Role of interleukin 6 and corticosteroids in the regulation of expression of glutathione S-transferases in primary cultures of rat hepatocytes

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The effect of recombinant interleukin 6 (rIL-6) on the transcript levels of rat glutathione S-transferase (GST) genes rGSTA2, rGSTP1, rGSTM1 and rGSTM2 was examined in primary cultures of rat hepatocytes. rIL-6 had little effect on the increase in expression of rGSTP1 that occurs in cultured hepatocytes. Dexamethasone (DEX), in contrast, prevented the expression of rGSTP1 by hepatocytes, and rIL-6 in combination with DEX had no additional effect. Neither rIL-6 nor DEX alone had a significant effect on the transcript levels of rGSTA2, rGSTM1 and rGSTM2 in cultured hepatocytes. However, when both were present (15 ng/ml rIL-6 and 10^{-7} M DEX) the transcript levels of rGSTA2, rGSTM1 and rGSTM2 decreased significantly ($P < 0.05$) after 48 h in culture. If the rIL-6 was removed from the cultures after 24 h, the levels of transcripts recovered and were the same at 48 h as cells cultured without rIL-6 for the entire period. Dose-response relationships of rIL-6 with 10^{-7} M DEX

were determined for transcripts of each GST isoenzyme and the IC_{50} values were between 1.5 and 7.5 ng/ml. Declines in transcript levels of rGSTA2 were observed with rIL-6 plus 10^{-8} or 10^{-7} M DEX but not with rIL-6 plus 10^{-9} , 10^{-6} , or 10^{-5} M DEX. To determine if the cytokine and glucocorticoid effects were mediated by sequences in the 5'-flanking sequence of rGSTA2, a plasmid construct containing a 1.6 kb fragment of the 5'-flanking sequence of the rGSTA2 gene and the chloramphenicol acetyltransferase (CAT) reporter gene was used to transfect rat hepatocytes in primary culture. The addition of rIL-6 and DEX to the culture medium caused a significant ($P < 0.05$) decrease in CAT activity after 48 h in culture. If rIL-6 was removed after 24 h in culture, CAT activity after an additional 24 h in culture was greater than the CAT activity in cells cultured for 48 h without rIL-6. Therefore cytokines and glucocorticoids may be important physiological regulators of GST expression.

INTRODUCTION

The glutathione S-transferases (GSTs; EC 2.5.1.18) are an important family of enzymes of detoxication and are grouped into four classes termed Alpha, Mu, Pi and Theta on the basis of amino acid sequences, substrate specificities and affinity for non-substrate ligands [1–11]. In the intact animal, levels of some of the GST isoenzymes can be increased by treatment with drugs such as phenobarbital or with antioxidants. The increases are mediated in part by response elements in the 5'-flanking sequence of the GST genes [8,12,13]. The physiological regulators of GST expression have not been characterized although products of oxidative stress may play a role [8,13].

Levels of GST may also change in cells cultured *in vitro*. For example, during primary culture of rat hepatocytes on collagen the levels of most forms of GST either decrease or remain unchanged [14–16]. The exception is rGSTP1 which is absent from freshly isolated hepatocytes. After 24 h in culture, however, transcript and protein levels of rGSTP1 in hepatocytes increase progressively [14–17]. The 5'-flanking sequence of the rGSTP1 gene contains a number of response elements that are binding sequences for transcription factors [18–22]. One of the response elements may bind liver activating protein (LAP) [also called nuclear factor–interleukin 6 (NF-IL6), interleukin 6 (IL-6)–D-site-binding protein or CCAAT/enhancer-binding protein β (C/EBP β)], a transactivating protein the expression of which is increased by IL-6 [23]. Deletion of the LAP response element

from reporter constructs containing the 5'-flanking sequence of the rGSTP1 gene leads to a 2-fold increase in the rate of reporter transcription which suggests that LAP functions as a negative regulator of rGSTP1 expression [24,25]. IL-6 is produced in the liver and is the principal effector of the acute-phase response [23]; significant levels of IL-6 are found in serum of individuals with and without liver disease [23,26,27]. Therefore the basal level of circulating IL-6 *in vivo* may inhibit rGSTP1 expression via the LAP response element. When hepatocytes are placed in culture, in the absence of IL-6, rGSTP1 expression may increase since its expression is no longer suppressed. To examine this possibility we cultured rat hepatocytes with and without recombinant IL-6 (rIL-6) in the medium and measured transcript levels of rGSTP1, rGSTA2, rGSTM1 and rGSTM2. In addition, dexamethasone (DEX) was added in some experiments because of previous observations that the spontaneous expression of rGSTP1 by hepatocytes in culture was blocked by this compound [28]. In the current study, rIL-6 in the medium of hepatocytes had little effect on the spontaneous increase in rGSTP1 expression, whereas DEX completely prevented the increase. Additions of rIL-6 or DEX alone to cultured hepatocytes had no significant effect on the transcript levels of rGSTA2, rGSTM1 or rGSTM2. However, when both rIL-6 and DEX were present, the transcript levels of all measured forms of GSTs were reduced significantly. The effect of rIL-6 and DEX on rGSTA2 transcript levels appeared to be mediated at least in part by regulatory elements in the 5'-flanking sequence of the rGSTA2 gene. In this paper the

Abbreviations used: GST, glutathione S-transferase; rIL-6, recombinant interleukin 6; DEX, dexamethasone; CAT, chloramphenicol acetyltransferase; AP-1, activating protein 1; NF-IL6, nuclear factor–interleukin 6; ATCC, American Type Culture Collection; C/EBP β , CCAAT/enhancer-binding protein β ; DMEM, Dulbecco's modified Eagle's medium; LAP, liver activating protein.

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nomenclature for rat GSTs that groups subunits by gene family and order of discovery is adopted [8].

MATERIALS AND METHODS

All chemicals used were of analytical grade and unless otherwise stated were purchased from Sigma or Fisher Scientific. Culture media were from Sigma or Mediatech; restriction enzymes were from Promega or New England Biolabs; random-primer DNA-labelling reagents and isotopes ($[\alpha\text{-}^{32}\text{P}]\text{dCTP}$ and $[\text{14C}]\text{chloramphenicol}$) were from Amersham or Du Pont-NEN; collagenase was from Worthington Biochemical Corp.; and murine rIL-6 was from R & D Systems.

Hepatocyte cultures for Northern-blot analysis

Hepatocytes were isolated from livers of male Sprague-Dawley rats by collagenase perfusion and centrifugation as described previously [17]. Livers were perfused *in situ* through the portal vein until cleared of blood with buffer A (137 mM NaCl, 5 mM KCl, 0.8 mM MgSO_4 , 0.35 mM Na_2HPO_4 , 0.42 mM KCl, 12 mM NaHCO_3 , 20 mM Hepes, 11 mM glucose, 5 mM EGTA; pH 7.4). Perfusion was then continued for 4–7 min with buffer B (buffer A with no EGTA plus 4 mM CaCl_2 and 0.05% collagenase). After perfusion the hepatocytes were dispersed manually in buffer A and filtered by gravity through stainless-steel mesh (40 mesh size). Dispersed cells were collected by centrifugation for 1 min at 50 g (r_{av} , 10 cm) and washed three times with buffer A. Cells were resuspended and plated in Medium 199 [29,30] that contained 10% calf serum, penicillin (100 units/ml) and insulin (10^{-7} M). Cells were plated at a density of approx. $1.4 \times 10^6/\text{ml}$ on 100 mm diam. plastic dishes coated with rat tail collagen. The cells were kept at 37 °C in humidified air with 5% CO_2 . At 4 h after plating the medium was changed and was then changed every 24 h. DEX, rIL-6 or both was added 4 h after plating and again each time the medium was changed. Hepatocyte viability was determined by Trypan Blue exclusion and found to be greater than 85% at the time of plating. Injury to the hepatocytes by the various treatments was determined by measuring lactate dehydrogenase leakage from damaged cells into the medium; none of the treatments induced cell injury. Cells were also examined by light microscopy and there were no differences noted in cell density or in appearance of the cells treated with rIL-6 plus DEX in comparison with control cells.

RNA preparation and Northern-blot hybridization

Total RNA was purified from cultured cells by lysis and extraction in guanidinium isothiocyanate/phenol/chloroform [31], and RNA concentration was determined from $A_{1\text{cm}, 260}$. Total RNA (15 μg) was denatured in 2.2 M formaldehyde/50% formamide and loaded on to 1% (w/v) agarose gels containing 2.2 M formaldehyde. The quality of the RNA was judged after staining of the gels with ethidium bromide. The RNA was transferred to Nylon membranes (Du Pont-NEN) by capillary blotting in 20 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) overnight. The membrane was then baked at 80 °C for 2 h and kept refrigerated until the time of hybridization.

The cDNAs for rGSTA2, rGSTM1 and rGSTM2 were from Dr. C. Pickett [32,33]. rGSTP1 was from Dr. Barch (Northwestern University, Chicago, IL, U.S.A.) [34], mouse albumin was from Dr. E. Morgan (Emory University, Atlanta, GA, U.S.A.) and ribosomal protein S14 (PCS14) was from the American Type Culture Collection (ATCC). Fragments of the cDNAs were purified from *Pst*I digests of the respective plasmids

(rGSTA2 = 0.53 kb, rGSTM2 = 1.0 kb and PCS14 = 0.59 kb). A 0.8 kb fragment of rGSTP1 was purified from an *Eco*RI–*Sal*I digest of the parent plasmid. The cDNA fragment for rGSTA2 hybridizes with both rGSTA1 and rGSTA2 and probably rGSTA3 messages [34,35]. To improve specificity, a different fragment was cut from the plasmid using *Bgl*II and *Eco*RI. This fragment was 300 bp and does not hybridize with rGSTA3 but does hybridize with rGSTA1 and rGSTA2 [32]. The cDNA for rGSTM2 is specific for rGSTM-class transcripts but does not distinguish between rGSTM1 and rGSTM2 transcripts because of extensive homology between them [33]. For some experiments a specific probe (268 bp) for rGSTM1 transcripts was made from the rGSTM1 cDNA as described previously [33]. The cDNA fragments were labelled with $[\text{32P}]\text{dCTP}$ by random-primer DNA labelling. Blots were hybridized with the cDNA at 65 °C overnight, washed three times for 30 min with 1 $\times$ SSC at 65 °C, and exposed to film. The autoradiograms were scanned in a densitometer as described previously [17]. Variability in the loading of RNA on to gels was controlled by normalizing the measured signal areas with those of PCS14 as an internal standard on each blot [17].

Hepatocyte cultures for transfection

Hepatocytes were prepared for transfection by a method similar to the above procedure with the following modifications. Rat livers were perfused for 5–10 min with buffer (126.8 mM NaCl, 6.7 mM KCl, 10 mM Hepes, 25 mM NaHCO_3 , 4.9 mM pyruvate, 4.9 mM fumarate, 4.9 mM glutamate, 11.5 mM glucose; pH 7.4). Collagenase (to 0.03%) and CaCl_2 (to 5 mM) were then added to the buffer and perfusion was continued for an additional 5–10 min. After perfusion, hepatocytes were dispersed manually in Dulbecco's modified Eagle's Medium (DMEM)/F12 (1:1, v/v) [29,30] with glutamine to which NaHCO_3 (29 mM), penicillin (100 units/ml), streptomycin (100 $\mu\text{g}/\text{ml}$) and 10% fetal bovine serum were added (pH 7.4). Hepatocytes were plated at a density of 1.5×10^6 cells/ml on 60 mm diam. collagen-coated dishes in the above medium.

Transfection plasmids

Plasmid pGTB1.6-cat was a gift from Dr. C. Pickett and contains 1.6 kb of the 5'-flanking sequence of the rGSTA2 subunit gene cloned into pSV0-cat [36]. Plasmid pSV0-cat lacks any promoter sequences and was obtained from the ATCC. Plasmid pSV2-cat, also from the ATCC, contains SV40 promoter and enhancer sequences and was used to monitor successful transfection of the cells.

Transfection of primary hepatocytes

After plating, hepatocytes were kept at 37 °C for 30 min and then placed in DMEM/F12 (1:1, v/v; pH 7.4) [29,30] that contained 3% fetal calf serum, 10^{-7} M DEX and 10^{-7} M insulin. After 3 h the cells were transfected using a calcium phosphate precipitation method [37]. Buffer (25 mM Hepes, 0.75 mM Na_2HPO_4 , 140 mM NaCl, 125 mM CaCl_2 ; pH 7.05; 300 μl) that contained 5 μg of plasmid DNA was added to the medium of each plate. Cells were cultured overnight in the transfection buffer at 37 °C and 3% CO_2 . Cells were then washed twice with PBS and placed in Medium 199 with 5 mM glucose, 4 mM NaHCO_3 , 10 mM Hepes, 100 $\mu\text{g}/\text{ml}$ penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, 10^{-7} M DEX, 10^{-7} M insulin and 10% calf bovine serum (pH 7.4). After transfection, plates of cells were randomly assigned to one of three treatment groups and cultured for a total of 48 h with fresh medium changes at 24 h: (1) no treatment (control);

(2) rIL-6 (15 ng/ml) for 48 h; (3) rIL-6 (15 ng/ml) for 24 h and then for 24 h without rIL-6. Cells were harvested after 48 h for measurement of chloramphenicol acetyltransferase (CAT) activity.

CAT assays

Cells were harvested after the plates had been washed twice in ice-cold PBS. Buffer (10 mM Tris/HCl, 10 mM NaCl, 1 mM EDTA; pH 7.5) was added and after 5 min the cells were scraped from the plates, transferred to centrifuge tubes, and centrifuged for 2 min at 8160 *g* (r_{av} , 7 cm). The supernatant was discarded and the cells were resuspended in 200 μ l of 250 mM Tris/HCl (pH 8.0) and lysed by three freeze-thaw cycles. Cellular debris was removed by centrifugation for 15 min at 11 750 *g* (r_{av} , 7 cm) and 75 μ l of the supernatant was transferred to tubes and heated to 65 °C for 10 min to inactivate endogenous acetylases. [¹⁴C]Chloramphenicol (0.25 μ Ci) and n-butyryl-CoA (final concentration 2.5 mM) were added and the reaction mixture was incubated overnight at 37 °C. Next, 2,6,10,14-tetramethylpentadecane/xylene (2:1, v/v; 200 μ l) was added to the reaction mixture which was vortexed and centrifuged for 15 min at 12 300 *g* (r_{av} , 5 cm). An aliquot of the upper (organic) phase was added to 5 ml of scintillation fluid and radioactivity was measured in a scintillation counter. For each treatment, triplicate plates were harvested separately for measurements of CAT activity. CAT activity was normalized to total cellular protein, and the CAT activities of cells transfected with pSV0-cat were subtracted from the CAT activities obtained from cells transfected with pGTB1.6-cat. The data from two separate transfection experiments were combined by expressing the CAT activity of treatment groups relative to the CAT activity of the no-treatment (control) groups which were arbitrarily set to 1 for each experiment.

Western blots

Cytosols from cultured cells and Western blots were made as described previously [17]. Protein concentrations of cytosols were measured by the Bio-Rad protein assay with BSA as the standard and 5 μ g of total protein from each cytosol was separated by SDS/PAGE and electroblotted to Immobilon membrane (Millipore). Alpha-class proteins were detected on Western blots with rabbit anti-(rat GST-Ya) serum (MED 26 YA, 1:2000; Biotrin, Dublin, Ireland), horseradish peroxidase-conjugated goat anti-rabbit serum (1:2000; Amersham) and enhanced chemiluminescence reagents (Du Pont-NEN or Amersham). rGSTA1 and rGSTA2 proteins co-migrate on SDS/PAGE and were resolved from rGSTA3 which was not quantified. The density of the resultant bands on films were quantified with a densitometer.

Data analysis

Each value represents the mean $\pm$ S.E.M. for separate experiments or animals. Statistical significance was determined by Student's *t* test at $P \leq 0.05$ using a two-tailed test.

RESULTS

Effect of rIL-6 and DEX on expression of rGSTP1

In untreated cells there was a marked increase in the transcript levels of rGSTP1 over 72 h of culture (Figure 1). Treatment with rIL-6 (15 ng/ml, approx. 100 units/ml) caused a slight decrease in transcript levels at 48 h but by 72 h rGSTP1 transcript levels were similar to those seen in cells cultured without rIL-6. When

Figure 1 Northern blot of RNA isolated from hepatocytes and probed for rGSTP1

Hepatocytes were cultured with or without rIL-6 (15 ng/ml) or DEX (10^{-7} M) in the medium. At the indicated times cells were harvested and RNA was purified and detected by Northern-blot analysis with cDNA for rGSTP1 as described in the Materials and methods section. RNA from fresh cells (0 h) was prepared from whole liver.

Table 1 Effect of rIL-6 and DEX on rGSTA2 and rGSTM2 transcript levels

Control cells were cultured without rIL-6; all culture media contained 10^{-7} M DEX. Results for the cells cultured for 24 h with rIL-6 (15 ng/ml) and then for an additional 24 h without it are in the final column [48 (-IL-6)]. Transcript levels are expressed as a percentage of the control levels. Results (mean $\pm$ S.E.M.) are from three to five separate culture experiments. rGSTA2 cDNA hybridizes with rGSTA1 and rGSTA2 transcripts and rGSTM2 hybridizes with rGSTM1 and rGSTM2 transcripts. * $P < 0.01$ compared with the control; † $P < 0.01$ compared with results for cells cultured with IL-6 for 48 h cells.

cDNA	Time in culture (h)...	Transcript level (%)			
		24	48	72	48 (-IL-6)
rGSTA2		76 $\pm$ 28	26 $\pm$ 5*	21 $\pm$ 6*	117 $\pm$ 23†
rGSTM2		69 $\pm$ 5	30 $\pm$ 8*	30 $\pm$ 9*	109 $\pm$ 9†

Figure 2 Dose-dependent effects of IL-6 on expression of rGSTA2

rGSTA2 transcript levels from hepatocytes cultured with (●) and without DEX (○). Hepatocytes were cultured in 10^{-7} M insulin $\pm$ 10^{-7} M DEX and various concentrations of rIL-6 for 48 h. Transcript levels were determined by densitometry using signal from PCS14 to control for variations in loading as described in the Materials and methods section. Transcript levels obtained from cells cultured without DEX were arbitrarily set at 100 and all other levels are expressed relative to these initial values. Values are means $\pm$ S.E.M. from cells obtained from three animals. * Indicates significant difference ($P < 0.05$) from cells cultured without rIL-6.

Figure 3 Northern blot of RNA obtained from cells cultured with DEX (10^{-7} M) and increasing concentrations of rIL-6

Blots were probed with cDNAs for PCS14 (A), albumin (B), rGSTA2 (C) and rGSTM1 (D) as described in the Materials and methods section.

cultured cells were treated with DEX (10^{-7} M) in the presence or absence of rIL-6 the expression of rGSTP1 was prevented.

Effect of DEX and rIL-6 on transcript levels of rGSTA2 and rGSTA1

In contrast with the results obtained for rGSTP1, neither DEX (10^{-7} M) nor rIL-6 (15 ng/ml) alone caused a significant decrease in rGSTA2 and rGSTAM2 transcript levels. However, there was a significant decrease in rGSTA2 and rGSTM2 transcript levels after treatment with rIL-6 plus DEX. The decrease was maximal after 48 h in culture and persisted for at least 72 h (Table 1). For this reason most of the subsequent studies were performed after 48 h in culture. In order to examine whether the decrease in transcript levels was reversible, hepatocytes were cultured with rIL-6 and DEX for 24 h. IL-6 was then removed from the medium and the cells were cultured for an additional 24 h with DEX alone. In the cultures in which rIL-6 was withdrawn, the transcript levels for both rGSTA2 and rGSTM2 were the same as those in control cells cultured without rIL-6 for 48 h (Table 1).

Dose-response effects of rIL-6 and DEX

rIL-6 alone at up to 30 ng/ml (approx. 200 units/ml) had no significant effect on rGSTA2 transcript levels although there was a trend for an increase at low concentrations of rIL-6 (< 15 ng/ml) (Figure 2). However, when cells were cultured in 10^{-7} M DEX and increasing concentrations of rIL-6, there was a progressive decrease in rGSTA2 transcript levels, with an IC_{50} value of about 7.5 mg/ml. Similar decreases in rGSTM1 (maximum decrease $38 \pm 3.2\%$ of control at 30 ng/ml IL-6 plus DEX;

Figure 4 Dose-dependent effects of rIL-6 on expression of rGSTM2 with and without DEX

All treatment and symbols are the same as in Figure 2 except that Northern blots are probed with cDNA for rGSTM2. Values are means $\pm$ S.E.M. from three animals. * Indicates significant difference ($P < 0.05$) from cells cultured without rIL-6.

Table 2 Effect of different concentrations of DEX or rGSTA2

Hepatocytes were cultured in medium containing rIL-6 (15 ng/ml) and various concentrations of DEX. Transcript levels were determined as described in the Materials and methods section using the rGSTA2 cDNA. Values are means $\pm$ S.E.M. from three animals. * Significantly different from control ($P < 0.05$).

DEX concentration (M)	Relative transcript level
0	100
10^{-9}	71 ± 19
10^{-8}	$30 \pm 5^*$
10^{-7}	$18 \pm 3^*$
10^{-6}	82 ± 27
10^{-5}	136 ± 52

Figure 3) and rGSTM2 (Figure 4) transcript levels were observed with increasing concentrations of rIL-6. Although a decline in rGSTM2 transcript levels was seen with rIL-6 alone, the greatest decline was observed with rIL-6 plus DEX (Figure 4).

When the concentration of DEX in the culture medium was varied from 0 to 10^{-5} M with rIL-6 (15 ng/ml), rGSTA2 transcript levels decreased at both 10^{-7} and 10^{-8} M DEX but not at lower (10^{-9} M) or higher (10^{-6} and 10^{-5} M) DEX concentrations (Table 2).

Effect of IL-6 on transcriptional activity

It is unclear from the above studies whether the decreases in GST transcript levels were due to changes in transcription rate or in message stability. We therefore transfected primary cultures of

Table 3 Effect of rIL-6 on expression of pGTB1.6-cat

Freshly isolated hepatocytes were transfected with pGTB1.6-cat as described in the Materials and methods section. Cells were cultured for 48 h with 10^{-7} M DEX $\pm$ 15 ng/ml rIL-6. For some cells rIL-6 was removed from the medium after 24 h and the cells were cultured for an additional 24 h. Data are expressed as means $\pm$ S.E.M. from two separate experiments in which CAT assays were performed on three separate plates of cells. CAT activities obtained in no-treatment (control) groups were set to 1 for each experiment to normalize data for transfection efficiency. * Significantly different ($P < 0.05$) from control cells and cells cultured with rIL-6 for 24 h.

Treatment	CAT activity relative to control
None	1.00 $\pm$ 0.24
rIL-6 (15 ng/ml) 48 h	0.38 $\pm$ 0.14*
rIL-6 (15 ng/ml) 24 h then withdrawn	1.68 $\pm$ 0.27

rat hepatocytes with a CAT reporter construct (pGTB1.6-cat) that contains a 1.6 kb portion of the 5'-flanking sequence of the rGSTA2 gene. The 1.6 kb fragment of the rGSTA2 gene contains all of the known regulatory elements and has been used previously to examine how xenobiotics and antioxidants regulate the expression of rGSTA2 [13]. After 48 h in culture with DEX, CAT activity of cells transfected with pGTB1.6-cat was about 12 times greater than that of cells transfected with pSV0-cat. Culturing the cells with both rIL-6 and DEX caused significant decreases in the levels of CAT activity (Table 3). When rIL-6 was removed after 24 h of culture, CAT activity at 48 h exceeded the level obtained in cells cultured for 48 h without rIL-6 (Table 3). The results obtained with the CAT construct mirrored the findings obtained when transcript levels of rGSTA2 were measured directly by Northern-blot analysis.

Effect of IL-6 and DEX on rGSTA1/A2 protein levels

Hepatocytes were cultured for 48 h with and without rIL-6 plus DEX in the medium. Cells were harvested and levels of Alpha-class GST determined using anti-rGSTA1/A2 serum. There were no changes in protein levels for rGSTA1/A2 as measured by Western-blot analysis (results not shown) under any of the treatment conditions.

DISCUSSION

Previous studies have shown that rGSTP1 expression increases spontaneously in freshly isolated rat hepatocytes during culture on collagen [14–18]. The 5'-flanking sequence of the rGSTP1 gene contains an activating protein 1 (AP-1)-like binding site termed the GPEI that lies 2.5 kb from the start site of the first exon which may function as a promoter [22]. During cell culture increased expression of Fos and Jun is observed and these transcription factors may bind to the GPEI site to induce the expression of rGSTP1. Studies in isolated rat hepatocytes, however, failed to demonstrate that the expression of rGSTP1 in these cells correlated with the transcription rates of *c-fos* and *c-jun* genes [22]. An alternative hypothesis is that the IL-6-sensitive silencer region (LAP-binding sequence) in the 5'-flanking sequence of the rGSTP1 gene is active *in vivo* but not *in vitro* where there is no IL-6 [25]. The current studies fail to support the alternative hypothesis because rIL-6 alone did not inhibit the expression of rGSTP1 in cultured cells. However, the results with the other GST isoenzymes suggests that rIL-6 significantly affects GST transcript levels only in the presence of corticosteroids.

Because DEX completely prevented the expression of rGSTP1 transcripts in the cultured cells, it was not possible to determine whether rIL-6 added to the DEX effect. Therefore, *in vivo*, IL-6 in the presence of corticosteroids may decrease the expression of rGSTP1 by hepatocytes, or corticosteroids alone may be sufficient to suppress rGSTP1 expression, but further studies are required to prove this hypothesis.

rIL-6 in combination with DEX had a significant effect on the transcript levels of rGSTA2, rGSTM1 and rGSTM2. Transcript levels declined in a dose-dependent manner and then rapidly recovered when rIL-6 was withdrawn. In general, the effect of cytokines on gene expression is mediated at the level of transcription [38]. However, post-transcriptional events may also be affected by cytokines [39]. In the current study the decrease in GST transcript levels appeared to be mediated, at least in part, by a decrease in rates of transcription. This conclusion is based on two observations. First, the greatest effect was seen when the hepatocytes were cultured with both rIL-6 and DEX, which are known to act in concert to alter rates of transcription [38]. Secondly, the effect of rIL-6 and DEX on expression of pGTB1.6-cat mirrored the change seen in rGSTA2 transcripts by Northern-blot analysis which suggests that the cytokine effect is mediated by elements in the 5'-flanking sequence of at least the rGSTA2 gene, i.e. transcriptional regulation. The current studies do not exclude the possibility that rIL-6 and DEX also affected post-transcriptional events but the data are most consistent with a decrease in transcription being the principal reason for the decrease in GST transcript levels.

The regulation of the rGSTA2 subunit gene has been examined extensively and its 5'-flanking sequence contains a number of response elements, although no IL-6-sensitive response elements (e.g. NF-IL6-binding sequences) have been described. We therefore examined the 5'-flanking sequence of the rGSTA2 gene to –1651 bp for possible NF-IL6-binding sites using the Wisconsin Sequence Analysis Package (version 8.1). At least one NF-IL6-binding site (attccagaa) is located at –1615 to –1607 bp and it overlaps a glucocorticoid response element at –1610 to –1596 bp. A second putative NF-IL6-binding site was identified at –1424 to –1417 bp (ttgacca). The occurrence of these potentially IL-6-sensitive response elements in the 5'-flanking sequence of the rGSTA2 gene supports our belief that rIL-6 affects transcript levels at the level of transcription. IL-6-induced NF-IL6 (or other IL-6-induced transcription factors) may also bind to other regulatory regions, such as AP-1 sites, altering gene expression [40]. Putative AP-1-binding sites are part of the rGSTA2 gene antioxidant response element [41] and IL-6 effects may be mediated by binding of NF-IL6 to the antioxidant response element or by association of NF-IL6 with other transcription factors, such as Fos and Jun, leading to a repression of transcriptional activity [40]. The glucocorticoid response element in the 5'-flanking sequence of the rGSTA2 gene was not thought to be active on the basis of studies in cultured cells [14]. However, the administration of DEX to rats increases transcript levels of rGSTA2 in the liver of rats which suggests that corticosteroids may affect rates of GST transcription *in vivo* [35]. The current studies provide further support that GST expression is influenced by corticosteroids, and this effect is probably mediated by regulatory elements in the 5'-flanking sequences of the genes.

Exposure of mouse myeloid leukaemia cells to rIL-6 increases the expression of a number of transcription factors including NF-IL6. NF-IL6 is a member of the C/EBP family and is also termed C/EBP β [23,39]. Studies by others [42] have shown that C/EBP α , which is not induced by IL-6, binds to the xenobiotic response element of the rGSTA2 gene and increases its rate of

transcription. Levels of C/EBP α and β messages and proteins have been examined during hepatic regeneration after partial hepatectomy in the rat [43]. Protein levels of C/EBP α increase 2-fold 1 h after partial hepatectomy and then decrease to normal levels by 6 h, whereas message and protein levels of C/EBP β increase 5- and 3-fold respectively within 3 h of partial hepatectomy and remain elevated at 9 h. Previous studies from this laboratory examined the effect of partial hepatectomy on rGSTA2 transcript levels and found that by 12 h they decreased and then recovered by 48 h after surgery [44]. The decrease in transcript levels was due in part to a decrease in rates of transcription at 6 h but not at 24 h after partial hepatectomy. The parallel changes between C/EBP β and rGSTA2 rates of transcription supports the hypothesis that rIL-6 decreases the expression of rGSTA2 via response elements in the 5'-flanking sequence of the gene. Similar results have been reported with cytochrome P-450-dependent mixed function oxidases [45], suggesting that during the acute-phase response the levels of a number of drug-metabolizing enzymes may decrease, perhaps leading to changes in the metabolism of xenobiotics by the liver. It therefore appears that GSTs are another family of proteins the physiological expression of which is regulated by cytokines.

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REFERENCES

- Mannervik, B. and Danielson, U. H. (1988) *CRC Crit. Rev. Biochem.* **23**, 283–337
- Pickett, C. D. and Lu, Y. H. (1989) *Annu. Rev. Biochem.* **58**, 734–764
- Chasseaud, L. F. (1979) *Adv. Cancer Res.* **29**, 175–274
- Boyer, T. D. (1989) *Hepatology* **9**, 486–496
- Tipping, E. and Ketterer, B. (1981) *Biochem. J.* **195**, 441–452
- Wolkoff, A. W., Goresky, C. A., Sellin, J., Gatmaitan, Z. and Arias, I. M. (1979) *Am. J. Physiol.* **236**, E638–E648
- Senjo, M., Ishibashi, T. and Imai, Y. (1985) *J. Biol. Chem.* **260**, 9191–9196
- Hayes, J. D. and Pulford, D. J. (1995) *CRC Crit. Rev. Biochem. Mol. Biol.* **30**, 445–600
- Boyer, T. D. and Olsen, E. (1991) *Biochem. Pharmacol.* **42**, 188–190
- Mannervik, B., Ålin, P., Guthenberg, C., Jensson, H., Tahir, M. K., Warholm, M. and Jornvall, H. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7202–7206
- Meyer, D. J., Coles, B., Pemble, S. E., Gilmore, K. S., Fraser, G. M. and Ketterer, B. (1991) *Biochem. J.* **274**, 409–414
- Rushmore, T. H. and Pickett, C. B. (1990) *J. Biol. Chem.* **265**, 14648–14653
- Rushmore, T. H., Nguyen, T. and Pickett, C. B. (1993) in *Structure and Function of Glutathione Transferases* (Tew, K. D., Pickett, C. B., Mantle, T. J., Mannervik, B. and Hayes, J. D., eds.), pp. 119–128, CRC Press, Boca Raton, FL.
- Abramovitz, M., Ishigaki, S. and Listowsky, I. (1989) *Hepatology* **9**, 235–239
- Vandenbergh, Y., Glaise, D., Meyer, D. J., Guillouzo, A. and Ketterer, B. (1988) *Biochem. Pharmacol.* **37**, 2482–2485
- Vandenbergh, Y., Tee, L., Morel, F., Rogiers, V., Guillouzo, A. and Yeoh, G. (1991) *FEBS Lett.* **284**, 103–108
- Lee, S. J., Friedman, S. L., Whalen, R. and Boyer, T. D. (1994) *Biochem. J.* **299**, 79–83
- Okuda, A., Sakai, M. and Muramatsu, M. (1987) *J. Biol. Chem.* **262**, 3858–3863
- Sakai, M., Okuda, A. and Muramatsu, M. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9456–9460
- Diccianni, M. B., Imagawa, M. and Muramatsu, M. (1992) *Nucleic Acids Res.* **20**, 5153–5158
- Morrow, C. S., Goldsmith, M. E. and Cowan, K. H. (1990) *Gene* **88**, 215–225
- Morimura, S., Okuda, A., Sakai, M., Imagawa, M. and Muramatsu, M. (1992) *Cell Growth Differ.* **3**, 685–691
- Chojkier, M. (1995) in *Progress in Liver Diseases* (Boyer, J. and Ockner, R. K., eds.), vol. 13, pp. 37–61, W. B. Saunders, Philadelphia
- Imagawa, M., Osada, S., Okuda, A. and Muramatsu, M. (1991) *Nucleic Acids Res.* **19**, 5–10
- Imagawa, M., Osada, S., Koyama, Y., Suzuki, T., Hirom, P. C., Diccianni, M. B., Morimura, S. and Muramatsu, M. (1991) *Biochem. Biophys. Res. Commun.* **179**, 293–300
- Le Moine, O., Marchant, A., Goldman, M., Dupont, E. and Deviere, J. (1994) *Acta Gastroenterol. Belg.* **57**, 245–254
- Napoli, J., Bishop, G. A. and McCaughan, G. W. (1994) *Gastroenterology* **107**, 789–798
- Sakai, M., Muramatsu, M. and Nishi, S. (1992) *Biochem. Biophys. Res. Commun.* **187**, 976–983
- Morton, H. J. (1970) *In Vitro* **6**, 89–108
- Rutzkey, L. P. and Pumper, R. W. (1974) *In Vitro* **9**, 468–469
- Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159
- Pickett, C. B., Telakowski-Hopkins, C. A., Ding, G. J., Argenbright, L. and Lu, A. Y. (1984) *J. Biol. Chem.* **259**, 5182–5188
- Ding, G. J.-F., Ding, V. D.-H., Rodkey, J. A., Bennett, C. D., Lu, A. Y. H. and Pickett, C. B. (1986) *J. Biol. Chem.* **261**, 7952–7957
- Sugouka, Y., Kano, T., Okuda, A., Sakai, M., Kitagawa, T. and Muramatsu, M. (1985) *Nucleic Acids Res.* **13**, 6049–6057
- Waxman, D. J., Sundseth, S. S., Srivastava, P. K. and Lapensens, D. P. (1992) *Cancer Res.* **52**, 5797–5802
- Rushmore, T. H., King, R. G., Paulson, K. E. and Pickett, C. B. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3826–3830
- Kingston, R. E. and Sheen, J. (1991) in *Current Protocols in Molecular Biology* (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K., eds.), pp. 9.6.6–9.6.9, John Wiley & Sons, New York.
- Fey, G. H. and Gauldie, J. (1990) in *Progress in Liver Diseases* (Popper, H. and Schaffner, F. eds.), vol. 9, pp. 89–116, W. B. Saunders, Philadelphia
- Baumann, H., Jahreis, G. P., Morella, K. K., Won, K.-A., Pruitt, S. C., Jones, V. E. and Prowse, K. R. (1991) *J. Biol. Chem.* **266**, 20390–20399
- Hsu, W., Kerppola, T. K., Chen, P.-L., Curran, T. and Chen-Kiang, S. (1994) *Mol. Cell. Biol.* **14**, 268–276
- Nguyen, T., Rushmore, T. H. and Pickett, C. B. (1994) *J. Biol. Chem.* **269**, 13656–13662
- Pimental, R. A., Liang, B., Yee, G. K., Wilhelmsson, A., Poellinger, L. and Paulson, K. E. (1993) *Mol. Cell. Biol.* **13**, 4365–4373
- Diehl, A. M. and Yang, S. Q. (1994) *Hepatology* **19**, 447–456
- Lee, S. J. and Boyer, T. D. (1993) *Biochem. J.* **293**, 137–142
- Chen, Y. L., Florentin, I., Batt, A. M., Ferrari, L., Giroud, J. P. and Chauvelot-Moachon, L. (1992) *Biochem. Pharmacol.* **44**, 137–148