Enhanced PDE4B expression augments LPS-inducible TNF expression in ethanol-primed monocytes: relevance to alcoholic liver disease

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Gobejishvili L, Barve S, Joshi-Barve S, McClain C. Enhanced PDE4B expression augments LPS-inducible TNF expression in ethanol-primed monocytes: relevance to alcoholic liver disease. Am J Physiol Gastrointest Liver Physiol 295: G718–G724, 2008. First published August 7, 2008; doi:10.1152/ajpgi.90232.2008.—Increased plasma and hepatic TNF-α expression is well documented in patients with alcoholic hepatitis and is implicated in the pathogenesis of alcoholic liver disease. We have previously shown that monocytes from patients with alcoholic hepatitis show increased constitutive and LPS-induced NF-κB activation and TNF-α production. Our recent studies showed that chronic ethanol exposure significantly decreases cellular cAMP levels in both LPS-stimulated and unstimulated monocytes and Kupffer cells, leading to an increase in LPS-inducible TNF-α production by affecting NF-κB activation and induction of TNF mRNA expression. Accordingly, the mechanisms underlying this ethanol-induced decrease in cellular cAMP leading to an increase in TNF expression were examined in monocytes/macrophages. In this study, chronic ethanol exposure was observed to significantly increase LPS-inducible expression of cAMP-specific phosphodiesterase (PDE)4B that degrades cellular cAMP. Increased PDE4B expression was associated with enhanced NF-κB activation and transcriptional activity and subsequent priming of monocytes/macrophages leading to enhanced LPS-inducible TNF-α production. Selective inhibition of PDE4 by rolipram abrogated LPS-mediated TNF-α expression at both protein and mRNA levels in control and ethanol-treated cells. Notably, PDE4 inhibition did not affect LPS-inducible NF-κB activation but significantly decreased NF-κB transcriptional activity. These findings strongly support the pathogenic role of PDE4B in the ethanol-primed priming of monocytes/macrophages and increased LPS-inducible TNF production and the subsequent development of alcoholic liver disease (ALD). Since enhanced TNF expression plays a significant role in the evolution of clinical and experimental ALD, its downregulation via selective PDE4B inhibitors could constitute a novel therapeutic approach in the treatment of ALD.

DYSREGULATED TNF METABOLISM in alcoholic hepatitis (AH) was first described by us almost two decades ago with the observation that cultured monocytes from patients with AH spontaneously produced TNF and produced significantly more TNF in response to an LPS stimulus than control monocytes (20). Furthermore, we and others have shown that there are increased serum levels of TNF, increased monocyte TNF production, and hepatic immunohistochemical staining for TNF in AH that frequently correlate with disease severity and mortality (2, 5, 12, 15, 19, 20, 22, 27). Although TNF plays an important role in the pathogenesis of liver injury and the clinical/biochemical abnormalities of AH (9, 20–22), the mechanism(s) by which ethanol enhances TNF expression, particularly in monocytes/macrophages, is only beginning to be understood (6, 14, 37, 38). We recently demonstrated that chronic ethanol exposure of monocytes/macrophages (including Kupffer cells) decreases both basal and LPS-stimulated cAMP levels and leads to the enhancement of LPS-inducible TNF production (6).

Increasing cellular cAMP levels with different types of cAMP enhancers has invariably resulted in the suppression of TNF production stimulated either in vitro or in vivo with LPS in monocytes/macrophages of both human and murine origin (39). Various studies have documented that, depending on the cell type, suppression of LPS-inducible TNF production by cAMP can occur in both NF-κB-dependent and -independent mechanisms (25, 28, 32, 34, 36). Our recent findings demonstrated that increasing the cellular cAMP levels with the use of a cell-permeable cAMP analog (dbcAMP) inhibits the synergistic enhancement in TNF-α expression caused by ethanol and LPS (6). To further extend our observations and clarify the mechanisms involved in the enhancement of LPS-inducible TNF expression in monocytes primed by ethanol exposure, we examined the role of phosphodiesterases (PDE), enzymes that degrade cAMP and play a critical role in cAMP-mediated signaling. Particularly, cAMP-specific PDE4, which is present in different tissues, is found to be the predominant PDE isoenzyme among 11 PDEs in human monocytes (4, 18).

Numerous studies have shown that LPS-inducible TNF production by monocytes is markedly decreased when PDE activity is blocked by PDE4-specific inhibitors (3, 4, 16, 23, 29, 30, 31, 33, 35). PDE4 isozyme has four isoforms, namely A, B, C, and D (18). Specifically, to monocytes/macrophages, it has been established that PDE4B is involved in Toll-like receptor signaling and is essential for LPS-induced TNF-α expression (10, 11). Accordingly, the present work examined the putative role of PDE4B expression and activity in the ethanol-mediated enhancement of LPS-inducible TNF expression in monocytes.

The data obtained from these studies support our earlier findings and further extend them by showing that chronic ethanol exposure significantly increases LPS-inducible PDE4B expression and activity, resulting in decreased cellular cAMP in monocytes. Selective inhibition of PDE4 by rolipram abrogated LPS-mediated TNF expression at both protein and mRNA levels in control and ethanol-treated cells, strongly supporting a causal role for PDE4B in priming and augmentation of TNF expression. Notably, PDE4 inhibition did not affect LPS-inducible NF-κB activation but significantly de-

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creased its transcriptional activity, thereby impacting TNF expression.

MATERIALS AND METHODS

Materials. RAW 264.7 murine macrophage and THP-1 human monocyte cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). LPS (Escherichia coli 0111:B4) was purchased from Difco Laboratories (Detroit, MI). Before use, LPS was dissolved in sterile, pyrogen-free water, sonicated, and diluted with sterilized PBS. Penicillin, streptomycin, DMEM and RPMI media, and fetal bovine serum were purchased from Invitrogen (Grand Island, NY); murine and human TNF-α ELISA kits were from BioSource International (Camarillo, CA). Rolipram and p65 antibody was from Biomol Research Laboratories (Plymouth Meeting, PA). Histone 3 antibody was from Cell Signaling Technology (Beverly, MA). Supershift antibodies for p50 and p65 were from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell culture. RAW 264.7 cells were cultured in DMEM, and undifferentiated THP-1 cells were cultured in RPMI media containing 10% (vol/vol) fetal bovine serum, 10 U/ml penicillin, and 10 μg/ml streptomycin at 37°C in a humidified 5% CO₂ atmosphere. Cells were chronically exposed to 25 mM ethanol for a period of 6 wk.

TNF-α ELISA. TNF-α in conditioned medium was quantified using ELISA kit in accordance with the manufacturer’s instructions. All assays were run in triplicate.

RNA isolation and real-time PCR assays. RT-PCR assays were used to assess TNF-α and PDE4B mRNA levels in RAW 264.7 and THP-1 cells. Total RNA was isolated from cells using TRIzol Reagent (Invitrogen). For real-time PCR, the first-strand cDNA was synthesized using TaqMan Reverse transcription reagents (Applied Biosystems, Foster City, CA). The reverse transcription was carried out with 1 μmol/μl random hexamer, 8 U of RNase inhibitor, and 25 U of Multiscribe Reverse Transcriptase with 200 ng of total RNA. The RT conditions were 10 min at 25°C, 30 min at 48°C, and 5 min at 95°C. Reactions in which the enzyme or RNA were omitted were used as negative controls. Real-time PCR was performed in triplicates with an ABI prism 7500 sequence detection system and SYBR green I dye reagents. The specific primers were designed for mouse GAPDH, TNF-α, and PDE4B with Primer3 software program. The following primers were used in real-time PCR: mGAPDH-FP, 5′TGGTG-GAAGGGCTCATGACC3′; mGAPDH-RP, 5′TTCTTCTGGGGCCAGTGTG3′; mTNF-α-FP, 5′GAAGTTCACATGTGGCCTC3′; mTNF-α-RP, 5′GTGAGGGTGCTGGCCATAGA3′; mPDE4B-FP, 5′GGACGAGAGGCCAGGAAAGAA3′; and mPDE4B-RP, 5′ACCCACCACTTGAAAGT3′. Human 18S, TNF-α, and PDE4B-specific primers were purchased from SuperArray Bioscience (Fredrick, MD).

The parameter threshold cycle (C₀) was defined as the fraction cycle number at which the fluorescence passed the threshold. The relative gene expression was analyzed using the 2⁻ΔΔC₀ method (17) by normalizing with GAPDH or 18S gene expression in all the experiments.

Nuclear protein extraction. Cells were washed twice with PBS and lysed by hypotonic shock in cytoplasmic extraction buffer (10 mM HEPES, pH 7.8, 1.5 mM MgCl₂, 0.5 mM EDTA, 0.1% Nonidet P-40, and 0.5 μM PMSF) and 1 μg/ml each of the protease inhibitors aprotinin, leupeptin, and lecinuthiol. The cells were collected by scraping, and the nuclei were separated from the cytosolic proteins by a low-speed centrifugation at 1,500 g for 5 min. The supernatant (cytoplasmic extract) was stored at −70°C. The nuclear pellet was resuspended in nuclear extraction buffer (20 mM HEPES, pH 7.8, 520 mM NaCl, 1.5 mM MgCl₂, 0.1 mM EDTA, 25% glycerol, 0.5 mM DTT, 0.1% Nonidet P-40, and 0.5 μM PMSF) and 1 μg/ml each of the protease inhibitors aprotinin, leupeptin, and lecinuthiol and incubated on ice for 60 min. The samples were centrifuged at 100,000 g for 15 min, and the supernatant containing nuclear proteins was stored in small aliquots at −70°C until further use. The protein concentration in nuclear extracts was measured with the use of the BioRad Dye Reagent (Bio-Rad, Hercules, CA) in accordance with the manufacturer’s protocol.

EMSA. Double-stranded oligonucleotide containing the binding site for NF-κB (5′-AGT-GTG-GAG-GAC-CTT-CCC-AGG-C-3′; Promega, Madison, WI) was end-labeled with [³²P] dATP using T4 polynucleotide kinase (Gibco, Grand Island, NY). Nuclear proteins (5 μg) were incubated in binding buffer (50 mM Tris, pH 7.5, 500 mM NaCl, 5 mM DTT, 5 mM EDTA, 20% (vol/vol) glycerol, and 0.4 μg/ml sonicated salmon sperm DNA) with 0.3 ng of [³²P]-labeled probe for 20 min at room temperature. For supershift analysis, nuclear extracts were preincubated with 1 μg of p65 and/or p50 monoclonal antibody for 15 min before addition of the radioactive probe. The resultant DNA/protein complexes were separated by electrophoresis using a 5% non-denaturing polyacrylamide gel in 0.5× Tris-borate-EDTA (50 mM Tris, pH 8.0, 45 mM borate, and 5 mM EDTA). The gels were dried and analyzed by autoradiography for ~18 h. The specificity of the reaction was evaluated by incubating the nuclear extract with a 100 molar excess of unlabeled wild-type (W) and mutant (M) NF-κB oligonucleotide.

Western blot analysis. Nuclear proteins (30 μg) were analyzed by SDS-polyacrylamide gel electrophoresis using a Bio-Rad electrophoresis system, followed by immunoblotting according to the manufacturer’s instructions. Immunoreactive bands were visualized using the enhanced chemiluminescence light detection reagents (Amersham, Arlington Heights, IL). Quantification (densitometry) of bands was performed using ImageQuantTLv2003.03. Histone 3 was used as a loading control.

Plasmids and transfections. NF-κB-luciferase reporter construct was obtained from BD Biosciences/Clontech (Palo Alto, CA). Transfections were carried out using FuGENE according to instructions from the manufacturer (Roche Diagnostics, Indianapolis, IN). To control for differences in transfection efficiency, transfected cells were scraped and re-plated after 24 h at a density of 1 × 10⁶ cells/well in 24-well plates and treated for 6 h.

Luciferase assay. Luciferase activity was measured using Luciferase Assay Reagent (Promega). After treatments, total cell lysates were prepared in reporter lysis buffer (Promega). Luciferase enzymatic activity was measured in a TD 20/20 luminometer using a specific substrate provided by Promega.

PDE4 enzymatic assay. PDE4 specific enzymatic activity was determined using PDE4 assay kit (FabGennix International, Frisco, TX) as described previously (13). Briefly, cells were lysed after treatment with SolObuffer (FabGennix International), and 25 μg protein was used per assay. Assay was carried in duplicate; the final concentration of cAMP was 2 μM. PDE4 activity was estimated from the difference between total and rolipram-resistant PDE activity.

Statistical analysis. All experiments were repeated at least three times. Data were presented as means ± SD for the indicated number of independently performed experiments. Student’s t-test was used for the determination of statistical significance. P < 0.05 was considered significant.

RESULTS

RAW 264.7 and THP-1 cells were continuously maintained in ethanol (25 mM)-supplemented medium, which was changed after every 12 h over a period of 6 wk. The concentration of ethanol chosen to treat the cells reflects the blood alcohol level that can be achieved during human alcohol consumption. Alcohol levels in media were routinely measured over the study duration using the Sigma Diagnostics Alcohol Reagent (Sigma Diagnostics, St. Louis, MO) (6). Typically, under the experimental conditions of ethanol treatment, a
reduction in ethanol concentration by ~12–15% occurred over the incubation period of 12 h. A minor reduction in the doubling time of both RAW 264.7 and THP-1 cells was observed in the first week only, with no further effect on either viability or proliferation (data not shown).

Ethanol exposure enhances PDE4 gene expression and activity in human THP-1 monocytes and murine RAW macrophages. We have previously shown that chronic ethanol exposure significantly decreases intracellular cAMP levels in monocytes and Kupffer cells and is responsible for priming them to LPS-inducible TNF overproduction. PDE4B is the predominant subtype in monocytes/macrophages that degrades cAMP and is critical for LPS signaling and TNF expression (10, 11). Therefore, we examined the effect of chronic ethanol exposure on PDE4B expression and activity in monocytes/macrophages. After ethanol exposure, cells were plated into 60-mm culture dishes at $1 \times 10^6$ cells/ml density and stimulated with LPS (100 ng/ml) for 2 h for gene expression analysis and 4 h for PDE4 activity measurement. The time points for TNF and PDE4B mRNA expression and PDE4 activity were selected on the basis of previously established expression optima (6, 10, 11). Ethanol treatment significantly augmented LPS-inducible PDE4B mRNA with increased TNF expression in both RAW 264.7 (murine macrophage) and THP-1 (human monocytic) cells (Fig. 1, A and B, respectively). Furthermore, ethanol-treated cells showed increased basal and LPS-inducible PDE4 activity compared with control cells (Fig. 2, A and B).

PDE4 inhibition results in decreased LPS-inducible TNF production caused by ethanol. Our previous work has demonstrated that ethanol-mediated decrease in cAMP levels plays a significant role in priming monocytes, resulting in increased LPS-stimulatable TNF production. Also, since PDE4B specifically degrades cAMP in response to LPS, its role in modulating cAMP-dependent regulation of TNF expression in ethanol-treated monocytes was examined. We used a PDE4-specific inhibitor, rolipram (10 μM), to inhibit PDE4 activity in all the experiments. At this concentration, rolipram has a negligible inhibitory effect on other PDEs (11). RAW cells exposed to ethanol were treated with rolipram for 30 min before LPS (100 ng/ml) stimulation for 6 h. Supernatants were collected and analyzed for TNF production by ELISA. As observed previously, ethanol primed the monocytes, leading to a significant increase in the LPS-inducible TNF production. Notably, inhibition of PDE4B by rolipram effectively inhibited the enhancement in the TNF-α expression caused by ethanol and LPS (Fig. 3A).

Effect of chronic alcohol exposure and PDE4 inhibition on LPS-induced TNF gene transcription. To address the mechanistic involvement of PDE4B in the effects of chronic ethanol exposure on TNF production, steady-state levels of TNF mRNA were quantified in cells pretreated with rolipram. Real-time PCR analysis of total RNA obtained from treated cells showed that the LPS-inducible steady-state TNF mRNA levels were significantly higher in ethanol-treated macrophages compared with control cells, corroborating our earlier findings and work done by others (Fig. 3B, open bars). Furthermore, this synergistic enhancement in the TNF mRNA levels caused by ethanol and LPS was significantly attenuated by PDE4 inhibition (Fig. 3B, solid bars).

![Fig. 1](attachment://fig1.png)

Fig. 1. Chronic alcohol exposure of monocytes/macrophages markedly upregulates LPS-inducible phosphodiesterase (PDE)4B and TNF-α mRNA expression. Cells were stimulated with LPS (100 ng/ml) and collected in 2 h for total RNA isolation. PDE4B and TNF-α mRNA were quantified using real-time PCR. Data are representative of 3 separate experiments. Data are presented as means ± SD (n = 3). A: RAW cells, *P < 0.05 compared with LPS-stimulated control. B: THP-1 cells, *P < 0.01 and **P < 0.05 compared with LPS-stimulated control. UT, untreated.

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The effect of ethanol on the LPS-inducible steady-state TNF mRNA expression in monocytes/macrophages could be due to an increase in the synthesis of TNF mRNA or an increase in the stability of TNF mRNA. We did not observe the difference in the half-life ($t_{1/2}$) of LPS-induced TNF mRNA between control and ethanol-treated cells (data not shown). We then examined the effect of PDE4 inhibition on stability of TNF mRNA. Ethanol-exposed cells with and without pretreatment with 10 μM rolipram for 30 min were stimulated with LPS (100 ng/ml) for 60 min followed by actinomycin D (1 μg/ml) up to 2 h. RNA was harvested at 0, 30, 60, and 120 min after actinomycin D addition and analyzed for TNF mRNA expression. The data obtained showed that PDE4 inhibition did not alter the stability of the LPS-induced TNF mRNA. The $t_{1/2}$ of LPS-induced TNF mRNA was ~25 min in both untreated as well as in rolipram-pretreated macrophages (Fig. 4). Thus pretreatment with PDE4 inhibitor, which abolished the enhancement in the LPS-inducible steady-state TNF mRNA expression in ethanol-treated macrophages, had no effect on the $t_{1/2}$ of LPS-induced TNF mRNA.

PDE4 inhibition does not alter LPS-inducible NF-κB nuclear translocation and DNA binding but attenuates transcriptional activity of NF-κB. LPS stimulation of monocytes/macrophages is known to induce TNF-α mRNA expression through a rapid and transient NF-κB activation leading to the induction of the TNF-α promoter. To assess whether the effect of PDE4 inhibition on LPS-stimulated TNF-α mRNA expression in ethanol-primed RAW cells was attributable to a decrease in NF-κB activation, nuclear levels of p65 and NF-κB DNA binding activity were examined in the nuclear proteins extracted from RAW cells pretreated with rolipram (10 μM for 30 min) followed by stimulation with LPS (100 ng/ml) for 30 min. Immunoblot analysis showed that pretreatment of cells with rolipram had no effect on the nuclear levels of LPS-

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**Fig. 2.** Chronic ethanol exposure significantly enhances PDE4-specific activity in monocytes/macrophages. Control and ethanol-treated cells were left untreated (UT) and/or stimulated with LPS (100 ng/ml) for 4 h. PDE4 activity was measured in 25-μg cell lysates and is represented as percent activity over control UT cells. Data are presented as means ± SD ($n = 3$). **A:** mouse macrophages (RAW), *$P < 0.01$ compared with control, UT; **$P < 0.05$ compared with control LPS and EtOH. **B:** human monocytes (THP-1), *$P < 0.05$ compared with control, UT. **$P < 0.01$ compared with control LPS.

**Fig. 3.** PDE4 inhibition significantly decreases TNF production at both protein and mRNA level in RAW 264.7 cells. At 30 min before LPS (100 ng/ml) stimulation, cells were treated with PDE4-specific inhibitor rolipram (Rol) (10 μM). **A:** samples were collected in 6 h, and TNF was quantified using ELISA. Data are presented as means ± SD ($n = 3$). *$P < 0.01$ compared with control LPS and EtOH; **$P < 0.01$ compared with control, LPS. **B:** cells were collected in 2 h after LPS stimulation for RNA extraction. NF-κB mRNA was quantified using real-time PCR. Data are representative of 3 separated experiments. Data are presented as means ± SD ($n = 3$). *$P < 0.05$ compared with LPS-stimulated control and **$P < 0.01$ with LPS-stimulated EtOH.

**Fig. 4.** PDE4 inhibition does not alter TNF mRNA half-life. RAW 264.7 cells chronically exposed to ethanol were pretreated with rolipram for 30 min and stimulated with LPS (100 ng/ml) for 1 h, followed by actinomycin D (ActD) (1 μg/ml), and total mRNA was isolated at times indicated above. The remaining TNF mRNA at each time point was quantified using real-time PCR.
inducible p65 (Fig. 5A); histone 3 was used as a loading control for the nuclear proteins.

EMSA was performed to assess the effect of PDE4 inhibition on NF-κB DNA binding to the consensus κB sequence. Nuclear extracts were isolated from ethanol-treated monocytes without or with rolipram followed by LPS treatment for 30 and 90 min. Correspondent to the p65 nuclear levels, rolipram did not have any effect on the ethanol- and LPS-mediated NF-κB DNA binding (Fig. 5B). NF-κB/Rel transcription factors are homo- or heterodimeric complexes that consist of various combinations of Rel subunits. To identify the subunits present in the LPS-inducible NF-κB complexes, supershift experiments were performed using antibodies against p65 and p50 (Fig. 5B). In nuclear extracts from ethanol-treated cells stimulated with LPS for 30 min with and without rolipram, the p50-specific antibody completely shifted complex C2 and reduced the extent of binding of complex C1 and generating lower mobility complexes S1 and S2, showing that both C1 and C2 contain p50. The p65-specific antibody significantly shifted complex C1 to S1 with no effect on complex C2, demonstrating that C1 contains the p65 subunit of NF-κB. A combination of the two antibodies fully shifted both C1 and C2 to form S1 and S2. Thus supershift analysis indicates that the NF-κB/Rel complexes induced by LPS consist of p65/p50 heterodimer (C1) and p50/p50 homodimer (C2) and are not affected by rolipram.

**PDE4 inhibition downregulates LPS-stimulated NF-κB transcriptional activity.** We then examined the effect of PDE4 inhibition on the LPS-stimulated NF-κB transcriptional activity by performing transient transfection in RAW 264.7 cells with a luciferase reporter construct under the control of an NF-κB promoter containing three tandem repeats of the κB sequence (κB-luc). LPS stimulation for 6 h of ethanol-treated cells increased the NF-κB transcriptional activity approximately fourfold. Rolipram pretreatment significantly attenuated the increases in LPS-stimulated NF-κB activity (decreased by 42%) (Fig. 6). Taken together, these data indicate that PDE4 inhibition has minimal to no effect on the LPS-inducible signaling components leading to NF-κB activation; however, it can significantly decrease gene expression mediated by NF-κB-dependent transactivation.

**DISCUSSION**

Our earlier work documented that decreased cAMP levels played a causal role in ethanol-mediated priming of mono-
cells/macrophages leading to an increase in LPS-inducible TNF expression (6). PDEs are the only superfamily of enzymes that catalyze the hydrolysis of the cyclic nucleotides, cAMP and cGMP, and they play a critical role in regulating the intracellular levels of these important second messengers. Different functional pools of these cellular cyclic nucleotides are regulated by distinct PDEs in a cell-dependent manner. Particularly in the context of cAMP-regulating LPS-inducible TNF expression in monocytes, work done by Conti and colleagues (10, 11) has demonstrated the essential role of PDE4 (subtype B, PDE4B), a cAMP-specific PDE isozyme. Hence, effects of chronic ethanol exposure on PDE4 expression and activity and its functional role in upregulating LPS-inducible TNF expression was examined in the present work.

A major finding of this study was that chronic ethanol exposure led to a significant increase in the LPS-inducible PDE4B expression and activity in both monocytes and macrophages. Since PDE4B is required for TNF expression in response to LPS in monocytes/macrophages, these findings strongly suggest that its augmentation induced by ethanol plays a critical role in priming the monocytes/macrophages to mount an exaggerated inflammatory response to endotoxin. The role of PDE4B in the ethanol-mediated priming of macrophages was examined by using the prototypical PDE4-selective inhibitor rolipram. Cellular responses modulated by rolipram involve PDE4 regulation of cAMP levels. In this study, rolipram inhibited the enhanced TNF production caused by the synergism of ethanol and LPS, thus strongly supporting a causal role for a PDE4B-regulated pool of cAMP in controlling LPS-responsive TNF expression in macrophages chronically exposed to alcohol. These findings are consistent with earlier observations that PDE4 is the major cAMP-hydrolyzing PDE isozyme that regulates the cAMP-dependent mechanisms that play a central role in the LPS-inducible TNF expression in monocytes/macrophages (4, 23, 29, 35, 39). Furthermore, in accordance with our earlier work, which showed that the noncleavable cAMP analog (dbcAMP) inhibits TNF transcription in ethanol-treated macrophages, rolipram was observed to downregulate the steady-state levels of TNF mRNA expression without affecting TNF mRNA stability.

Numerous studies have demonstrated the critical role of NF-κB in the LPS-inducible transcriptional induction of the TNF gene (1, 7, 24). Our previous work (8) demonstrated that overproduction of TNF observed in peripheral blood mononuclear cells of alcoholic patients is associated with increased NF-κB activation and elevated TNF mRNA levels. In this regard, similar to our observations with dbcAMP, rolipram treatment had no effect on LPS-inducible NF-κB activation (nuclear translocation), as indicated by the nuclear p65 levels and DNA binding in ethanol-treated cells. However, functional studies in ethanol-treated monocytes demonstrated that rolipram significantly inhibited LPS-inducible transcriptional activation of the NF-κB reporter plasmid. Since the p50/p50 complex is not affected by rolipram pretreatment, it is unlikely that the observed repression of NF-κB activity caused by rolipram is occurring via the enhancement of p50/p50-mediated competition with the transcriptionally active p50/p65 heterodimer. These data and our previous observations suggest that chronic ethanol exposure may not only lead to an increase in LPS-inducible NF-κB activation but also further enhance the functional activity of nuclear NF-κB/Rel complexes because of decreased PDE4-regulated cellular pools of cAMP levels, resulting in overproduction of TNF by “ethanol-primed” monocytes/macrophages. This inference is also supported by several observations in different cell types, including monocytes, that show that cAMP represses NF-κB-mediated transcription and TNF mRNA expression without affecting NF-κB activation/DNA binding activity (25, 28, 32, 37). The exact mechanisms by which cAMP levels regulate NF-κB activity are unclear and are presently being investigated. In contrast to NF-κB binding sites, an activator protein-1 (AP-1)/cAMP response element (CRE)-like site in the proximal promoter region has been demonstrated to play an essential role in the cAMP-mediated downregulation of TNF gene transcription in murine macrophages (26). Hence, it is likely that the decreased levels of cAMP caused by enhanced PDE4B expression and activity in monocytes chronically exposed to ethanol could 1) increase NF-κB-dependent transactivation of the TNF promoter and 2) decrease AP-1/CRE-mediated suppression of TNF promoter activity, ultimately leading to overproduction of TNF.

Initial work by our laboratory showed increased priming of both human monocytes from patients with alcoholic hepatitis as well as Kupffer cells (macrophages) from rats chronically fed ethanol. Notably, our recent work has demonstrated that ethanol-mediated decrease in cAMP levels in cultured monocyte/macrophages and hepatic Kupffer cells plays a critical role in the priming of these cells leading to an exaggerated TNF response. Thus monocytes and macrophages, including hepatic Kupffer cells, play an important role in the dysregulated cytokine metabolism and pathogenesis in alcoholic liver disease (ALD). Our present data strongly suggest that decreased cellular cAMP levels in monocytes/macrophages chronically exposed to ethanol occur because of an increase in PDE4B expression. Importantly, the findings show that ethanol-mediated increase in PDE4B expression in monocytes/macrophages synergizes with LPS to upregulate the induction of TNF gene, leading to excessive TNF production. In terms of mechanisms, it is highly likely that chronic ethanol treatment decreases PDE4B-regulated pool of cAMP and influences cAMP signaling involved in the regulation of NF-κB-dependent and -independent LPS-inducible TNF expression. Overall, these data strongly support the pathogenic role of PDE4B in the ethanol-mediated priming of monocytes/Kupffer cells for increased...
LPS-inducible TNF production and the subsequent development of ALD. Importantly, since enhanced TNF expression plays a significant role in the evolution of clinical and experimental ALD, its downregulation via selective PDE4B inhibitors could constitute a novel therapeutic approach in attenuating or preventing the development/progression of ALD.

REFERENCES


