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Interleukin 6 regulates metallothionein gene expression and zinc metabolism in hepatocyte monolayer cultures

(cytokines/trace elements/liver/cytoprotection)

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ABSTRACT **Attention has focused on the cytokine interleukin 6 (IL-6) as a major mediator of acute-phase protein synthesis in hepatocytes in response to infection and tissue injury. We have evaluated the effects of IL-6 and IL-1α as well as extracellular zinc and glucocorticoid hormone on metallothionein gene expression and cellular zinc accumulation in rat hepatocyte monolayer cultures. Further, we have evaluated the teleological basis for cytokine mediation by examining cytoprotection from CCl_4-induced damage. Incubation of hepatocytes with IL-6 led to concentration-dependent and time-dependent increases in metallothionein-1 and -2 mRNA and metallothionein protein. The level of each was increased within 3 hr after the addition of IL-6 at 10 ng/ml (10 hepatocyte-stimulating factor units/ml). Maximal increases in metallothionein mRNA and metallothionein protein were achieved after 12 hr and 36 hr, respectively. In contrast, IL-1α concentrations as high as 20 ng/ml (1000 lymphocyte-activating factor units/ml) had no effect. Concomitant with the up-regulation of metallothionein gene expression, IL-6 also increased cellular zinc. Responses to IL-6 required the synthetic glucocorticoid hormone dexamethasone and were optimized by increased extracellular zinc. In addition, IL-6 with dexamethasone, dexamethasone alone, and increased extracellular zinc each reversed, in decreasing potency, the deleterious effects of CCl_4 on hepatocyte viability as measured by cell protein and lactate dehydrogenase activity of the medium. Thus, IL-6 is a major cytokine mediator of metallothionein gene expression and zinc metabolism in hepatocytes and provides cytoprotection from CCl_4-induced hepatotoxicity via a mode consistent with dependence upon increased cellular metallothionein synthesis and zinc accumulation.**

Tissue injury, stress, and infection cause the release of the cytokine interleukin 1 (IL-1) from macrophages, monocytes, and other cell types. Once released, IL-1 triggers the up-regulation of a broad spectrum of systemic acute-phase responses involved in host defense (1). IL-1α administered to rats induces tissue-specific synthesis of the metal-binding protein metallothionein (MT) in a fashion similar to other acute-phase proteins (2, 3).

MT is intimately involved in the metabolism of zinc (4). Not only does MT bind zinc but MT expression is transcriptionally regulated by dietary zinc. In addition, induction of MT synthesis by a variety of hormones, including glucocorticoids, epinephrine, and glucagon, as well as IL-1, results in an increased transfer of zinc from plasma to the liver (4, 5). This has been demonstrated in kinetic experiments using intact rats following metallothionein induction by dibutyryl cAMP (6). MT may serve to provide zinc to metalloenzymes and to "zinc finger" motifs of DNA-binding transcription factors or may act to buffer intracellular zinc concentrations for these or a variety of other functions (2, 7). Also, MT may play a cytoprotective role as a radical scavenger (8) or as a donor of zinc for stabilization of membranes (9).

The mechanism by which IL-1 regulates MT gene expression and zinc metabolism in hepatocytes is not clear. A direct effect at the cellular level could involve multiple modes of signal transduction (6, 10, 11). Some evidence suggests that the process either does not require glucocorticoids or requires only a permissive effect (12). In addition, considerable evidence indicates that IL-1 stimulates glucocorticoid release via its corticotropin-releasing activity on pituitary cells (13). Therefore, IL-1 could affect MT and attendant functions, in part, via glucocorticoid hormones. Alternatively, another cytokine, IL-6 [also referred to as interferon β_2 and hepatocyte-stimulating factor (HSF)] could mediate IL-1 effects on MT at the level of the hepatocyte. IL-1 induces IL-6 synthesis in fibroblasts (14). IL-6 has been shown to regulate synthesis and secretion of a variety of acute-phase proteins (15) and, synergistically with IL-3, to increase proliferation of hematopoietic progenitor cells (16).

The present study was designed to evaluate the effects of recombinant human (rh)IL-1α and rhIL-6 as well as extracellular zinc and the synthetic glucocorticoid hormone dexamethasone on MT expression and zinc metabolism by rat hepatocytes. Further, we evaluated the teleological basis for cytokine mediation by examining cytoprotection from CCl_4-induced cellular damage.

MATERIALS AND METHODS

Cytokine Preparations. rhIL-1α was provided by Hoffmann–La Roche. Specific activity was 2×10^7 lymphocyte-activating factor (LAF) units (10^9 D10 units)/mg of protein. rhIL-6 was provided by Genetics Institute (Cambridge, MA). Specific activity was 10^6 HSF units (7×10^6 CESS units)/mg of protein.

Hepatocyte Preparation and Culture. Hepatocytes were isolated from male rats (Sprague–Dawley strain; University of Florida Breeding Facility) by collagenase perfusion (17). Viability of isolated hepatocytes was >85% as judged by trypan blue exclusion. Hepatocytes were suspended in Waymouth's MB 752/1 medium containing 10% fetal bovine serum (GIBCO) and insulin (1 μg/ml; Sigma). Aliquots containing 2.5×10^6 hepatocytes were inoculated into 60-mm collagen-coated culture dishes and viable parenchymal cells were allowed to attach selectively over a 3-hr period at 37°C. Following attachment, the medium was removed and cells were maintained with Waymouth's MB 752/1 medium supplemented with bovine serum albumin (BSA, 2 mg/ml; Sigma) for 21 hr. Thereafter, fresh medium containing rhIL-

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Abbreviations: MT, metallothionein; IL, interleukin; rhIL, recombinant human IL; HSF, hepatocyte-stimulating factor; LAF, lymphocyte-activating factor; LDH, lactate dehydrogenase; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide.

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1α, rhIL-6, and/or dexamethasone was added. The basal zinc concentration of the culture medium was 1 μM. For some experiments, the zinc concentration was adjusted to 16 μM or 48 μM by adding zinc sulfate.

MT, Cell Zinc, and Protein Measurements. MT was measured by the cadmium binding assay (18) using ^{109}Cd (DuPont/NEN) with a specific activity of 20 μCi/μg of Cd (1 μCi = 37 kBq). The zinc content of hepatocytes was measured directly by atomic absorption spectrophotometry after the cells were digested with 0.2% SDS in 0.2 M NaOH. Cell protein concentrations were determined by the Lowry method (19).

MT mRNA. Total RNA was extracted from hepatocytes by the method of Chomczynski and Sacchi (20). Briefly, hepatocytes from three to five dishes were homogenized (Polytron P-10 generator, Brinkmann) in guanidinium thiocyanate solution, protein was removed using a phenol/chloroform/isoamyl alcohol mixture, and RNA was precipitated with ethanol. RNA was dissolved in sterile distilled deionized water and the concentration of each sample was calculated using A_{260}. Dot blot and Northern blot analyses were conducted as described (21), except 60-mer oligonucleotide probes specific for MT-1 and -2 genes and corresponding to bases 16–76 from the 5′ terminus (22) were used for hybridization. The probes were 5′-end-labeled with [γ-^{32}P]ATP (DuPont/NEN) using T4 polynucleotide kinase (Bethesda Research Laboratories) and were purified by chromatography (Sephadex G-50, Sigma) prior to hybridization. The specific activity of each probe was routinely 3.0 μCi/pmol as measured by Cerenkov counting (Beckmann LS 7500). A labeled β-actin oligonucleotide probe was used to verify uniformity of hybridization.

For the Northern blot, total RNA was electrophoresed in a 1.1% agarose gel and transferred to a nitrocellulose filter (BA85; Schleicher & Schuell). Dot blot analysis was used to quantitate MT mRNA. After hybridization, the ^{32}P content of each dot was measured by liquid scintillation counting (2, 5). Molecules of MT mRNA per cell were calculated (21) using an RNA/DNA ratio of 4.0 and 6.4 pg of DNA per cell.

Cytoprotection Studies. Hepatocytes were cultured for 24 hr with hormones and/or added zinc as described above. Then hepatocytes from each treatment group were cultured for up to 18 hr with medium containing 5 mM CCl_4 first dissolved in dimethyl sulfoxide (DMSO). These and control cultures contained 140 mM DMSO. Cell survival curves were constructed by expressing the amount of cell protein remaining on culture dishes after exposure to CCl_4 as a percentage of that on control dishes. Cell leakage curves were constructed using measurements of lactate dehydrogenase (LDH) activity leaked into medium from hepatocytes exposed to CCl_4. LDH activity was measured spectrophotometrically as the increase in NADH from the oxidation of lactate (23). Activity was normalized on the basis of cell protein.

Statistical Analyses. Data were subjected to analysis of variance and Duncan's multiple range test (24).

RESULTS

IL-6 Increases MT Gene Expression. To determine the effects of rhIL-1α and rhIL-6 on MT gene expression, hepatocytes were cultured for 24 hr in Waymouth's medium supplemented with various concentrations of the two cytokines (Fig. 1). The medium was also supplemented with 1 μM dexamethasone, since other studies have shown that glucocorticoid hormones are often required for cytokine effects (25, 26). Clearly, incubation of hepatocytes with rhIL-6 led to concentration-dependent increases in MT mRNA (Fig. 1A). A maximal increase in MT mRNA, ≈3 times that of control, was achieved with rhIL-6 at 10 HSF units/ml (10 ng/ml). In

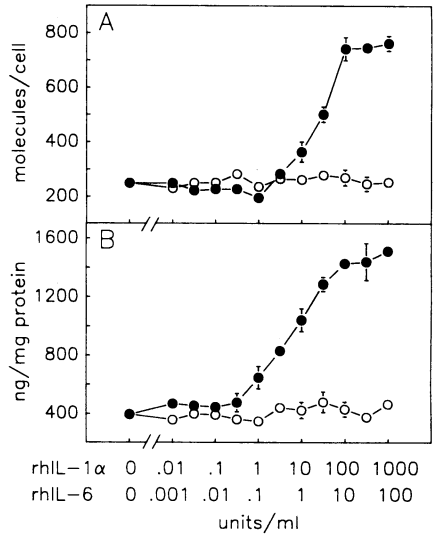

FIG. 1. Concentration dependence of cytokine regulation of MT expression in rat hepatocyte monolayer cultures. Twenty-four-hour hepatocyte cultures were incubated with Waymouth's medium containing BSA (2 mg/ml), 1 μM zinc, 1 μM dexamethasone, and either rhIL-1α (○) or rhIL-6 (●) as indicated. After 24 hr the hepatocytes were harvested and MT mRNA (*A*) or MT protein (*B*) was measured. Each point represents the mean ± SEM (n = 4).

contrast, rhIL-1α at concentrations as high as 1000 LAF units/ml (20 ng/ml) had no effect on MT mRNA. We also examined the effects of these cytokines on MT protein 24 hr after addition to the culture medium (Fig. 1*B*). Similar to its effect on the mRNA, rhIL-6 increased MT protein levels in a concentration-dependent manner. Again, a maximal increase of ≈3.5 times that of control cells was achieved with rhIL-6 at 10 HSF units/ml (10 ng/ml). In contrast, increasing amounts of rhIL-1α had no effect on MT concentrations.

Temporal effects of rhIL-6 on expression are shown in Fig. 2. Hepatocytes were cultured for up to 48 hr with either Waymouth's medium alone, medium supplemented with dexamethasone, or medium supplemented with dexamethasone and rhIL-6 at 10 HSF units/ml (10 ng/ml). Cultures with rhIL-6 exhibited time-dependent increases in both MT-1 (Fig. 2*A*) and MT-2 (Fig. 2*B*) mRNA. rhIL-6 up-regulated expression such that mRNA levels for both MT-1 and MT-2 were increased over those in both control and dexamethasone-treated hepatocytes within 3 hr of culture. The maximal increase in each mRNA was achieved after 12–18 hr. In addition, induction of MT-2 mRNA reached levels ≈3 times that of MT-1 mRNA. Specificity of the individual oligonucleotide probes is shown by adding the values at 24 hr, 150 and 600 molecules per cell, respectively. The sum agrees with the value of 750 molecules per cell for the combined probe shown in the concentration–response experiment (Fig. 1*A*). Time-dependent increases in MT protein were also produced by rhIL-6 (Fig. 2*C*). MT was increased within 3 hr of culture but did not reach maximal levels until ≈36 hr.

Extracellular Zinc and Glucocorticoid Affect IL-6-Induced MT Expression and Cell Zinc Concentration. Zinc metabolism can be modulated via regulation of MT synthesis by glucocorticoids (17, 27, 28) and dietary zinc (21). To examine the extent to which extracellular zinc and glucocorticoid hormone affect IL-6-induced MT expression, hepatocytes were harvested after 24 hr of culture with various treatment combinations and RNA was extracted for Northern blot analysis (Fig. 3). A control experiment using a β-actin oligonucleotide showed a uniform abundance of β-actin mRNA (data not shown). Therefore, changes in the intensity of the 550-base band representing MT mRNA correspond to differences in treatments. Abundance of the mRNA was

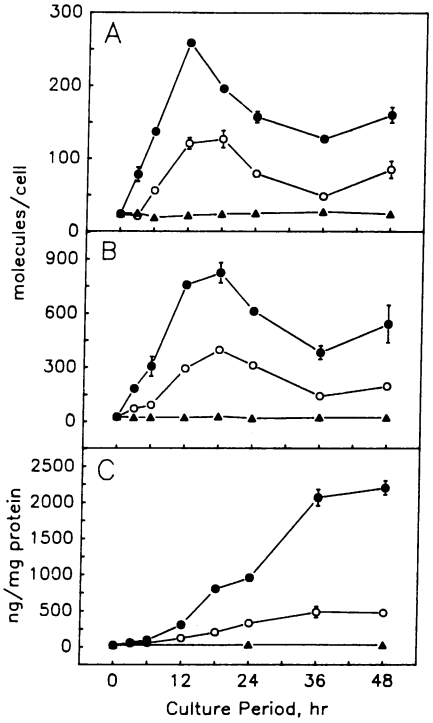

FIG. 2. Time course of induction of MT-1 and -2 mRNA and MT protein by rhIL-6. Twenty-four-hour rat hepatocyte monolayer cultures were incubated for up to 48 hr with either Waymouth's medium (▲), medium containing 1 μM dexamethasone (○), or medium with 1 μM dexamethasone and 10 HSF units of rhIL-6 per ml (●). All media contained BSA (2 mg/ml) and 1 μM zinc. At various times after these additions, hepatocytes were harvested and MT-1 mRNA (*A*), MT-2 mRNA (*B*), or MT protein (*C*) was measured. Each point represents the mean ± SEM (n = 4).

increased by the addition of zinc to the culture medium. At both 1 μM and 16 μM zinc, the addition of rhIL-6 alone had little or no effect, whereas dexamethasone (1 μM) increased the MT mRNA level dramatically. However, when rhIL-6 was added (10 HSF units/ml) with dexamethasone, levels were increased above that of the corresponding dexamethasone control cultures. Thus, MT mRNA was most abundant in hepatocytes cultured with a combination of added zinc, dexamethasone, and rhIL-6.

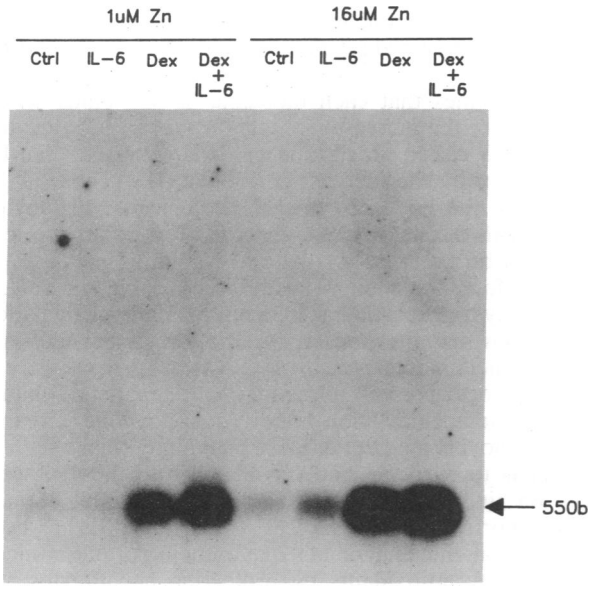

FIG. 3. Northern blot illustrating the effects of combinations of zinc, dexamethasone, and rhIL-6 on MT mRNA in rat hepatocyte monolayer cultures. Twenty-four-hour hepatocyte cultures were incubated with Waymouth's medium containing BSA (2 mg/ml) and the treatment combinations indicated: Ctrl, control; Dex, dexamethasone (1 μM); IL-6, rhIL-6 [10 HSF units (10 ng)/ml]. After 24 hr the hepatocytes were harvested and total RNA was extracted for Northern blot analysis. The 550-base band represents MT mRNA.

The trends in MT mRNA observed in the Northern blot were confirmed by dot blot analysis (Table 1). Levels increased 8.2- and 11.2-fold in response to rhIL-6 and dexamethasone in cells cultured with 1 and 16 μM zinc, respectively. MT protein levels and cellular zinc concentrations are also shown. Clearly, the same trends are reflected such that the highest levels of MT and cell zinc were observed in hepatocytes cultured with a combination of added zinc, dexamethasone, and rhIL-6.

IL-6 Protects Hepatocytes from CCl_4-Induced Damage. The teleological basis for cytokine mediation of MT expression was studied by examining cytoprotection of hepatocytes against CCl_4 toxicity. Hepatocytes were treated for 24 hr with combinations of added zinc, dexamethasone, and rhIL-6 and were subsequently cultured for up to 18 hr with the hepatotoxin CCl_4. Cell protein per dish and LDH activity in the medium were used as indices of cytoprotection.

Survival curves constructed from cell protein measurements are shown in Fig. 4*A*. Hepatocytes pretreated with low extracellular zinc (1 μM) were the most susceptible to CCl_4-induced damage, with only 25% surviving the first 6 hr of exposure. The addition of rhIL-6 alone had no effect, while the addition of 48 μM zinc or of dexamethasone provided partial protection, significantly improving survival to 65% and 89%, respectively. In contrast, full protection was provided by a combination of rhIL-6 and dexamethasone without additional zinc.

The trends observed in survival were reflected by the leakage of LDH into the culture medium (Fig. 4*B*). Hepatocytes pretreated with low extracellular zinc (1 μM) exhibited the greatest leakage. The addition of rhIL-6 alone provided no protection, while the addition of 48 μM zinc or of dexamethasone reduced LDH leakage by 55% and 80%, respectively, over 18 hr of exposure to CCl_4. Again, the addition of rhIL-6 plus dexamethasone provided full protection, completely eliminating CCl_4-induced leakage over 18 hr of exposure.

Table 1. Zinc and glucocorticoid dependence for IL-6 stimulation of MT expression and cellular zinc accumulation

Treatment	MT mRNA, molecules per cell	MT, ng/mg of protein	Cell Zn, nmol/mg of protein
1 μM Zn	60^{a}	66^{a}	1.8^{ab}
+ IL-6	57^{a}	69^{a}	1.7^{a}
+ Dex	275^{b}	305^{a}	2.2^{c}
+ Dex + IL-6	495^{c}	1415^{b}	2.6^{d}
16 μM Zn	115^{ab}	179^{a}	2.0^{bc}
+ IL-6	115^{ab}	202^{a}	1.9^{ab}
+ Dex	576^{c}	933^{b}	3.4^{e}
+ Dex + IL-6	1289^{d}	3964^{c}	5.3^{f}
(Pooled SEM)	(54; n = 3)	(196; n = 3)	(0.1; n = 4)

Twenty-four-hour rat hepatocyte monolayer cultures were incubated with Waymouth's medium containing BSA (2 mg/ml) and the treatment combinations indicated: Dex, dexamethasone (1 μM); IL-6, rhIL-6 [10 HSF units (10 ng)/ml]. After 24 hr the hepatocytes were harvested and MT mRNA, MT protein, and cell zinc content were measured. Each value represents the mean ± pooled SEM (n = 3 or 4). Values with differing superscript letters are significantly different ($P < 0.05$).

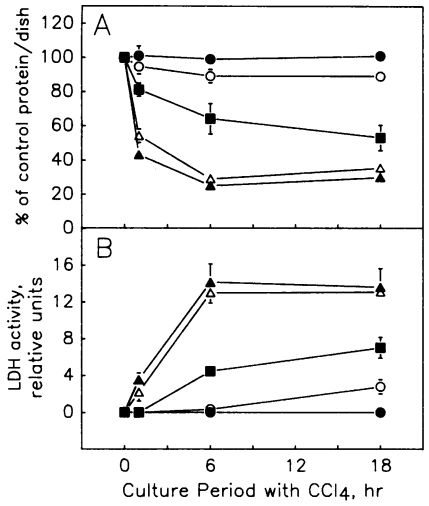

FIG. 4. Cytoprotection against CCl_4 hepatotoxicity. Twenty-four-hour hepatocytes were treated for 24 hr with BSA-supplemented (2 mg/ml) Waymouth's medium containing either 1 μM zinc (▲), 1 μM zinc and rhIL-6 (△), 48 μM zinc (■), 1 μM zinc and dexamethasone (○), or 1 μM zinc, dexamethasone, and rhIL-6 (●). Dexamethasone and rhIL-6 were at 1 μM and 10 HSF units/ml, respectively. After pretreatments, hepatocytes were cultured for up to 18 hr with BSA-supplemented Waymouth's medium containing 5 mM CCl_4 and 140 mM DMSO or 140 mM DMSO alone. Cell survival curves (*A*) were constructed by expressing the amount of cell protein remaining on dishes exposed to CCl_4 as a percentage of that on control dishes. Cell leakage curves (*B*) were constructed using measurements of LDH activity leaked into the medium by hepatocytes exposed to CCl_4. Each point represents the mean ± SEM (n = 4).

DISCUSSION

A great deal of interest has been generated regarding the mechanisms that account for enhanced expression of acute-phase hepatic proteins in response to tissue injury, stress, and infection. IL-1, a cytokine produced by activated macrophages and other cell types, triggers the acute-phase response *in vivo* (1). Administration of rhIL-1α to rats induces the synthesis of MT (2, 3). The increase in synthesis of this zinc-binding protein produces a tissue-specific redistribution of zinc with a transient depression of zinc in the plasma and concomitant uptake of zinc by the liver, bone marrow, and thymus. Similar changes are triggered by dibutyryl cAMP, endotoxin, and other mediators with IL-1-like activity (4) and have been verified by simulation and modeling techniques (6). The present study, utilizing rat hepatocyte monolayer cultures, shows that, at the level of the hepatocyte, IL-6 rather than IL-1 is a mediator of MT production and changes in zinc metabolism. Further, IL-6-induced changes provide cytoprotection from CCl_4-induced hepatotoxicity in a manner consistent with dependence upon increased cellular MT.

In many instances the results of studies using cultures of various cell types to assess the effects of cytokines on acute-phase protein synthesis seem to conflict. It has been pointed out that these differences in responsiveness to individual cytokines are due to factors related to specific cell types or lines and to differences in evaluation criteria such as measurement of a specific protein versus its mRNA (29). In the present study, we found that IL-1 had no effect on MT mRNA in isolated hepatocytes (Fig. 1), whereas Karin *et al.* (30) have shown that IL-1 increases MT mRNA in Hep G2 hepatoma cells. We investigated the possibility that rat hepatocytes do not contain functional receptors for human IL-1α. However, this was not the case since rhIL-6 and rhIL-1α each increased expression of the ceruloplasmin gene (with J. Gitlin; data not shown). These observations are consistent with those of others (15, 31), who found that IL-6 affects the synthesis of a broad spectrum of acute-phase proteins in hepatocytes whereas IL-1 regulates only a few.

We found that rhIL-6 gave a maximal increase in MT expression at 10 HSF units/ml (10 ng/ml). This concentration agrees well with the value of 30 HSF units/ml reported for maximal induction of acute-phase proteins (15). The increase in MT expression is probably dependent upon changes initiated at the level of transcription since MT mRNA was maximally induced by the same level of rhIL-6.

Glucocorticoids may play an important role in regulation of the acute-phase response. Both IL-1 and IL-6 stimulate the release of corticotropin from cultured pituitary cells, suggesting that these cytokines increase glucocorticoid levels *in vivo* (13). Previous studies have demonstrated the ability of glucocorticoids to stimulate MT synthesis in hepatocytes both *in vivo* (28) and *in vitro* (17, 27). This ability is due to the presence of glucocorticoid-responsive elements in the promoter region of the MT genes (32). In the present study, glucocorticoid (dexamethasone) was required for rhIL-6 to up-regulate MT synthesis. Glucocorticoid dependency has also been recognized for rhIL-6 regulation of other acute-phase proteins (25, 26). Presumably, glucocorticoids influence either rhIL-6 receptors on hepatocytes or a component of intracellular signal transduction directed by IL-6. The glucocorticoid dependency for IL-6 regulation of some acute-phase proteins in primary cultures of hepatocytes may reflect a need for a basal level of glucocorticoids, which normally bathe the liver *in vivo*, to facilitate expression of some liver functions. Alternatively, it has been suggested that a possible effect of increased levels of circulating glucocorticoids on the liver during inflammation may be to help shift the target tissues of IL-6 from cell populations such as monocytes to other cells such as hepatocytes (33, 34).

The ability of increased levels of extracellular zinc to facilitate rhIL-6-induced MT production and cellular zinc accumulation (Table 1) can be attributed to two mechanisms. (*i*) Zinc binds to and stabilizes apometallothionein so that the protein's turnover is reduced (4). (*ii*) The promoter regions of the MT genes contain regulatory elements that are responsive to zinc (32). Presumably, transcription is increased by zinc via a putative trans-acting nuclear protein that binds zinc and interacts with the metal regulatory element of the DNA (32, 35).

If one assumes that each molecule of MT binds seven atoms of zinc, the increases in cellular zinc in hepatocytes cultured with either added zinc or dexamethasone can be accounted for by the increase in cellular MT (Table 1). In comparison, the increases in cellular zinc of hepatocytes cultured with dexamethasone and rhIL-6 together are less than would be expected for the corresponding increases in MT. Therefore, the addition of dexamethasone and rhIL-6 together may trigger a change in the intracellular distribution of zinc such that the portion of zinc not associated with metallothionein is reduced.

The teleological reason for acute-phase hepatic MT induction and zinc accumulation is not yet clear. One potential benefit of moving zinc out of plasma and into organs such as the liver is to enhance zinc's availability to these tissues. Based on zinc's role in stabilizing membranes (9, 36) and MT's purported role as a radical scavenger (8), it has been proposed that zinc and/or MT may play important intracellular roles as antioxidants by protecting hepatocytes and other cells during infection when host-generated cytotoxic oxygen species are produced in large quantities (37–39). In the present study, treatments that increased hepatocyte MT and zinc also reversed the deleterious effects of CCl_4 on hepatocyte viability. These results fit the theoretical framework of a functional role of zinc and/or MT in membrane stabilization. MT can be envisioned as providing stabilization during oxidative stress, either directly as a radical scavenger

or indirectly as a zinc donor to membrane sites or to specialized components such as the cytochrome P-450 system. Zinc has been shown to inhibit this system by stabilizing NADPH (40–42). If MT and/or zinc have important physiological functions of this type, a dietary zinc deficiency compounded by chronic infection, stress, or tissue injury could have an adverse effect on liver metabolism. Further, in conditions of chronic elevation of IL-6, the dietary zinc supply may be an important determinant in the physiological manifestations induced by this cytokine.

Thus, the results of this study demonstrate that IL-6, in the presence of glucocorticoid hormone, is a major physiological determinant of MT gene expression and zinc metabolism in hepatocytes. Further, IL-6-induced changes provide cytoprotection in a manner consistent with dependence upon increased cellular MT and/or zinc. Since IL-1 did not elicit changes in expression, measures of MT mRNA, MT protein, and cell zinc concentrations in hepatocytes may provide sensitive bioassays to functionally discriminate between IL-1 and IL-6 activity. In addition, our findings, taken together with the results of other studies, may provide a mechanism to explain how IL-1 triggers the up-regulation of MT and zinc accumulation in hepatocytes during the acute-phase response. Tissue injury, stress, and infection cause the release of IL-1 from macrophages (1). Then IL-1 stimulates the release of corticotropin, which causes adrenal steroidogenesis (13). Glucocorticoids can act on hepatocytes to increase MT production as well as to feedback-inhibit the release of IL-1 from macrophages (13). In addition to activation of the pituitary–adrenal axis, IL-1 also stimulates the synthesis of IL-6 by fibroblasts and other cell types (1, 14). In the presence of glucocorticoids, IL-6 increases MT production and zinc uptake by hepatocytes. These IL-6 effects are optimized by increased levels of extracellular zinc. The functional role of increased hepatic MT production and zinc accumulation may be to provide cytoprotection.

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