

Regulation of microsomal triglyceride transfer protein mRNA expression by endotoxin and cytokines

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Abstract We studied the effect of endotoxin (LPS), and cytokines (TNF, IL-1, and IL-6) on hepatic microsomal triglyceride transfer protein (MTP) mRNA levels *in vivo* in Syrian hamsters and *in vitro* in HepG2 cells. LPS, interleukin-1 (IL-1), and to a lesser extent tumor necrosis factor (TNF) significantly decreased MTP mRNA levels in hamster liver. These effects required several hours. Furthermore, IL-1 and IL-6 significantly decreased MTP mRNA levels in HepG2 cells. This decrease appeared soon after IL-1 administration (8 h) and at very low doses (0.1 ng/ml). MTP activity and protein levels of the large subunit of MTP also decreased modestly in HepG2 cells with prolonged cytokine treatment. IL-1 reduced the expression of an MTP promoter luciferase construct to a similar degree as seen with MTP mRNA, indicating that transcriptional regulation plays a major role in the decrease of MTP gene expression. Deletional analysis of the MTP promoter identified the region -121 to -88 bp upstream to the coding sequence as the site of the negative regulation by IL-1. This region contains an insulin response element (IRE), activating protein 1 (AP-1), hepatic nuclear factor 1 (HNF-1) and hepatic nuclear factor 4 (HNF-4) consensus sequences; mutations of the IRE and HNF-4 sites did not affect the response to IL-1. In contrast, mutating AP-1 or HNF-1 sites led to a marked decrease in basal expression and the loss of the IL-1 effect, suggesting that an intact AP-1 and/or HNF-1 regulatory element are crucial for the IL-1 regulation of MTP gene expression. However, prolonged incubation with IL-1 did not alter HepG2 apolipoprotein B secretion suggesting that MTP mRNA down-regulation does not contribute significantly to the cytokine-induced effects on lipid metabolism.—Navasa, M., D. A. Gordon, N. Hariharan, H. Jamil, J. K. Shigenaga, A. Moser, W. Fiers, A. Pollock, C. Grunfeld, and K. R. Feingold. **Regulation of microsomal triglyceride transfer protein mRNA expression by endotoxin and cytokines.** *J. Lipid Res.* 1998. 39: 1220–1230.

Supplementary key words tumor necrosis factor • interleukins • apolipoprotein B • hepatic nuclear factor-1 • activating protein-1

Infection, inflammation, and trauma frequently induce changes in lipid metabolism (1–3). Multiple cytokines, in-

duced during the host response, are capable of altering lipid metabolism, suggesting that the changes in serum lipid and lipoprotein levels are part of the acute phase response (1–3). Cytokines bind to hepatocyte receptors leading to transcriptional induction or repression of specific sets of acute-phase response genes (4).

The induction of acute phase proteins has been divided into two major groups (4, 5). Class 1 acute phase proteins are stimulated by interleukin-1 (IL-1) type cytokines and include C reactive protein, serum amyloid A, and α 1 acid glycoprotein. Class 2 acute phase proteins are stimulated by the IL-6 family of cytokines and include haptoglobin, fibrinogen, and α -1-antichymotrypsin. The IL-1-induced activation of NF-IL-6 and NF- κ B is thought to mediate the increase in acute phase protein gene transcription stimulated by IL-1 while activation of NF-IL-6 and the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway is thought to mediate the IL-6 family stimulation of acute phase protein gene expression. Much less is known about the transcriptional repressors of the negative acute-phase protein genes and there does not appear to be a universal inhibitor that accounts for the observed decreases in expression.

Microsomal triglyceride transfer protein (MTP), which catalyzes the transfer of triglyceride, cholesteryl ester and phosphatidylcholine between membranes and lipoproteins, is located within the lumen of microsomes isolated from the liver and intestine (6). MTP is a heterodimer with two subunits of apparent molecular mass 58,000 and 88,000 daltons. The small subunit has been identified as the multifunctional protein, protein disulfide isomerase (PDI).

Abbreviations: TNF, tumor necrosis factor; IL, interleukin; MTP, microsomal triglyceride transfer protein; LPS, endotoxin; IRE, insulin response element; AP, activating protein; HNF, hepatic nuclear factor; JAK, Janus kinase; STAT, signal transducer and activator of transcription; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MEM, minimum essential media; PC, phosphatidylcholine; PDI, protein disulfide isomerase; APO, apolipoprotein.

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The unique large subunit confers the catalytic property to the protein complex. It has been shown that patients with abetalipoproteinemia have defects in the sequences encoding the MTP gene that result in an absence of the large subunit, which in turn lead to a defect in the secretion of apolipoprotein B-containing lipoproteins (7). The central role played by MTP in lipoprotein assembly and secretion has been recently shown in studies demonstrating that the secretion of apolipoprotein B-containing lipoproteins can be induced in a non lipoprotein-producing cell line (HeLa cells) by expression of MTP (8).

Studies on the transcriptional regulation of human and hamster MTP genes have shown that the MTP promoters of human and hamster are organized similarly (9). MTP is primarily expressed in the liver and intestine with AP-1, HNF-1, and HNF-4 regulatory elements located between -120 and -90 bp from the transcription start site. This activation region showed no effect on transcription from the heterologous SV 40 minimal promoter, suggesting that this region is not an enhancer but an integral part of the MTP promoter (9). The promoter also has a modified sterol regulatory element (-175 to -164) and a negative insulin response element (-123 to -112). As a consequence, the human gene is positively regulated by cholesterol and negatively regulated by insulin (9, 10). Given the changes cytokines induce in lipid metabolism, it is possible that cytokines could regulate MTP transcription.

EXPERIMENTAL PROCEDURES

Animal procedures

Male Syrian hamsters (approximately 140–160 g) were purchased from Simonsen Laboratories (Gilroy, CA). The animals were maintained in a reverse light cycle room (3 a.m. to 3 p.m. dark, 3 p.m. to 3 a.m. light), and were provided with rodent chow and water ad libitum. Anesthesia with isoflurane was induced and the animals were injected intraperitoneally (ip) with LPS, TNF, IL-1, and TNF + IL-1 at the indicated doses in 0.5 ml 0.9% saline or with saline alone. Human TNF α with a specific activity of 5×10^7 U/mg was kindly provided by Genentech, Inc. (South San Francisco, CA). Recombinant human IL-1 β with a specific activity of 1×10^9 U/mg was generously provided by Immunex (Seattle, WA). The doses of TNF, IL-1, and TNF + IL-1 used in these experiments were based on previous studies demonstrating that these doses altered serum lipid and lipoprotein levels (11, 12). Subsequently, because LPS and cytokines may induce anorexia, food was withdrawn from both control and treated animals.

Isolation of RNA and Northern blotting

Poly A⁺ mRNA was isolated from hamster liver by a variation of the guanidinium thiocyanate method and Northern blots were hybridized with the human MTP cDNA probe and exposed to X-ray film for various durations to ensure that measurements were on linear portion of the curve (11). Bands were quantified by densitometry. Northern blots obtained from hamster liver were not adjusted for loading by using "housekeeping" genes. We and others have found that LPS and cytokines increase mRNA levels of actin in liver (11, 13). LPS also increases hepatic mRNA levels for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and cyclophilin (14). Therefore, the mRNA levels of actin, GAPDH, and cyclophilin, which are widely used for normalizing data, can-

not be used to study LPS-induced regulation of proteins in liver. However, the differing direction of the changes in mRNA levels for specific proteins after LPS and cytokines, the magnitude and consistency of the alterations, and the relatively small standard error of the mean make it very unlikely that the changes observed are due to unequal loading of mRNA. In the case of Northern blots obtained from HepG2 cells, mRNA levels of GAPDH were used to normalize MTP mRNA levels.

Cell culture and cytokine treatment

HepG2 cells were obtained from American Type Culture Collection and maintained in minimum essential medium (MEM, Mediatech, Inc., Herndon, VA) supplemented with 10% fetal bovine serum under standard culture conditions (5% CO₂, 37°C). Cells were seeded into 100-mm culture dishes and allowed to grow to 80% confluence. Immediately prior to the experiment, cells were washed with serum free MEM and the experimental medium (MEM + 0.1% BSA) containing TNF, IL-1, or IL-6 at the indicated concentrations was added. In separate experiments cells were also treated with MEM containing 5% or 8% human serum albumin. Cells were incubated at 37°C for the indicated time. RNA purification and Northern blotting were performed according to previously described methods (15).

MTP activity assay

Cell homogenates were treated with deoxycholate to release MTP from the microsomal fraction as described previously (16). Soluble protein fractions were isolated from the tissue homogenates by ultracentrifugation, dialyzed in 15 mM Tris, pH 7.4, 40 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 0.02% NaN₃ overnight, and assayed for MTP activity as described previously (16). MTP activity was determined by the transfer of radiolabeled triolein from donor small unilamellar vesicles (40 nmol of egg phosphatidylcholine, 0.08 nmol of [¹⁴C]triglyceride, 2 nmol of cardiolipin) to acceptor small unilamellar vesicles (240 nmol of egg PC, 0.48 nmol of TG) at 37°C for 1 h.

MTP protein mass determination

MTP large subunit and PDI (small subunit) mass were determined by Western blot analysis (16). Cell homogenates (100 μ g of protein) and purified bovine MTP standard were fractionated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and probed with anti-bovine MTP large subunit polyclonal antibody or PDI polyclonal antibody. Horseradish peroxidase-conjugated IgG was used as the secondary antibody. Bands corresponding to the MTP large subunit or PDI were visualized by a colorimetric reaction and quantitated directly by densitometry.

Apolipoprotein B secretion

Cells were treated with medium containing 1 ng/ml or 3 ng/ml IL-1 for 7 days at 37°C. Cells were then incubated in fresh medium with IL-1 for an additional 24 h. Medium was collected and apolipoprotein B (apoB) levels were quantified by an ELISA as described previously (10). Briefly, human apoB was quantitated by a sandwich ELISA using a monoclonal anti-human apoB antibody, goat anti-human apoB polyclonal antibody, and rabbit anti-goat IgG conjugated to alkaline phosphatase as primary, secondary, and tertiary antibodies, respectively. The amount of apoB was quantitated using the *p*-nitrophenyl-phosphate colorimetric reaction. Samples of unknown concentration were measured in triplicate against a standard curve of purified human LDL. Concentrations of standard ranged from 1.25 to 40.0 ng LDL protein in a 2-fold dilution series. Within this concentration range, the absorbance of the assay was linear. Unknowns were diluted to concentrations within the linear range.

Promoter-luciferase reporter gene constructs

Human and hamster MTP promoter fragments, containing varying lengths of 5'-flanking sequences and the non-translated region of exon 1, were generated by polymerase chain reaction as described previously (9). Briefly, the polymerase chain reaction fragments were tailored to contain a Kpn I site at the 5' end and a BamHI site at the 3' end. After ethanol precipitation, fragments were digested with these enzymes, gel purified through Gene Clean (Bio101, La Jolla, CA), and cloned 5' to the promoterless luciferase reporter gene into KpnI and BgHI sites in the pGL2 basic vector (Promega, Madison, WI). In addition, normal and mutated regions of the human promoter corresponding to -129 bp to -76 bp were synthesized with KpnI restriction enzyme recognition ends (GenoSys, Woodlands, TX). This oligonucleotide was cloned into the KpnI site present 5' to the MTP -69 bp basal promoter, the -30 bp TATA box minus promoter, and the SV40 early minimal promoter in a pGL2Pro vector (Promega, Madison, WI). All promoter deletion constructs and site-specific mutant constructs were confirmed by sequencing in an automater sequencer (ABI, Foster City, CA). The RSV β -Gal reporter gene was used for normalizing the transient transfection. RSV-B gal expression was not altered by IL-1. In contrast, the expression of both CMV-B gal and SV40-B gal was increased 1.5- to 2.5-fold by IL-1 treatment.

Transient transfection

HepG2 cells were grown overnight in 35-mm plates and washed twice with serum-free medium. DNA-lipofectin complex, containing 1.5 μ g/ml MTP promoter-luciferase vector, 0.75 μ g/ml RSV β -GAL vector, and 5 μ g/ml lipofectin (Life Technologies, Gaithersburg, MD), was allowed to form at room temperature for 15 min. The cells were overlaid with the DNA-lipofectin complex and incubated for 4-6 h at 37°C. After washing the cells with serum-free medium, fresh growth medium containing 10% FBS was added. After 48 h of incubation at 37°C cells were washed with serum free MEM and the experimental media with or without IL-1 at the indicated concentrations was added. After 24 h cells were harvested, washed twice with ice-cold phosphate-buffered saline (PBS), treated in lysis buffer (Promega, Madison, WI), and aliquots of the lysates were assayed for luciferase and β -galactosidase enzyme activities. The luciferase enzyme activity was determined according to the manufacturer's instructions (Promega, Madison, WI), and was quantified in a Monolight 2010 luminometer (Analytical Luminescence Laboratory, Ann Arbor, MI). The β -galactosidase activity of cell lysates was determined as described (17) and values were used to normalize variability in the efficiency of transfection.

Statistics

Results are expressed as mean \pm SEM. Comparisons among groups were performed by the Student's *t*-test for unpaired data

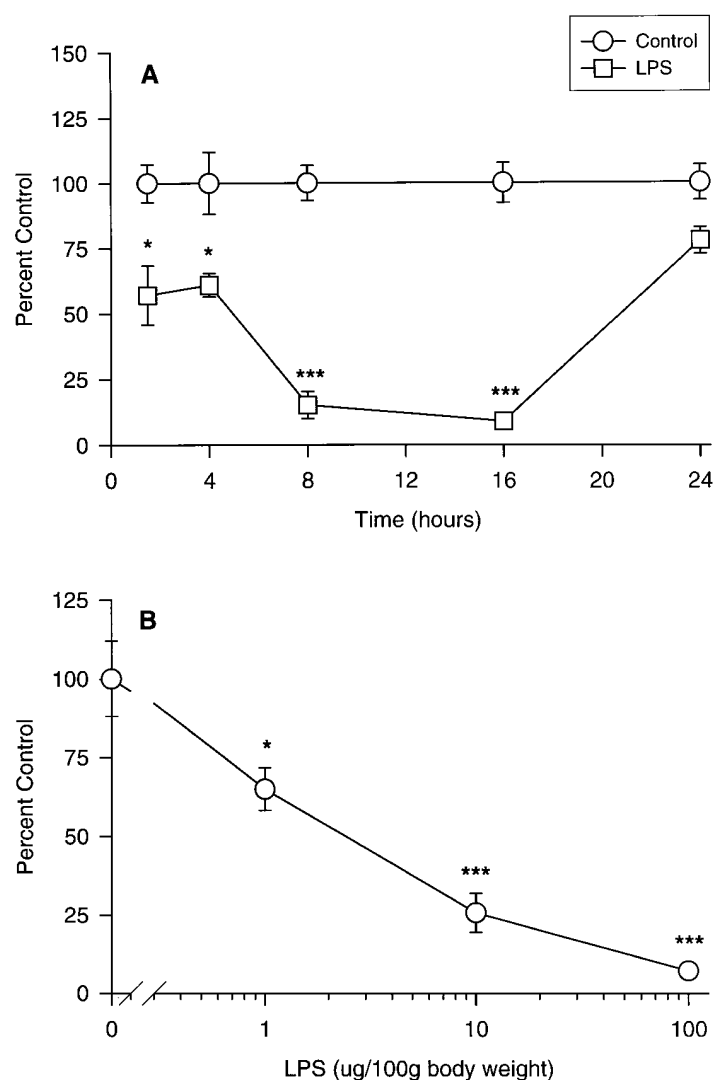


Fig. 1. Time course and effect of different doses of LPS on MTP mRNA levels in Syrian hamster liver. Animals were injected ip with 100 μ g/100 g bw of LPS or saline (controls). At 4, 8, 16, and 24 h, animals were killed, and livers were obtained and frozen immediately until RNA isolation (A). Animals were injected ip either with saline (control) or LPS at the doses indicated; 16 h later, animals were killed and livers were obtained and frozen immediately until RNA isolation (B). Hepatic MTP mRNA levels were determined as described in Material and Methods. Data are presented as percent change vs. controls (mean \pm SEM). $n = 10$ for time course and $n = 5$ for dose response curve, * $P < 0.05$, *** $P < 0.001$.

with Bonferroni correction for multiple comparisons. Significance was established at P value less than 0.05.

RESULTS

Effect of LPS, TNF, and IL-1 on hepatic MTP mRNA levels in Syrian hamsters

Hamsters were treated with a single dose of LPS (100 μ g/100 g bw) and the time course of the effect on hepatic MTP mRNA levels is presented in Fig. 1A. LPS rapidly decreased hepatic MTP mRNA levels (40% decrease by 2 h). By 8 h, LPS had decreased MTP mRNA by 80%, with a maximum effect at 16 h (90% decrease). MTP mRNA levels returned to control values by 24 h.

We chose the 16 h point (maximum effect) after LPS administration to characterize the dose-response curve of LPS on hepatic MTP mRNA levels. LPS induced a dose-dependent decrease in hepatic MTP mRNA levels (Fig. 1B). The maximum effect was reached at 100 μ g LPS/100 g bw (90% decrease). However, hepatic MTP mRNA were significantly decreased by lower doses of LPS, and only 1.2 μ g/100 g bw was required to produce a 50% decrease in hepatic MTP mRNA levels in hamsters.

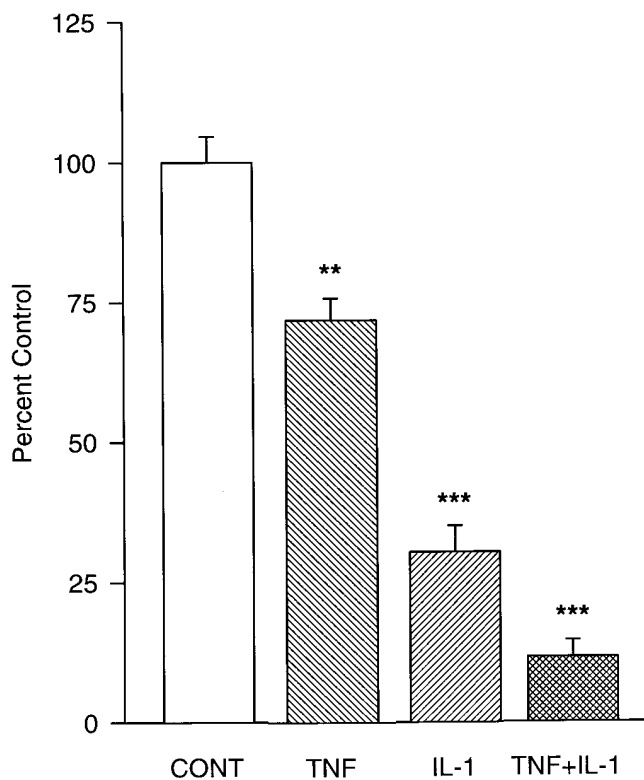


Fig. 2. Effect of TNF and IL-1 on MTP mRNA levels. Animals were injected ip either with saline (controls), TNF (17 mg/100 g bw), IL-1 (0.5 μ g/100 g bw), or TNF + IL-1 (17 mg TNF/100 g bw + 0.5 mg IL-1/100 g bw). Eight hours later, animals were killed and livers were obtained and frozen immediately until RNA isolation. Hepatic MTP mRNA levels were determined as described in Material and Methods. Data are presented as percent change vs. controls (mean \pm SEM); $n = 5$; ** $P < 0.01$, *** $P < 0.001$ vs. other cytokine groups.

The effect of TNF (17 mg/100 g bw), IL-1 (0.5 μ g/100 g bw), and TNF + IL-1 (17 mg TNF/100 g bw + 0.5 μ g IL-1/100 g bw) on hepatic mRNA levels is shown in Fig. 2. TNF induced a moderate but significant decrease in MTP mRNA levels (-25%); IL-1 administration caused a 60% reduction in MTP mRNA. Furthermore, there was an additive effect with both TNF and IL-1, as administration of the combination of cytokines induced a further significant decrease in MTP mRNA levels (-85%).

Effect of TNF, IL-1, and IL-6 on MTP mRNA in HepG2 cells.

The effects of TNF, IL-1, and IL-6 on MTP mRNA levels in HepG2 cells at 24 h are shown in Fig. 3. TNF and IL-1 treatment caused a decrease in MTP mRNA levels in HepG2 cell (Fig. 3), which paralleled that observed in hamster liver (Fig. 2). TNF (100 ng/ml) caused a moderate, but nonsignificant decrease in MTP mRNA levels, while IL-1 (100 ng/ml) caused a significant 60% decrease. The decrease in MTP mRNA levels after IL-6 (100 ng/ml) administration was approximately 50%.

The time course of the effect of IL-1 on MTP mRNA levels in HepG2 cells is shown in Fig. 4. IL-1 caused a significant reduction in MTP mRNA levels at 8 h (40% decrease) and reached a 60% decrease by 24 h. This effect was very prolonged and persisted for more than 60 h with continuous treatment with cytokine. The reduction in MTP mRNA was not due to IL-1-induced toxicity. Cells remained via-

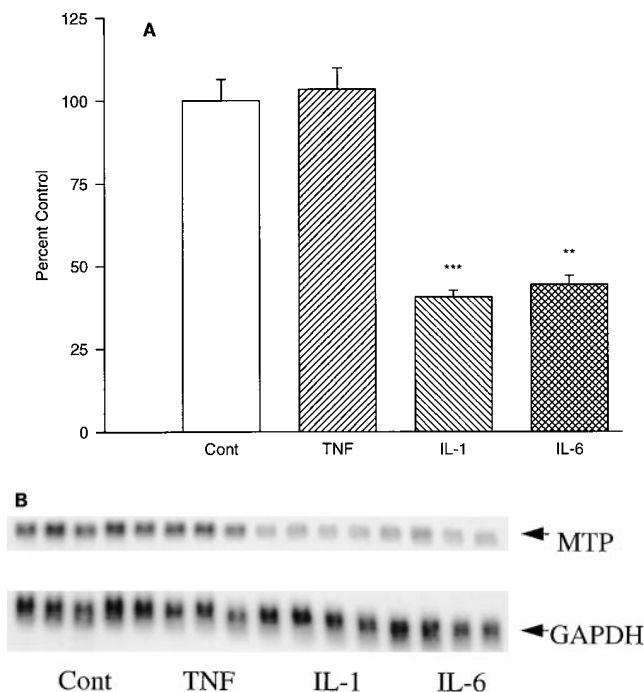


Fig. 3. Effects of TNF, IL-1, and IL-6 on MTP mRNA levels in HepG2 Cells. Cells were incubated at 37°C for 24 h in media alone (controls) or media plus TNF (100 ng/ml), IL-1 (100 ng/ml), or IL-6 (100 ng/ml). Thereafter, cells were lysed and MTP mRNA levels were determined as described in Material and Methods. Data are normalized to GAPDH mRNA levels and presented as percent change vs. controls (mean \pm SEM); $n = 6$; ** $P < 0.05$, *** $P < 0.001$.

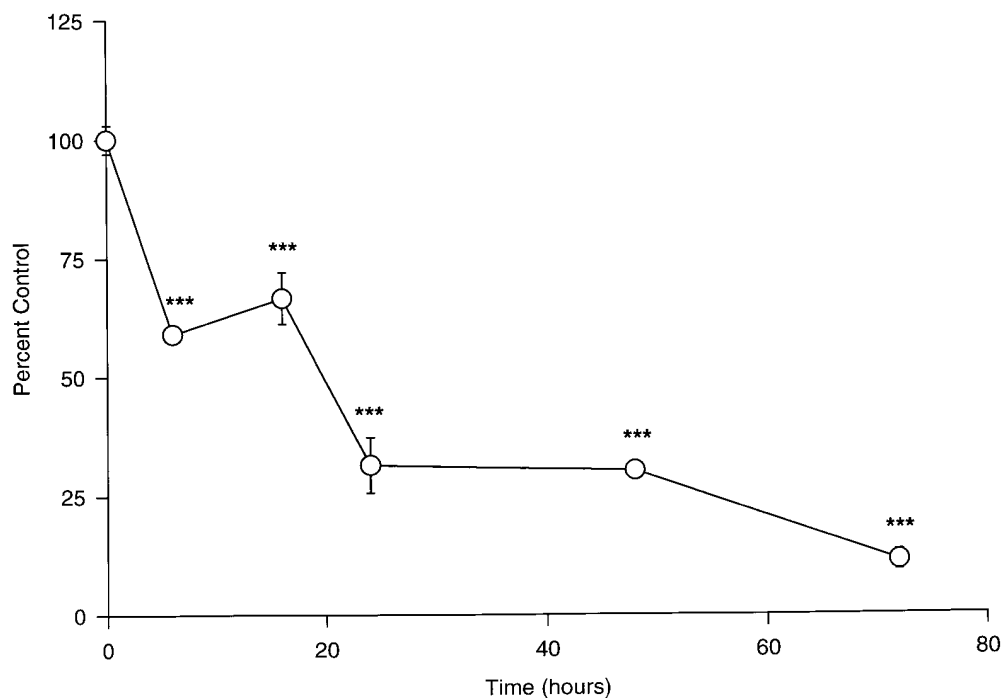


Fig. 4. IL-1 time course in HepG2 cells. Cells were incubated at 37°C in media alone (controls) or with IL-1 (100 ng/ml) for 8, 16, 24, 48, and 72 h. Thereafter, cells were lysed and MTP mRNA levels were determined as described in Material and Methods. Data are normalized to GAPDH mRNA levels and presented as percent change vs. controls (mean ± SEM); n = 6; ****P* < 0.001.

ble, using the trypan blue exclusion test. During these experiments mRNA levels of the positive acute-phase protein, serum amyloid A (18), increased after IL-1 administration (13.5-fold increase), further indicating that the viability of the cells was not affected. Based on these data we chose the 24 h point to study the dose-response curve of IL-1, which is shown in Fig. 5A. IL-1 was able to decrease MTP mRNA levels at very low doses (0.1 ng/ml) and the dose required to produce a 50% decrease in MTP mRNA levels was only 0.11 ng/ml. Between 1.0 ng/ml and 100 ng/ml of IL-1 there was a maximal reduction in MTP mRNA levels of 60%.

The IL-6 dose-response curve at 24 h is shown in Fig. 5B. In contrast to IL-1, the minimum dose of IL-6 required to cause a significant reduction in MTP mRNA levels in HepG2 cells was 100 times greater (10 ng/ml). A 50% decrease in MTP mRNA levels was the maximum effect seen and this decrease required high concentrations of IL-6 (100 ng/ml).

Effect of cytokines on MTP activity and protein levels

MTP protein has a long half life (10) and therefore, to determine whether the cytokine-induced decrease in mRNA levels affects activity, we carried out chronic experiments. We initially determined the effect of incubation with IL-1 or IL-6 for 7 days on MTP activity. As shown in Fig. 6A, both IL-1 and IL-6 decreased MTP activity by 30% in HepG2 cells. Moreover, as shown in Fig. 6B, both IL-1 and IL-6 decreased MTP large subunit protein mass by 50%. In contrast, neither IL-1 nor IL-6 decreased MTP small subunit mass (PDI). Additionally, total protein was not af-

ected by either IL-1 or IL-6 treatment (controls, 1.99 ± 0.19; IL-1, 2.29 ± 0.24; IL-6, 2.12 ± 0.32 mg).

Effect of IL-1 on apolipoprotein B secretion

We next determined whether the modest decrease in MTP activity induced by prolonged incubation with IL-1 would affect apoB secretion. ApoB secretion into the media was not affected by incubation for 7 days with either 1 ng/ml or 3 ng/ml IL-1 (control, 0.385 ± 0.232; 1 ng IL-1, 0.380 ± 0.008; 3 ng IL-1, 0.398 ± 0.013 ng total LDL/μg total protein). Thus, while prolonged incubation with IL-1 has modest effects on MTP activity, these changes do not alter apoB secretion.

Effect of IL-1 on transcriptional regulation of the MTP gene

It has previously been shown that the -612 bp upstream from the coding sequence contains regulatory elements for expression of the MTP gene (9). We transfected the 612 bp and other truncated constructs coupled to the coding sequence for luciferase to determine the location of the elements required for the decrease in gene expression induced by IL-1. For these experiments, luciferase values were normalized to β-galactosidase activity from a cotransfected vector without the MTP promoter. Data are expressed in relation to basal expression of the 612 bp promoter construct.

Using the 612 bp human MTP promoter construct, we found strong basal expression that was significantly reduced by 60% by IL-1 (Fig. 7), paralleling the effects on MTP mRNA levels in HepG2 cells and hamster liver.

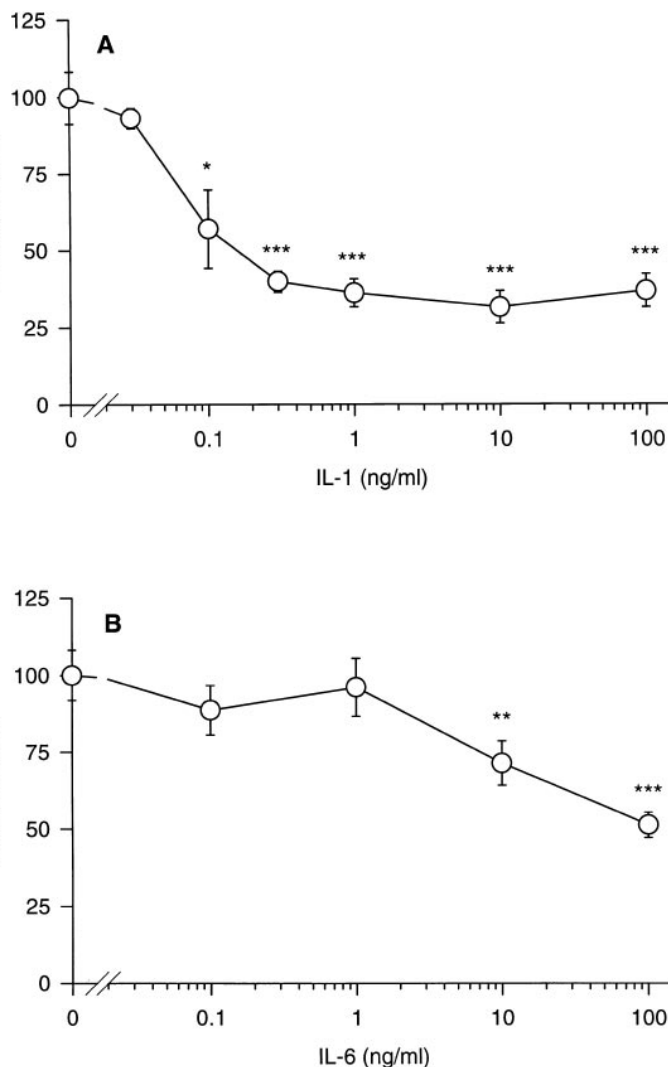


Fig. 5. IL-1 and IL-6 dose-response curve in HepG2 cells. Cells were incubated at 37°C for 24 h in media alone (controls) or in media containing the indicated concentrations of IL-1 (A) or IL-6 (B) (from 0.05 to 100 ng/ml). Thereafter, cells were lysed and MTP mRNA levels were determined as described in Material and Methods. Data are normalized to GAPDH and presented as percent change vs. controls (mean \pm SEM); n = 6; * P < 0.05, ** P < 0.01, *** P < 0.001.

There was an increase in the basal luciferase activity in the deletion mutants of the promoter starting at -379 , -239 , -142 and -121 bp in accordance with previous results that showed an increase in the promoter activity after deletion of sequences 5' to -239 bp (9). In each of these deletion mutants of the MTP promoter, IL-1 caused a significant reduction in luciferase activity. As seen previously (9), further deletions from -121 to -88 bp or even smaller constructs reduced the promoter activity in HepG2 cells by over 80% as compared to the -612 bp construct (Fig. 8). Constructs with deletions starting at less than -121 bp were also associated with the loss of the down-regulation of the promoter activity by IL-1 (Fig. 8), suggesting that MTP promoter contains a sequence at -121 to -88 bp that is negatively regulated by IL-1 (this area contains an insulin response element, an AP-1, an HNF-1, and an HNF-4 site).

As described in detail previously, the human and hamster MTP gene are similar in structure (9). We therefore next evaluated the effect of IL-1 on hamster MTP constructs (Fig. 8). Similar to our observations using human MTP constructs, IL-1 resulted in a significant decrease in expression in constructs containing the major regulatory

elements (-580 , -123 , -81 , and -58 constructs) of the hamster promoter. In -81 and -58 constructs, that did not contain the insulin response element, AP-1 site, HNF1, or HNF4 regulatory elements, basal expression was decreased but still substantial. Moreover, IL-1 did not alter MTP gene expression in these smaller hamster constructs suggesting that regulatory elements between -81 and -123 are crucial for IL-1 gene repression.

Mutational analysis of the MTP region negatively regulated by IL-1

To more definitively determine the role of the regulatory elements between -121 and -88 (insulin response element, AP-1, HNF1, HNF4) we next determined the effect of mutations within the individual putative regulatory elements in the -121 to -88 bp MTP region in the human gene. Random nucleotide substitutions were introduced in the four putative elements, generally following the A to C and G to T rule. The mutant oligonucleotides with changes in the IRE (M1), AP-1 (M2), HNF-1 (M3), or HNF-4 (M4) sites were cloned 5' to the -69 bp human minimal promoter and the effect of the mutations on transactivation in basal conditions and after IL-1

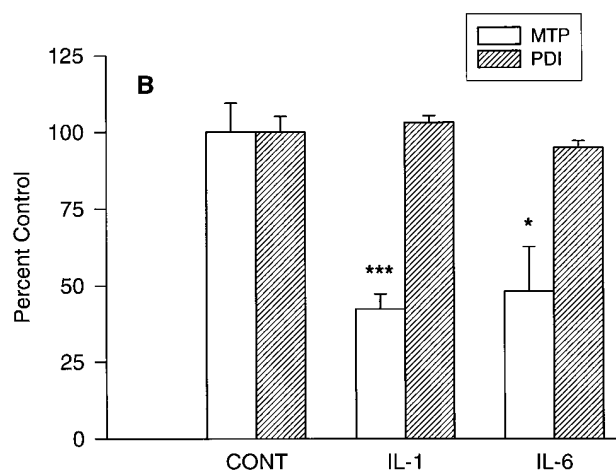
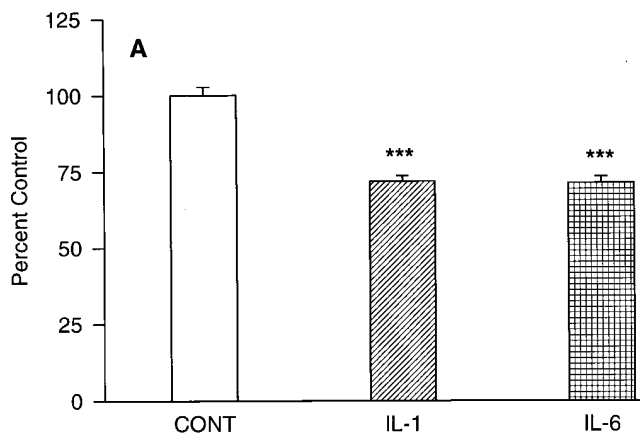


Fig. 6. Effect of IL-1 and IL-6 on MTP activity and large subunit MTP and PDI protein mass in HepG2 cells. Cells were incubated at 37°C for 7 days in media alone (controls) or media plus IL-1 (100 ng/ml), or IL-6 (100 ng/ml). Thereafter, cells were scraped and harvested in PBS and MTP activity (A) and protein mass for MTP large subunit and PDI (B) were determined as described in Materials and Methods. Data are presented as percent change vs. controls (mean \pm SEM); $n = 5$; * $P < 0.05$, *** $P < 0.001$. Control MTP activity was 19.7 ± 0.7 percent triglyceride transferred per h per mg of cell protein.

administration was analyzed by transient transfection in HepG2 cells. As previously described and shown in Fig. 9, the promoter activity of the IRE mutant (M1) was higher than the wild type while the AP-1 mutant (M2) and HNF-1 mutant (M3) had very low basal activity (9). The expression of the HNF-4 mutant (M4) was reduced slightly as compared to the wild type. Of note is that IL-1 administration caused the same percentage decrease in luciferase activity in the mutated IRE (M1) and HNF-4 (M4) as observed in the wild type or in the -121 or -612 construct promoters, indicating that mutation of these two elements (IRE and HNF-4) does not affect the response to IL-1. In contrast, mutating the AP-1 (M2) or HNF-1 (M3), in addition to the loss of basal expression, leads to the absence of an IL-1 effect, suggesting that AP-1 and/or HNF-

1 are also the key sites for IL-1 repression of MTP gene expression.

Effect of albumin on MTP mRNA levels

The acute phase response is known to decrease HNF-1 expression (19). Similarly, increasing the albumin concentration in medium of HepG2 has also been shown to decrease HNF-1 expression (20). We therefore next determined the effect of incubating HepG2 cells in 5% or 8% human serum albumin on MTP mRNA levels. As shown in Fig. 10, increased concentration of albumin in the medium reduced MTP mRNA levels, suggesting a role for HNF-1 in regulating MTP mRNA levels.

DISCUSSION

The hepatic response to infection, inflammation, and trauma results in a marked alteration in the synthesis of a large number of hepatic proteins (5, 21) including profound alterations in lipid metabolism and serum lipoprotein levels (1-3). In Syrian hamsters both serum triglyceride and cholesterol levels increase after LPS or cytokine treatment due to increases in VLDL and LDL, while HDL cholesterol levels decrease (11, 12). These changes in lipid and lipoprotein metabolism can be considered part of the acute phase response and studies have shown that these changes are beneficial to the host (1, 2). Our laboratory and others have shown that the proteins involved in lipid metabolism may be increased or decreased during the acute phase response, due to changes in mRNA levels. For example, hepatic mRNA levels for HMG-CoA reductase (11), the rate-limiting enzyme in cholesterol synthesis, and apolipoprotein J (22) increase, while mRNA levels for cholesterol 7 α -hydroxylase (23), the rate-limiting enzyme in bile acid synthesis, cholesteryl ester transfer protein (CETP) (24), lecithin:cholesterol acyltransferase (LCAT) (25), and apolipoprotein E (11) decrease during the acute phase response. Little is known regarding the mechanisms by which cytokines regulate the expression of these genes except for recent studies involving apolipoprotein E by Berg, Calnek, and Grinnell (26) who have demonstrated that treatment of HepG2 cells with IL-1 or IL-6 results in the phosphorylation of BEF-1, a member of the NF-1 family of nuclear factors, to an isoform designated B1. An increase in the B1 isoform was associated with a concomitant and proportional decrease in the levels of apolipoprotein E mRNA (26), but the precise mechanism for this inhibition remains to be defined. Both BEF-1 and B1 bind with equal affinity to the apolipoprotein E regulatory regions; it is possible that B1 cannot stimulate transcription.

The present study indicates that MTP is a negative acute phase protein: LPS, IL-1, and TNF administration decrease hepatic MTP large subunit mRNA levels in vivo in Syrian hamsters and IL-1 and IL-6 decrease MTP large subunit mRNA levels in HepG2 cells. Notably, IL-1 was able to decrease MTP mRNA levels at very low concentrations and the IL-1 dose required to produce a 50% de-

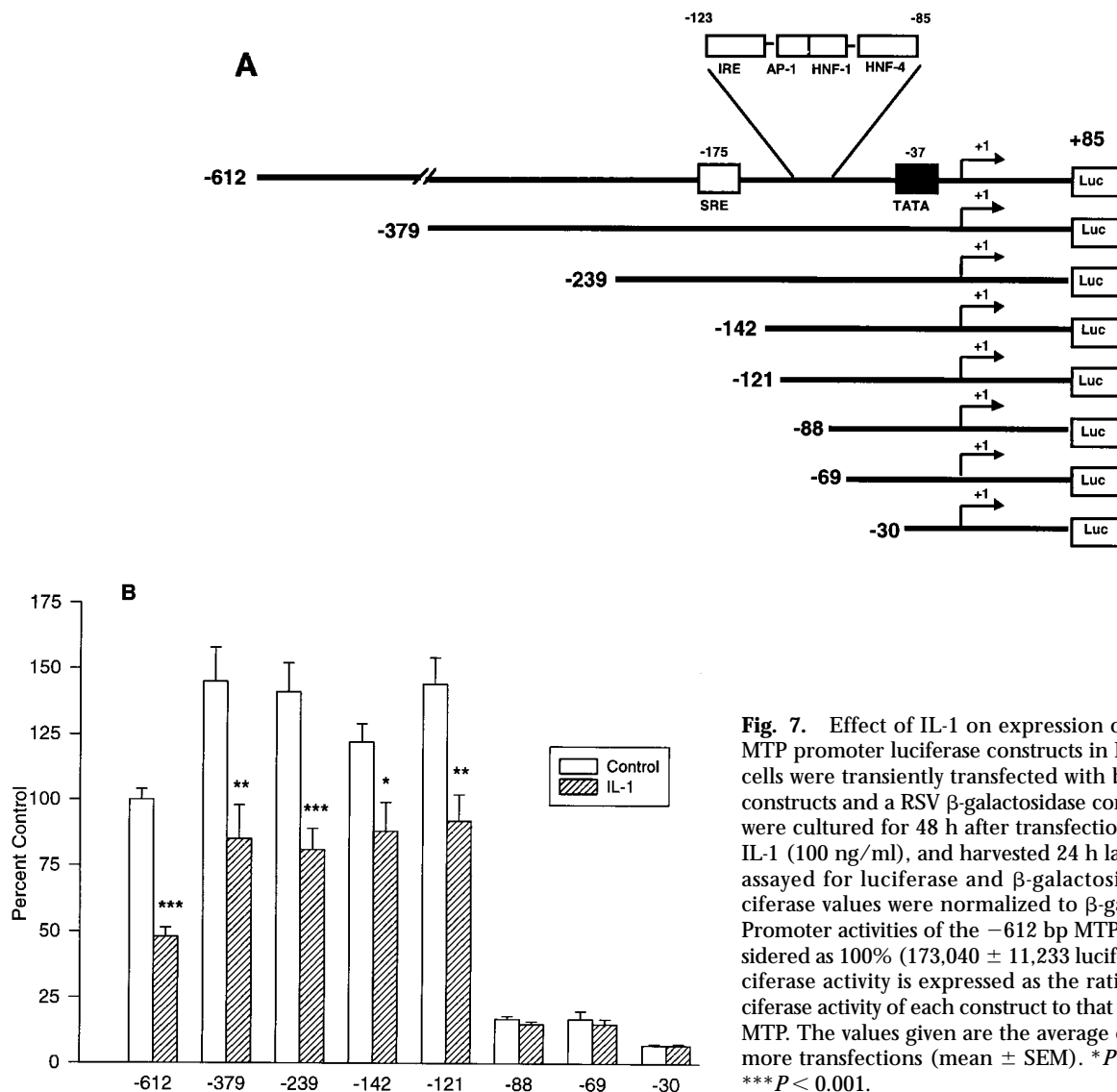


Fig. 7. Effect of IL-1 on expression of truncated human MTP promoter luciferase constructs in HepG2 cells. HepG2 cells were transiently transfected with both MTP-luciferase constructs and a RSV β -galactosidase control construct. Cells were cultured for 48 h after transfection, then treated with IL-1 (100 ng/ml), and harvested 24 h later. Cell lysates were assayed for luciferase and β -galactosidase activities. Luciferase values were normalized to β -galactosidase activity. Promoter activities of the -612 bp MTP construct were considered as 100% ($173,040 \pm 11,233$ luciferase/ β gal). The luciferase activity is expressed as the ratio of normalized luciferase activity of each construct to that of the basal -612 bp MTP. The values given are the average of data from four or more transfections (mean \pm SEM). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

crease was only 0.11 ng/ml. Because of the long half life of MTP protein, only prolonged incubation with either IL-1 or IL-6 resulted in a decrease in both the protein mass of the large subunit of MTP and MTP activity in HepG2 cells. In contrast, PDI protein mass (small subunit) was not altered by either IL-1 or IL-6.

Further, we have shown that this decrease in MTP mRNA is likely due to a decrease in transcription as our studies demonstrate that IL-1 decreases the expression of an MTP promoter (612 bp) luciferase construct. Previous studies have characterized the MTP promoter (9). In the present study, by using MTP promoter luciferase constructs of various lengths, we were able to localize the inhibitory effect of IL-1 on gene transcription to a section of the promoter between -121 to -88 bp from the transcription start site. This region of the promoter contains an insulin response element, an AP-1 site, an HNF-1 site, and an HNF-4 site (9). Specific mutations in these response elements demonstrate that neither the insulin response element nor the HNF-4 site were required for IL-1 inhibition of transcrip-

tion. Mutations in either the AP-1 or the HNF-1 site resulted in a marked decrease in basal transcription and an absence of an IL-1 effect. Whether the absence of inhibition by IL-1 was due to the low basal transcription or indicates a requirement for these regulatory elements cannot be clearly differentiated by these studies. Nevertheless, the present study localizes the inhibitory effect of IL-1 to approximately 12 bp of the MTP promoter (-99 to -110).

Studies by other investigators have shown that both AP-1 and HNF-1 expression and/or activation are altered during the acute phase response (19, 27-30). The acute phase response results in a decrease in HNF-1 mRNA levels in the liver within 30 min after injury and this decrease is sustained for over 24 h (19). Similarly, increasing the oncotic pressure of the medium has also been shown to decrease HNF-1 expression (20). In the present studies we demonstrate that the addition of 5% or 8% human serum albumin to the medium decreases HepG2 MTP mRNA. One can therefore postulate that a decrease in HNF-1 syn-

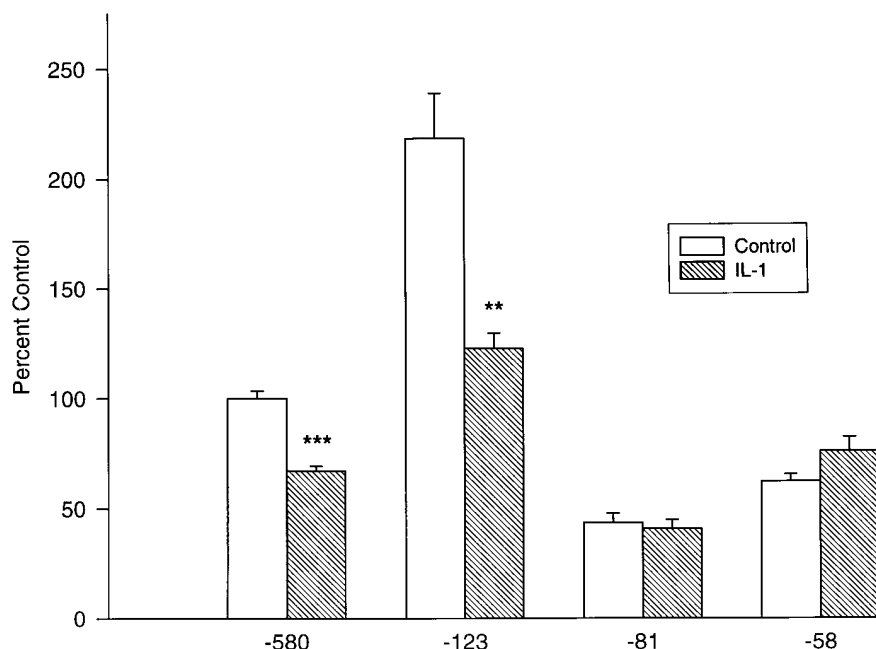


Fig. 8. Effect of IL-1 on expression of truncated hamster MTP promoter luciferase constructs in HepG2 cells. Cells were transiently transfected with both MTP-luciferase constructs and a RSV β -galactosidase control construct. Cells were cultured for 48 h after transfection then treated with IL-1 (100 ng/ml) for 24 h. Cell lysates were assayed for luciferase and β -galactosidase activities. Luciferase values were normalized to β -galactosidase activity. Promoter activities of the -580 bp MTP construct were considered as 100% (7,835 \pm 434 luciferase/ β gal). The luciferase activity is expressed as the ratio of normalized luciferase activity of each construct to that of the basal -580 bp MTP. The values given are the average of data from six transfections (mean \pm SEM). ** P < 0.01, *** P < 0.001.

A	IRE	AP-1	HNF-1	HNF-4
WT	-129AACTGCAGCCCACCTACG	T TTAATC	ATTAAT	A GTGAGCCCTTCAG TGAACCTTAG-76
M1	-129AACTGACTAAACAAGCAT	T TTAATC	ATTAAT	A GTGAGCCCTTCAG TGAACCTTAG-76
M2	-129AACTGCAGCCCACCTACG	T <u>GACCGA</u>	ATTAAT	A GTGAGCCCTTCAG TGAACCTTAG-76
M3	-129AACTGCAGCCCACCTACG	T TTAATC	<u>CGGACG</u>	A GTGAGCCCTTCAG TGAACCTTAG-76
M4	-129AACTGCAGCCCACCTACG	T TTAATC	ATTAAT	A <u>GTGCTAAAGGACG</u> TGAACCTTAG-76

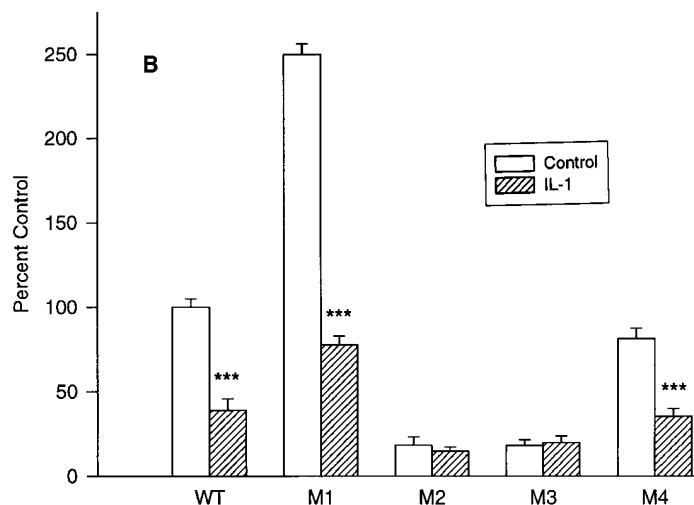


Fig. 9. Effect of IL-1 on expression of MTP promoter response element mutant constructs in HepG2 cells. Cells were transiently transfected with both MTP-luciferase constructs and an RSV β -galactosidase control construct. Cells were cultured for 48 h after transfection and treated with IL-1 (100 ng/ml) for 24 h. Cell lysates were assayed for luciferase and β -galactosidase activities. Luciferase values were normalized to β -galactosidase activity. Promoter activities of the wild type (WT) MTP construct were considered as 100% (23,473 \pm 2,227 luciferase/ β gal). The luciferase activity is expressed as the ratio of normalized luciferase activity of each construct to that of the basal WT MTP construct. The values given are the average of data from six transfections (mean \pm SEM). *** P < 0.001. M1, insulin response element mutation; M2, AP-1 element mutation; M3, HNF-1 element mutation; and M4, HNF-4 element mutation.

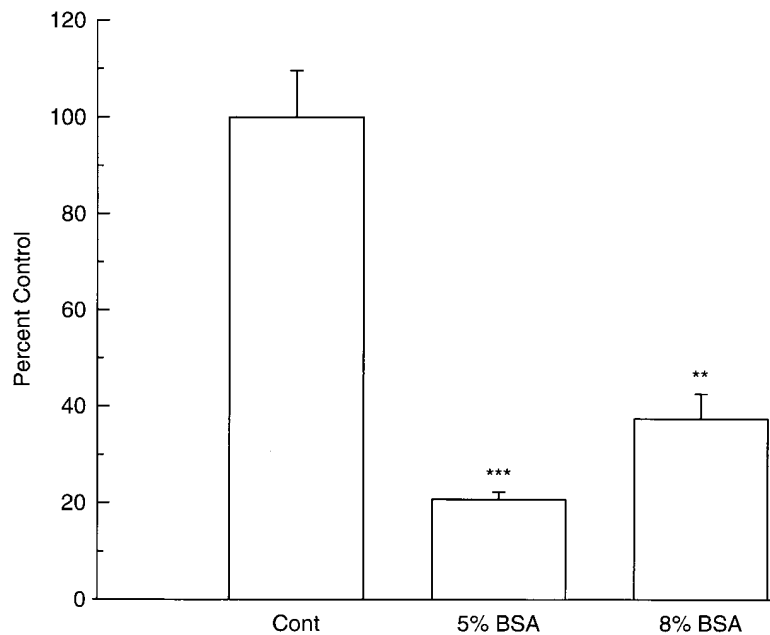


Fig. 10. Effect of albumin on MTP mRNA levels in HepG2 cells. Cells were incubated at 37°C for 24 h in media alone (controls) or media plus 5% or 8% human serum albumin. Thereafter, cells were lysed and MTP mRNA levels were determined as described in Materials and Methods. Data are normalized to G-3PD mRNA levels and presented as percent change versus controls (mean ± SEM). n = 4.

thesis after LPS or cytokine administration could result in an inhibition of MTP mRNA transcription. In contrast, LPS and cytokines increase c-jun, Jun B, and c-fos mRNA levels, increasing AP-1 binding activity and the transcription of an AP-1 reporter gene (27–30). It is possible that because of the close proximity of the AP-1 and HNF-1 sites that an increase in the binding of AP-1 inhibits HNF-1 binding and thereby decreases gene transcription. This type of competitive inhibition has been previously shown to account for the decrease in transferrin expression induced by the non-productive binding of PPAR-RXR to the transferrin promoter at a site which inhibited the binding of HNF-4 (31). It is, of course, also possible that another unidentified IL-1 induced factor binds to the AP-1/HNF-1 region and thereby inhibits MTP gene transcription. The relative contributions of these potential mechanisms to decreasing MTP mRNA transcription during the acute phase remain to be elucidated.

While endotoxin and cytokines rapidly reduced MTP mRNA levels, their effect on MTP activity and protein levels was modest and required a prolonged period of time. Moreover, conditions that reduced MTP activity by 30% had no effect on apoB secretion. These observations indicate that the changes in MTP mRNA that occur do not contribute to the alterations in lipid metabolism seen during the acute phase response. It is likely that inhibition of MTP mRNA during the acute phase serves a purpose other than to regulate lipid metabolism. The mRNA levels of other proteins, particularly those with long half lives such as albumin, also decrease in the liver during the acute phase response (21, 32). It is pertinent that both MTP and albumin transcription are regulated by HNF-1 (9, 20) which decreases during the acute phase response (19). Decreases in HNF-1 and/or other transcription factors may allow for coordinate decreases in the synthesis of a number of hepatic proteins that are not acutely required, thereby allowing the cell

machinery to more effectively translate mRNA for the synthesis of the positive acute phase proteins required for host defense. \square

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