then at 75°C for 10 minutes, and then chilled on ice. Then 2 μL of each sample was added to 20 μL of 1.5 mmol/L $MgC1_2$, 50 mmol/L KC1, 10 mmol/L Tris-HC1 (pH 8.3), 0.2 mmol/L of each deoxynucleoside triphosphate and 0.01% gelatin, 5 U/100 μL *Taq* DNA polymerase (Perkin Elmer Cetus, Norwalk, CT), and 50 pmol of sense primer and 10 pmol of antisense primer. The sequences of primer pair, 5' and 3' and the predicted size of the amplified polymerase chain fragments have been previously published; for CINC, MCP-1, MIP-2, MIP-1α, and RANTES primers used by Tang et al.[29] were used, for TNF-α and β actin,[30] ENA-78,[31] IFN-γ, IL-12, IL-10, IL-4,[32,33] and IL-13.[34] Amplification was performed in an automated thermal cycler at 94°C for 60 seconds, 50°C for 90 seconds, and 72°C for 2 minutes for 35 cycles, followed up by 72°C for 10 minutes. To normalize signals from the different RNA samples, we amplified 2 μL of the same reverse transcriptase reaction with β-actin–specific primers. Polymerase chain reaction (PCR) products and molecular weight markers were subjected to electrophoresis on 1% agarose gels and visualized by means of ethidium bromide staining. The gels were analyzed by laser scanning densitometry using a Molecular Dynamics Densitometer and Image Quant software (Molecular Dynamics, Sunnyvale, CA). To compare the abundance of specific messenger RNAs (mRNAs) in RNA pools from different treatment groups, equal amounts of total RNA of each of the groups were transcribed into complementary DNA in parallel. All amplification reactions of 1 experiment were performed in parallel in the same heating block to ensure compatible conditions.

Varying the numbers of PCR cycles from 30 to 40 did not change the relative differences between samples, indicating that our PCR conditions were not within the plateau phase of amplification. Each experiment included a negative control (sample RNA that had not been subjected to reverse transcription [RT]). This sample did not yield a PCR product, confirming the absence of extraneous genomic DNA or PCR product contaminating the samples.

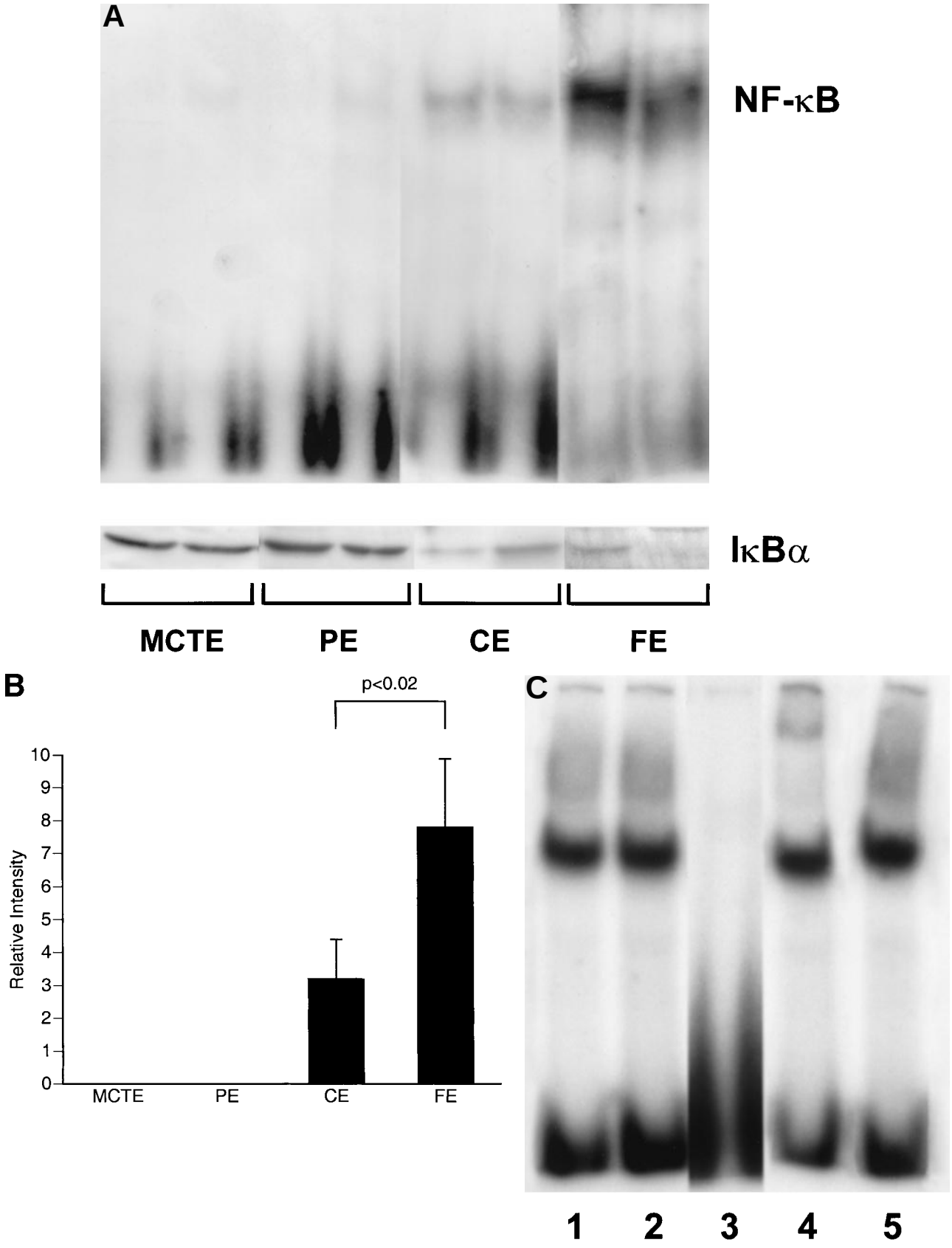
Measurements of Cytokines in Plasma. The cytokines measured in plasma were IL-1β, MIP-2, and IL-10 (Biosource) using kits that used the enzyme-linked immunosorbent assay technique. The intra-assay variation was less than 7%.

Statistical Analysis. All data are expressed as means ± SD unless otherwise indicated. Statistical significance was evaluated using the unpaired Student's *t* test for comparison between 2 means. Differences among more than 2 groups were analyzed using analysis of variance. Statistical significance was set at $P < .05$.

RESULTS

There was no significant difference in weight gain among the different experimental groups (data not shown). There

FIG. 1. (A) Representative EMSA for NF-κB activation in nuclear extracts and Western blot analysis for IκBα protein in cytosolic extracts in liver tissue from rats in the different experimental groups. Extracts from rats fed MCTE and PE showed low binding activity. Increased binding activity was seen in rats fed CE with the highest activity seen in the FE group. Preservation of IκBα was associated with absent or low NF-κB binding in the MCTE and PE groups in which much lower levels of IκBα were seen in the group in which activation of NF-κB was seen (CE and FE groups). p65 Was detected in the MCTE and PE groups but was absent in the CE and FE groups (not shown). (B) Quantitation of NF-κB activity was performed by densitometric analysis of band intensity. There were 5 animals in each group. Activation of NF-κB was either not detectable or was at extremely low levels in rats fed MCTE or PE. The degree of NF-κB activation was significantly greater ($P < .02$) in rats fed FE compared with those fed CE. (C) EMSA for NF-κB activation in liver nuclear extracts from rats fed FE for 2 weeks (lanes 1 and 2). Competition of NF-κB binding with 100 × molar excess of cold probe (lane 3) and supershift assays using an antibody against p50 (lane 4) show the specificity of DNA binding. Addition of an unrelated oligonucleotide (STAT 5) had no effect on the formation of the complex (lane 5).


A
NF-κB
IκBα
MCTE
PE
CE
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B
p<0.02
Relative Intensity
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MCTE
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was also no significant difference in levels of blood alcohol levels (mg/dL; mean ± SE) in the ethanol-fed groups, which are as follows: MCTE, 228 ± 28; PE, 236 ± 21; CE, 248 ± 24; FE, 230 ± 26.

Histopathology and ALT Activity. The most severe pathological changes were seen in the FE-fed rats (Table 1). In particular, the degree of necrosis and inflammation was more severe than in the other ethanol-fed groups. Dextrose-fed rats and rats fed MCTE showed no pathology. The highest levels of ALT were seen in the CE and FE groups (Table 1).

Endotoxin and Lipid Peroxidation. The highest levels of endotoxin in plasma and lipid peroxidation (conjugated dienes and thiobarbituric acid reactive substances) were seen in the rats showing the most severe liver injury (CE and FE groups) (Table 2).

Activation of NF-κB and Degradation of IκBα With Pathological Liver Injury. To evaluate activation of NF-κB, EMSA of nuclear extracts from whole livers were performed. Nuclear localization of NF-κB was increased in the CE and FE groups (Fig. 1). The degree of NF-κB activation was significantly higher ($P < .02$) in rats fed FE compared with those fed CE (Fig. 1). These 2 ethanol-fed groups (*i.e.*, CE and FE) showed evidence of necrosis and inflammation with the pathological changes being more severe in the FE group (Table 1). Activation of NF-κB was absent in the MCTE and PE groups in which there was no necrosis or inflammation. There also was no activation of NF-κB in the dextrose-fed rats.

To determine whether activation of NF-κB might be the result of degradation of IκBα, protein expression of IκBα in whole liver homogenates was measured by Western blot analysis. In livers from the CE and FE group, a marked decrease and/or absence of IκBα protein was observed (Fig. 1). In the MCTE and PE group, no change in IκBα protein levels was found; the levels were similar to those in dextrose-fed controls. Results similar to that seen with IκBα were also seen with p65. Thus, preservation of IκBα protein in the MCTE and PE groups coincided with absence of activation of NF-κB; in contrast, loss of IκBα protein coincided with NF-κB activation in the CE and FE groups.

To address the question of whether activation of NF-κB occurred before or concurrently with pathological liver injury, nuclear and cytoplasmic extracts were studied from rats fed FE for 2 weeks. In addition, appropriate controls (fish oil and dextrose) were also used. Our results showed that activation of NF-κB was seen in rats fed FE for 2 weeks (Fig. 1); there was no evidence of pathological liver injury at this time period. Also, no activation of NF-κB was seen in rats fed fish oil and dextrose for 2 weeks; these results are similar to those seen in FD-fed rats after 1 month.

The protein/DNA complex was further characterized by using competition and supershift assays. A 100-fold excess of nonradioactive NF-κB or STAT 3 oligonucleotide was added to an EMSA-binding reaction containing the nuclear extracts from FE-fed rats. Although addition of the NF-κB oligonucleotide completely abrogated complex formation, addition of STAT 3 oligonucleotide had no effect. Antibodies against p50 were used for supershift assays to show specificity of the NF-κB complex (Fig. 1).

Expression of Proinflammatory Cytokines Is Upregulated in Association With Inflammatory Changes. We examined liver tissue for steady state mRNA levels of TNFα, IL-1β, IFN-γ, and IL-12 p40 by RT-PCR. All of these cytokines showed increased levels in the groups exhibiting necrosis and inflammation (Fig. 2). The pattern of TNFα expression was similar to that observed previously.[30,35,36] An approximately 2- to 3-fold increase in IFN-γ was seen in CE and FE rats over controls and the MCTE group. A similar increase was also seen for IL-1β mRNA in the CE and FE groups. IL-12 p40 mRNA was detected in the PE group with much higher levels seen in the CE and FE groups (Fig. 2).

Evaluation of Chemokine Expression. We next evaluated the levels of intrahepatic chemokines. The C-C subfamily of chemokines included MCP-1, MIP-1α, and RANTES. MIP-1α mRNA was detected only in the CE and FE groups. An increase in MCP-1 mRNA compared with dextrose-fed control rats was seen in the CE and FE groups; the levels of MCP-1 mRNA in these 2 groups were also significantly higher than in the MCTE and PE groups. Ethanol increased RANTES expression in all groups; however, the highest expression was seen in the CE and FE groups (Fig. 3).

Chemokines of the C-X-C subfamily that were studied in the livers of rats included MIP-2, IFN-inducible protein 10 kd (IP-10), epithelial neutrophil activating protein (ENA)-78, and CINC. MIP-2 and IP-10 mRNA was detected only in rats exhibiting pathological changes (PE, CE, and FE) with higher levels seen in the CE and FE groups (Fig. 4). ENA-78 mRNA was detected only in the CE and FE groups; the level in the FE group was significantly higher than in the CE group. CINC mRNA was detected in all dietary groups; a significant increase with ethanol treatment was seen in the CE and FE groups. Thus, an increase in all of the C-X-C chemokine mRNAs studied were seen mainly in the 2 groups (CE and FE) that exhibited necrosis and inflammation in the liver (Fig. 4).

Anti-inflammatory Cytokine Expression. The 3 anti-inflammatory cytokines whose expression was studied included IL-10, IL-4, and IL-13. Ethanol administration caused significant increases in IL-10 mRNA levels in the MCT and palm oil groups. In marked contrast, in the CE and FE groups, IL-10 mRNA levels were decreased compared with dextrose-fed control rats and the MCTE and PE groups (Fig. 5). Similar to what was seen with IL-10, ethanol administration increased IL-4 mRNA in the MCT and palm oil–fed groups; ethanol did not alter IL-4 mRNA in the corn oil and fish oil groups. A faint band for IL-13 mRNA was seen in all groups; there were no differences between any of the groups studied.

Plasma Levels of Cytokines. To corroborate the findings of our results for mRNA expression for the various cytokines in liver tissue, we measured plasma levels of selected cytokines or chemokines based on the availability of reagent kits for measurements in rat plasma. The cytokines/chemokines evaluated were IL-1β, MIP-2, and IL-10. The levels of IL-1β and MIP-2 were increased in the CE and FE groups compared with the MCTE group (Fig. 6). For MIP-2, the concentration of MIP-2 was highest in the FE group. The results for IL-10 in plasma were consistent with the results obtained by RT-PCR analysis; the levels of IL-10 were significantly lower in the CE and FE groups compared with the MCTE group, with the lowest level seen in the FE group (Fig. 6).

DISCUSSION

Considerable attention has been devoted to delineating some of the factors involved in the pathogenesis of alcoholic liver injury. One hypothesis is that endotoxin and reactive oxygen intermediates increase NF-κB binding activity in liver cells. The exact causal relationship between activation of

NF-κB and alcoholic liver injury remains speculative but an obvious mechanism for such a relationship is that NF-κB, acting as an intermediate, increases the expression of cytokines and chemokines. An important finding in the study is that the activation of NF-κB was associated with necrosis and inflammation in the liver.

Activation of NF-κB. Our data suggest that nuclear translocation of the transcription factor NF-κB is associated with the development of necroinflammatory changes in the liver in alcohol-fed rats. Activation of NF-κB occurs secondarily to the proteolytic degradation of IκBα, allowing free NF-κB to translocate to the nucleus and initiate gene transcription.[10-12] Our findings support a role for IκBα in alcoholic liver injury because activation of NF-κB in the presence of inflammatory changes in the liver was accompanied by a loss of IκBα in the CE-fed and FE-fed rats. The inhibition of NF-κB activation in the MCTE and PE groups, in which inflammation was absent, was accompanied by preservation of IκBα protein expression. The mechanisms involved in the stabilization of IκBα and suppression of NF-κB activation in the groups of rats fed saturated fatty acids remains to be elucidated, but a role for the anti-inflammatory cytokines, such as IL-10, has been suggested.[37] It is also important to point out that the levels of endotoxin and lipid peroxidation were lower in the groups in which ethanol was administered with saturated fatty acids (MCT and palm oil). Lower levels of these 2 proinflammatory stimuli could contribute to the absence of NF-κB activation. Although we did not, in the present study, identify the specific cell types expressing NF-κB and cytokines, based on our previous studies[35] and those of others,[27] we expect that the Kupffer cell is the major cell type showing activation of NF-κB. We cannot, however, exclude the contribution of endothelial cells and hepatocytes to NF-κB activation. Both of these cells respond to proinflammatory stimuli; the hepatocyte, in addition, shows induction of NF-κB in response to regenerative stimuli.[38,39]

Increased Proinflammatory Cytokines in Rats With Necrosis and Inflammation in the Liver. Increased levels of TNFα, IFN-γ, IL-1, and IL-12 have been shown to play pivotal roles in injury caused by endotoxemia.[40-43] The Kupffer cell is the predominant immune-effector cell in the liver that, in response to inflammatory mediators, generates TNFα and other proinflammatory cytokines.[44] A combined role for TNFα and Cox-2 in alcoholic liver injury in the intragastric feeding rat model has been previously shown.[45] Increased levels of IL-1 have also been shown in alcoholic liver injury. Monocytes from patients with alcoholic liver disease secrete more IL-1 in response to lipopolysaccharide than monocytes from control patients. The mechanisms whereby TNFα and IL-1 promote liver injury have been extensively reviewed.[13] IFN-γ occupies a special place among immunoregulatory cytokines in affecting virtually all cell types involved in the immune response.[46] An important function of IFN-γ is the

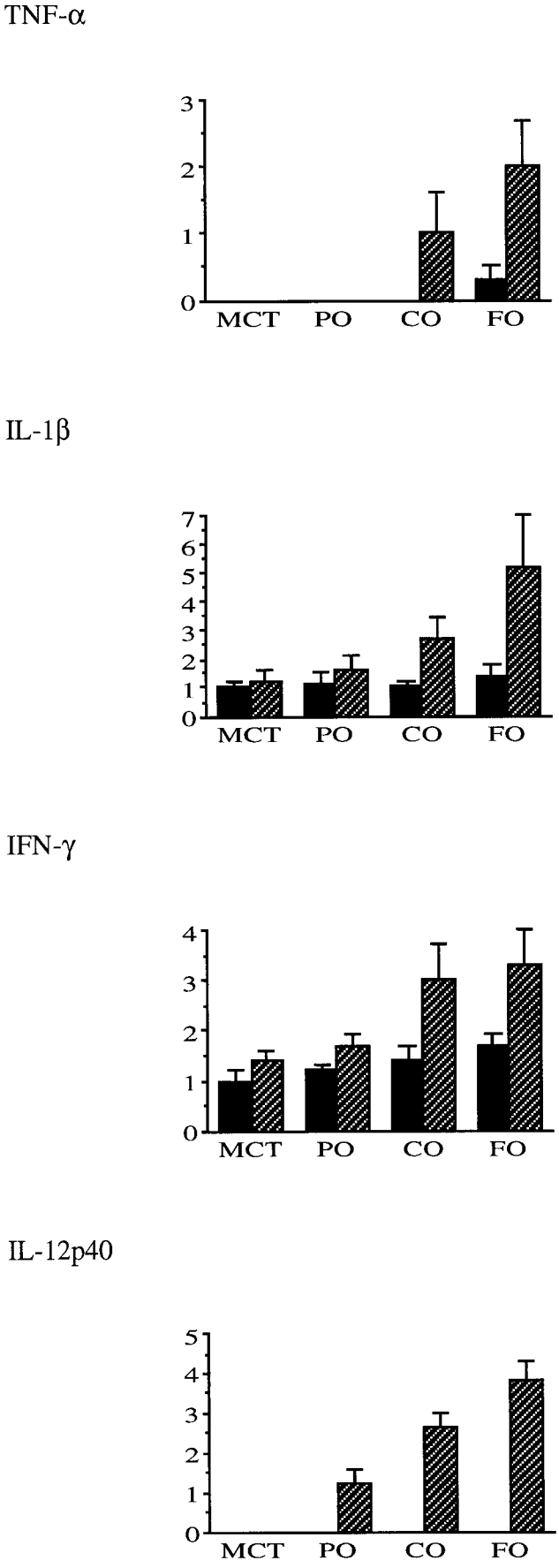

FIG. 2. RT-PCR analysis of TNFα, IL-1β, IFN-γ, and IL-12 p40 in the different experimental groups. All mRNA levels were standardized to β-actin mRNA in the same groups. TNFα mRNA was detected only in the rats fed FE, FD, and CE. The level of TNFα mRNA was significantly higher in the FE groups (2.0 ± 0.7) versus the FD groups (0.3 ± 0.2). IL-1β was similar in all dextrose-fed groups with significantly higher levels ($P < .05$) seen in the CE and FE groups. The highest levels of IFNγ were seen in the CE and FE groups ($P < .02$). IL-12 p40 was not detected in the dextrose-fed rats. The highest level ($P < .01$) was seen in the FE group. ■ dextrose, ▨ ethanol.

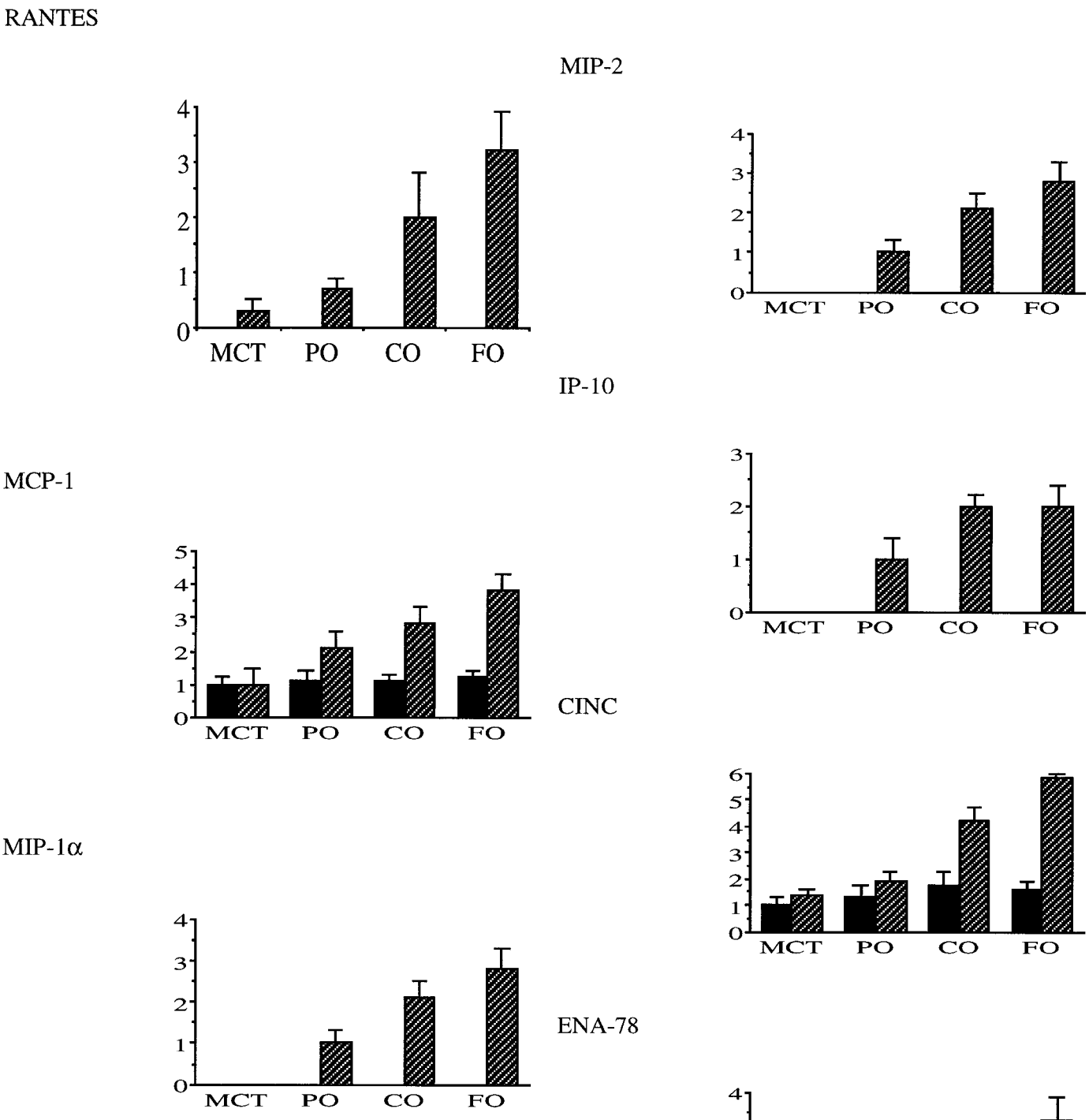

FIG. 3. RT-PCR of the C-C subfamily of chemokines in the different experimental groups. RANTES and MIP-1α were detected only in ethanol-fed rats with the highest levels in the corn oil and fish oil groups ($P < .01$). Ethanol administration significantly increased MCP-1 mRNA in the corn oil and fish oil groups. ■ dextrose, ▨ ethanol.

modulation of cytokine production by monocytes and macrophages. IFN-γ enhances TNFα synthesis and downregulates IL-10 synthesis.[47] Thus, at least part of the changes seen with TNFα and IL-10 in the CE and FE rats in the present study may be a result of increased IFN-γ production.

The heterodimeric cytokine IL-12 is produced by mononuclear cells and increases in response to inflammatory stimulation with endotoxin and IFN-γ.[48] IL-12 is also a

FIG. 4. RT-PCR analysis of the C-X-C subfamily of chemokines (MIP-2, IP-10, CINC, and ENA-78) in the different experimental groups. The pattern for expression of MIP-2, IP-10, and ENA-78 was similar with the highest levels seen in the CE and FE groups. CINC mRNA was detected in all dextrose-fed groups but was significantly increased by ethanol ($P < .01$) in the corn oil and fish oil groups. ■ dextrose, ▨ ethanol.

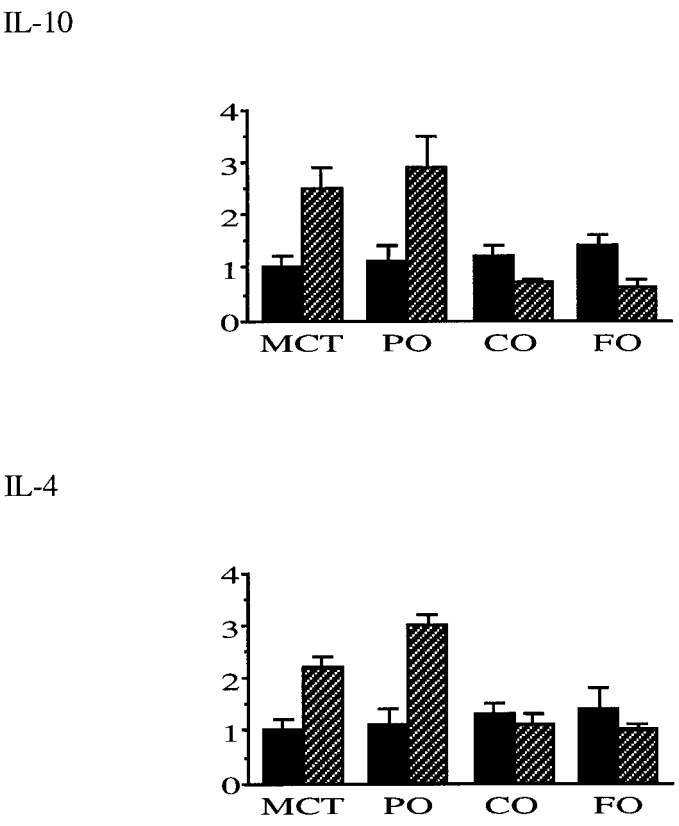

FIG. 5. RT-PCR of the anti-inflammatory cytokines in the different experimental groups. Ethanol administration led to significant increases ($P < .01$) in IL-10 mRNA in the MCT and palm oil groups but led to decreases in the corn oil and fish oil groups ($P < .05$). A similar pattern was also seen for IL-4 mRNA although the ethanol-induced decreases in the corn oil and fish oil groups were not as dramatic as those for IL-10. A faint-band for IL-13 mRNA was seen in all groups (not shown). ■ dextrose, ▨ ethanol.

potent inducer of IFN-γ by T cells.[49] Additionally, IL-12 also downregulates IL-10 and IL-4 production.[49] Increased IL-12 expression is also associated with increased cell recruitment to inflammatory sites and increased chemokine expression.[48] Thus, IL-12 may participate in a cycle of cell recruitment and chemokine production by infiltrating cells and thus contributing to liver injury.

Chemokines. Recently a group of cytokines, called chemokines, has been described that exhibits strong chemoattractant activities.[17] The C-X-C chemokines (MIP-2, IP-10, CINC, ENA-78) attract primarily neutrophils, the C-C chemokines (MIP-1α, MCP-1, RANTES) attract monocytes and T lymphocytes.[17,18] Of note is that the inflammatory infiltrate in the intragastric feeding model is composed mainly of mononuclear leukocytes whereas neutrophils, although present, are fewer in number.

Hepatic and plasma levels of IL-8 are markedly increased in patients with alcoholic hepatitis.[15] Although no homologue to IL-8 has been identified in the rat, several C-X-C molecules have been identified in the inflamed rat liver, including CINC and MIP-2.[50,51] CINC, an 8-kd polypeptide, is the analogue of IL-8 in the rat, and both hepatocytes and Kupffer cells can produce CINC after exposure of these cells to ethanol. Increased levels of MIP-2, similar to that observed in the present study, have been shown in inflammatory liver injury in rats.[51] Isolated Kupffer cells from alcohol-fed rats produce large amounts of MIP-2.[51] MIP-2 is also cytotoxic to isolated hepatocytes previously exposed to alcohol *in vivo*.[51] IP-10, in contrast to other C-X-C chemokine family members, is a chemoattractant for monocytes and activated T lymphocytes.[52] IP-10 production is stimulated by IFN-γ. ENA-78 has been found to be produced by a variety of immune or nonimmune cells in response to TNF or IL-1.[31] Thus, both immune and nonimmune cells of the liver may be involved in the cytokine cascade that enhances the recruitment of neutrophils into the liver parenchyma.

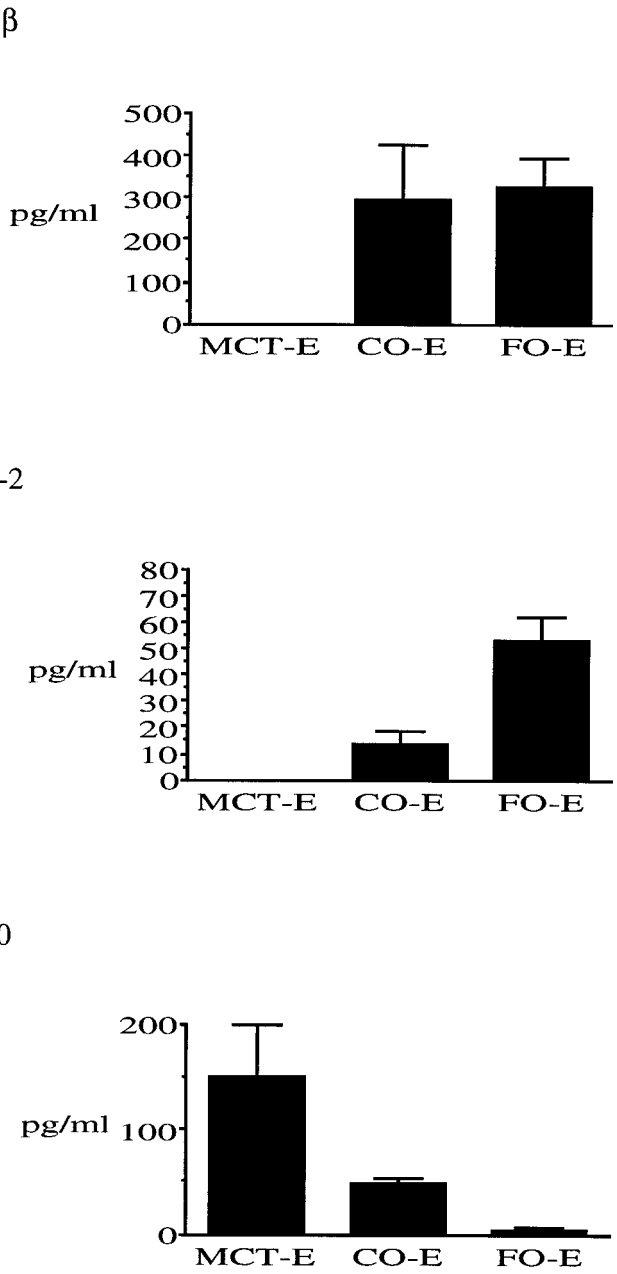

FIG. 6. Plasma levels for IL-1β, MIP-2, and IL-10 in rats in the different ethanol-fed groups. Levels of IL-1β were significantly higher ($P < .01$) in the CE and FE rats compared with those fed MCTE. Similarly, MIP-2 levels were also increased in the CE and FE groups ($P < .01$) with higher levels in the FE group ($P < .01$). In contrast, the highest level of IL-10 was seen in the MCTE group ($P < .01$) with significantly lower ($P < .01$) levels in the CE and FE groups, with the lowest level seen in the FE rats ($P < .01$ vs. CE). The levels of the cytokines were below the limit of detection in all the control dextrose-fed groups.

All of the C-C chemokines have also been shown to be involved in inflammatory tissue injury. MIP-1α is an endotoxin-inducible chemokine that is a strong chemoattractant for monocytes.[53] Both MCP-1 and RANTES also preferentially attract monocytes and lymphocytes.[17,18] The role for MCP-1 is less well studied in liver disease models than in other models of tissue injury; however, MCP-1 also appears to be involved in mononuclear cell recruitment into the liver. *In vitro*, RANTES is nearly as potent a chemoattractant for monocytes as MCP-1. A role for RANTES in inflammatory liver injury has not been previously documented.

The exact causal relationship between enhanced chemokine expression and liver injury is uncertain. Based on the pattern of expression of the various chemokines and the high degree of homology shared by the C-X-C chemokines, and the fact that redundant receptors exist for the C-C chemokines, each chemokine to a varying extent may contribute to the pathogenesis of the inflammatory response in the liver.

Anti-inflammatory Cytokines. Regulation of inflammation by cytokines involves an intricate balance between pro- and anti-inflammatory cytokines.[54] Indeed, inhibition of the endotoxin-induced cascade of proinflammatory cytokines is the primary mechanism through which anti-inflammatory mediators, such as IL-10, IL-4, and IL-13, confer protection against the effects of endotoxin.[54,55]

The decrease in TNFα production by IL-10 is secondary to suppression of NF-κB activation.[37] IL-4 enhances cytokine mRNA degradation.[37] Although IL-13 has also been shown to decrease cytokine production,[56] our results do not show any difference in IL-13 mRNA levels among the different groups. This does not, however, rule out a combined role for IL-10, IL-4, and IL-13 because these cytokines, acting in concert, are able to inhibit NF-κB activation.[56] Decreased synthesis of IL-10 in monocytes has been described in alcoholic cirrhosis.[57] The mechanisms leading to decreased IL-10 and IL-4 production in rats exhibiting inflammatory liver injury is unknown but based on the biological activity of these anti-inflammatory cytokines, we hypothesize that decreased levels of IL-10 and IL-4 are important in determining the pathological outcome of liver injury. Thus, our results, taken in the context of protection experiments conducted with IL-4 and IL-10,[58,59] strengthen the idea that these cytokines are capable of antagonizing the production and effects of proinflammatory cytokines and, at least in rats fed saturated fats and ethanol, protect against the development of necroinflammatory changes. Our findings would predict a synergistic effect of IL-10 and IL-4 in protecting against the effect of proinflammatory cytokines.

CONCLUSION

An important experimental issue addressed by the present study was to extend the observation relating the saturation of dietary fat and alcoholic liver disease to underlying mechanisms contributing to tissue injury. Although the causal relationships between the events described in this study and pathological changes is uncertain, the activation of NF-κB constitutes one mechanism by which endotoxin and oxidative stress promote liver injury. Our study yields results that NF-κB activity is increased by the combination of unsaturated fats and ethanol and that the balance between pro- and anti-inflammatory cytokines is important in alcoholic liver injury. Not all of the cytokines and chemokines evaluated in the present study are affected by NF-κB activation.[60] Thus, other factors, independently of NF-κB, may affect the production of pro- or anti-inflammatory cytokines.[60] We recognize that RT-PCR is a complex multistep technique with potential problems and variability in yield. Although there are criticisms of PCR-based approaches to the quantitation of specific mRNAs, we attempted to optimize each step in the technique and believe that the results of measurements of certain cytokines in plasma show that the mRNA results of these cytokines are probably valid. Regardless of the limitations imposed by the measurements performed in this study, our findings underscore the critical role for NF-κB activation and cytokine imbalance in alcoholic liver injury. The elucidation of factors that regulate the balance between pro- and anti-inflammatory cytokines should provide further insight into the pathogenesis of tissue injury in alcoholic liver disease as well as provide opportunities for therapeutic intervention. For example, blockage of activation of NF-κB, as is seen with acetaldehyde, markedly decreases cytokine production and ameliorates inflammation and necrosis.[61,62]

Acknowledgment: The authors thank Dr. Hidezaku Tsukamoto for helpful comments, and Diana Peters and Timothy Cloutier for technical assistance.

REFERENCES

1. Nanji AA, Zakim D. Alcoholic liver disease. In: Zamkin D, Td, eds. Hepatology: A Textbook of Liver Disease. 3rd ed. Philadelphia: Saunders, 1996;891-961.
2. Nanji AA, Khettry U, Sadrzadeh SMH, Yamanaka T. Severity of liver injury in experimental alcoholic liver disease. Correlation with plasma endotoxin, prostaglandin E_2, leukotriene B_4 and thromboxane B_2. Am J Pathol 1993;142:367-373.
3. Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. Gastroenterology 1993;108:218-224.
4. Lieber CS. Alcohol and the liver: 1994 update. Gastroenterology 1994;106:1085-1105.
5. Nanji AA, Khwaja S, Tahan SR, Sadrzadeh SMH. Plasma levels of a novel noncyclooxygenase-derived prostanoid (8-isoprostane) correlate with severity of liver injury in experimental alcoholic liver disease. J Pharmacol Exp Ther 1994;269:1280-1285.
6. Fukui H, Brauner B, Bode JC, Bode C. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: re-evaluation with an improved chromogenic assay. HEPATOLOGY 1991;12:162-168.
7. Bode C, Kugler V, Bode JC. Endotoxemia in patients with alcoholic and non-alcoholic cirrhosis and in subjects with no evidence of chronic liver disease following acute ethanol excess. J Hepatol 1987;4:8-13.
8. Reinke LA, Lai EK, DuBose CM, McCay PB. Reactive free radical generation in vivo in heart and liver of ethanol-fed rats: correlation with radical formation in vitro. Proc Natl Acad Sci U S A 1987;84:9223-9227.
9. Kamimura S, Gaal K, Britton S, Bacon BR, Triadafilopoulos G, Tsukamoto H. Increased 4-hydroxynoneral levels in experimental alcoholic liver disease: association of lipid peroxidation with liver fibrogenesis. HEPATOLOGY 1992;16:448-453.
10. Baeuerle PA, Baltimore D. NF-kB: ten years after. Cell 1996;87:13-20.
11. May MJ, Ghosh S. Rel/NF-kB and IkB proteins: an overview. Semin Cancer Biol 1997;8:63-73.
12. Barnes PJ, Karin M. Nuclear factor kB-a pivotal factor in chronic inflammatory diseases. N Engl J Med 1997;336:1066-1071.
13. McClain CJ, Hill D, Schmidt J, Diehl AM. Cytokines and alcoholic liver disease. Semin Liver Dis 1993;13:170-182.
14. Hill D, Diehl AM, Tsukamoto H, Shedlofsky S, McClan CJ. Cytokines and alcoholic liver disease. In: Remick DL ed. Cytokines in Health and Disease. 2nd ed. New York: Marcel Dekker, 1997; p 401.
15. Hill DB, Marsano LS, McClain CJ. Increased plasma interleukin-8 concentrations in alcoholic hepatitis. HEPATOLOGY 1993;18:576-580.
16. Maher JJ. Rat hepatocytes and Kupffer cells interact to produce interleukin-8 (CINC) in the setting of ethanol. Am J Physiol 1995;269: G518-G523.
17. Furle MB, Randolph GJ. Chemokines and tissue injury. Am J Pathol 1995;146:1287-1301.

18. Baruch AB, Michiel DF, Oppenheim JJ. Signals and receptors involved in recruitment of inflammatory cells. J Biol Chem 1995;270:11703-11706.
19. Tsukamoto H, Towner SJ, Ciofalo LM, French SW. Ethanol-induced fibrosis in rats fed a high fat diet. HEPATOLOGY 1986;6:814-822.
20. Tsukamoto H, Gaal K, French SW. Insight into the pathogenesis of alcoholic liver necrosis and fibrosis. HEPATOLOGY 1990;12:599-608.
21. Nanji AA, Mendehall CL, French SW. Beef fat prevents alcoholic liver disease in the rat. Alcohol Clin Exp Res 1989;13:15-19.
22. Nanji AA, French SW. Dietary linoleic acid is required for development of experimental alcoholic liver disease. Life Sci 1989;44:223-227.
23. Morimoto M, Zern MA, Hagbjork AL, Ingelman-Sundberg M, French SW. Fish oil, alcohol and liver pathology: role of cytochrome P4502E1. Proc Soc Exp Biol Med 1994;207:197-205.
24. Nanji AA, Zhao S, Sadrzadeh SMH, Dannenberg AJ, Tahan SR, Waxman DJ. Markedly enhanced cytochrome P4502E1 induction and lipid peroxidation is associated with severe liver injury in fish oil-ethanol-fed rats. Alcohol Clin Exp Res 1994;18:1280-1285.
25. Polavarapu R, Spitz DR, Sim JE, Follansbee MH, Oberley LW, Rahemtulla A, Nanji AA. Increased lipid peroxidation and impaired antioxidant enzyme function is associated with pathological liver injury in experimental alcoholic liver disease in rats fed diets high in corn oil and fish oil. HEPATOLOGY 1998;27:1317-1323.
26. Liu SL, Esposti SD, Yao T, Diehl AM, Zern MA. Vitamin E therapy for acute CCl_4-induced hepatic injury is associated with inhibition of nuclear factor kappa B binding. Hepatology 1995;22:1474-1481.
27. Lin M, Rippe RA, Niemela O, Britteham G, Tsukamoto GH. Role of iron in NF-KB activation and cytokine gene expression by rat hepatic macrophages. Am J Physiol 1997;272:G1355-G1364.
28. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1997;162:156-159.
29. Tang WW, Yin S, Wittwer A, Qi M. Chemokine gene expression in anti-glomerular basement membrane antibody glomerulonephritis. Am J Physiol 1995;269:F323-F330.
30. Nanji AA, Zhao S, Sadrzadeh SMH, Waxman DJ. Use of reverse transcription PCR to evaluate in vivo cytokine gene expression in rats fed ethanol for long periods of time. HEPATOLOGY 1994;19:1483-1487.
31. Colletti LM, Kunkel SM, Walz A, Burdick MD, Kunkel RG, Wolke CA, Strieter RM. Chemokine expression during hepatic ischemia/reperfusion-induced lung injury in the rat. The role of epithelial neutrophil activating protein. J Clin Invest 1995;95:134-141.
32. Kotake S, Schumacher HR, Wilder RL. A simple nested RT-PCR method for quantitation of the relative amounts of multiple cytokine mRNAs in small tissue samples. J Immunol Methods 1996;199:193-203.
33. Sokolowski AR, Mottram PL, Jensen TJ, Satoskar A, Russell ME. Heart transplants in interferon-8, interleukin-4 and interleukin-10 knockout mice. Recipient environment alters graft rejection. J Clin Invest 1997;100: 2449-2456.
34. Katagiri K, Itami S, Hatano Y, Takayasu S. Increased levels of IL-13 mRNA, but not IL-4 mRNA are found in vivo in peripheral blood mononuclear cells in patients with atopic dermatitis. Clin Exp Immunol 1997;108:269-294.
35. Nanji AA, Miao L, Thomas P, Rahemtulla A, Khwaja S, Zhao S, Peters D, et al. Enhanced cyclooxygenase-2 gene expression in alcoholic liver disease in the rat. Gastroenterology 1997;112:943-951.
36. Nanji AA, Griniuviene B, Yacoub LK, Fogt F, Tahan SR. Intercellular adhesion molecule-1 in experimental alcoholic liver disease: relationship to endotoxemia and TNFα messenger RNA. Exp Mol Pathol 1995;62:42-51.
37. Wang P, Wu P, Siegel ML, Egan RW, Billah MM. Interleukin (IL)-10 inhibits nuclear factor kB (NF-kB) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. J Biol Chem 1995;270:9558-9563.
38. Mirza A, Liu SL, Frizell E, Zhu J, Maddukuri S, Martinez J, Davies P, et al. A role for tissue transglutaminase in hepatic injury and fibrogenesis and its regulation by NF-Kappa B. Am J Physiol 1997;272:G281-G288.
39. Iimuro Y, Nishiura T, Hellerbrand C, Behrns KE, Schoonhoven R, Grisham JW, Brenner DA. NF-KB prevents apoptosis and liver dysfuction during liver regeneration. J Clin Invest 1998;101:802-811.
40. Heinzel FP. The role of IFN-gamma in the pathology of experimental endotoxemia. J Immunol 1990;145:2920-2922.
41. Remick DG, Strieter RM, Eskandari MK, Nguyen DT, Genord MA, Raiford CL, Kunkel SL. Role of tumor necrosis factor-α in lipopolysaccharide-induced pathologic alterations. Am J Pathol 1990;136:49-60.
42. Doherty GM, Lange JR, Langstein HN, Alexander HR, Buresh CM, Norton JA. Evidence for IFN-γ as a mediator of the lethality of endotoxin and tumor necrosis factor-α. J Immunol 1992;149:1666-1670.
43. Heinzel FP, Rerko RM, Ling P, Hakimi J, Schoenhaut DS. Interleukin-12 is produced in vivo during endotoxemia and stimulates synthesis of gamma interferon. Infect Immun 1994;62:4244-4249.
44. Laskin DL. Nonparenchymal cells and hepatotoxicity. Semin Liver Dis 1990;10:293-304.
45. Nanji AA, Miao L, Thomas P, Rahemtulla A, Khwaja S, Zhao S, Peters D, et al. Enhanced cyclooxygenase-2 gene expression in alcoholic liver disease in the rat. Gastroenterology 1997;112:943-951.
46. Farrar MA, Schrieber RD. The molecular cell biology of interferon gamma and its receptor. Annu Rev Immunol 1993;11:571-611.
47. Shakov AN, Woerly G, Car BD, Ryffel B. Interferon-γ enhances tumor necrosis factor-α production by inhibiting early phase interleukin-10 transcription. Eur Cytokine Netw 1996;7:741-750.
48. Brunda MJ. Interleukin-12. J Leukoc Biol 1994;55:280-287.
49. Pearlman E, Lass JH, Bardenstein DS, Diaconu E, Hazlett FE, Albright J, Higgins AW, et al. IL-12 exacerbates helmith-mediated corneal pathology by augmenting inflammatory cell recruitment and chemokine expression. J Immunol 1997;158:827-833.
50. Deutschman CS, Haber BA, Andrejko K, Cressman DE, Harrison R, Elenko E, Taub R. Increased expression of cytokine-induced neutrophil chemoattractant in septic rat liver. Am J Physiol 1996;271:R593-R600.
51. Bautista AP. Chronic alcohol intoxication induces hepatic injury through enhanced macrophage inflammatory protein-2 production and intercellular adhesion molecule-1 expression in the liver. HEPATOLOGY 1997;25: 335-342.
52. Wang X, Yue TL, Ohlstein EH, Sung CP, Feuerstein GZ. Interferon-inducible protein involves vascular smooth muscle cell migration, proliferation and inflammatory response. J Biol Chem 1996;271:24286-24293.
53. Davatelis G, Tekamp-Olson P, Wolpe SD, Hermsen K, Luedke C, Gallegos C, Coit D, et al. Cloning and characterization of acDNA for murine macrophage inflammatory protein (MIP); a novel monokine with inflammatory and chemokinetic properties. J Exp Med 1988;167: 1939-1944.
54. Jaeschke H. Chemokines and liver inflammation: the battle between pro- and anti-inflammatory mediators. HEPATOLOGY 1997;25:252-253.
55. Bogdan C, Vodovotz Y, Nathan C. Macrophage deactivation by interleukin 10. J Exp Med 1991;174:1549-1553.
56. Lentsch AB, Shanley TP, Sarma V, Ward PA. In vivo suppression of NF-kB and preservation of IkBα by interleukin-10 and interleukin-13. J Clin Invest 1997;100:2443-2448.
57. La Moine O, Marchant A, DeGroote D, Azar C, Goldman M, Deviere J. Role of defective monocyte interleukin-10 release in tumor necrosis factor-alpha overproduction in alcoholic cirrhosis. HEPATOLOGY 1995;22: 1436-1439.
58. Howard M, Muchamuel T, Andrade S, Menon S. Interleukin 10 protects mice from lethal endotoxemia. J Exp Med 1993;177:1205-1208.
59. Santucci L, Frorucci S, Chiorean M, Brunori PM, Matteo FM, Sidoni A, Migliorati G, et al. Interleukin-10 reduces lethality and hepatic injury induced by lipopolysaccharide in galactosamine sensitized mice. Gastroenterology 1996;111:736-744.
60. Firestein GS, Manning AM. Signal transduction and transcription factors in rheumatic disease. Arthritis Rheum 1999;42:609-621.
61. Lindros KO, Jokelainen K, Nanji AA. Acetaldehyde prevents nuclear factor-Kappa β activation and hepatic inflammation in ethanol-fed rats. Lab Invest 1999;79:799-806.
62. Jokelainen K, Thomas P, Lindros K, Nanji AA. Acetaldehyde inhibits NF-Kβ activation through IKβ alfa preservation in rat Kupffer cells. Biochem Biophys Res Commun 1998;253:834-836.