Modulation of Glutathione S-Transferase Subunits A2, M1, and P1 Expression by Interleukin-1β in Rat Hepatocytes in Primary Culture*

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The influence of various cytokines on the expression of glutathione S-transferases (GSTs) was investigated in rat hepatocytes in primary culture. Only treatment of hepatocytes with interleukin-1β (IL-1) was effective, resulting in a marked decrease in GSTs. Steady-state mRNA levels of rGSTA2 and M1 were strongly down-regulated by IL-1 in a dose-dependent manner after a 24-h exposure while rGSTP1 mRNA level was increased by a 48-h treatment. Similar effects of IL-1 were observed at the protein levels. The response to IL-1 appeared to be specific for each subunit within GST gene families. In addition, IL-1 strongly suppressed the induction of rGSTA2 by 3-methylcholanthrene, oltipraz (a synthetic derivative of 1,2-dithiole-3-thione), and phenobarbital and that of rGSTM1 by oltipraz and phenobarbital, whereas it was ineffective on rGSTP1 induction by these compounds. Using in vitro nuclear run-on transcription assay and Northern blot analysis of α-amanitin-treated cells, IL-1-mediated rGSTM1 mRNA decrease was found to result from mRNA destabilization.

These results provide the first demonstration that IL-1 regulates some major GST subunits in hepatocytes by a post-transcriptional mechanism.

The response of organisms to inflammation and infection is characterized by a number of changes that include release of proinflammatory cytokines, increased secretion of some plasma proteins in the liver, referred to as acute phase proteins (1), and impairment of drug metabolism capacity (2, 3). Several in vivo and in vitro studies have shown that administration of proinflammatory cytokines results in the decrease of hepatic drug metabolism and various cytochrome P-450 (CYP)1-related activities (4, 5).

The detoxication of many drugs and carcinogens is due to the combined effects of mixed function oxygenation, brought about mainly by the CYPs and conjugation of the oxidized products by enzymes such as the glutathione S-transferases (GSTs), NADPH:quinone oxidoreductase, epoxide hydrolases, sulfotransferases, and UDP-glucuronoyl-transferases (6). Little is known about the effects of cytokines on conjugating enzymes. We are chiefly concerned with the GSTs that catalyze the conjugation of glutathione to a wide range of electrophiles and thus play a key role in the protection of cells from toxic and carcinogenic electrophiles as well as from lipid hydroperoxides generated by an oxidative stress (7). GSTs belong to a supergene family consisting of four main multigene families, i.e. alpha, mu, pi, and theta (8). In the rat, the GST alpha class is composed of subunits A1, A2, A3, A4, and A5; the GST mu class contains subunits M1, M2, M3, M5, and M6; the GST pi is represented only by subunit P1; and GST theta class comprises subunits T1, T2, and T3 (9). The level of expression of GSTs is a crucial factor in determining the sensitivity of cells to a broad spectrum of toxic chemicals, and induction of GSTs by environmental compounds (drugs or diet) is thought to represent an important mechanism of protection against carcinogens in rodents (10).

The present study was conducted to determine whether several cytokines, used at a concentration known to affect plasma protein patterns and CYPs, could modulate the constitutive and inducible expression of GSTs. To investigate the effects of different cytokines (IL-1, IL-2, IL-4, IL-6, IL-10, interferon (IFN)α and γ, tumor necrosis factor α (TNFα) and transforming growth factor β (TGFβ)) separately, we used rat hepatocytes in primary culture in the absence or presence of these cytokines and analyzed the expression of rat GST subunits at both the mRNA and protein levels. We present the first evidence that IL-1, a major proinflammatory cytokine, regulates some subunits of different classes of GSTs. Because the levels of GSTs in the organism may determine the potential for detoxication of xenobiotics, it is important to analyze the effects of both positive and negative regulators of GST gene expression. Besides health status, exogenous chemicals may greatly influence GST-mediated drug detoxication capacity of the liver. As observed for CYPs (5, 11, 12), we found that IL-1 decreased induction of some GST subunits by 3-methylcholanthrene (MC, a polycyclic aromatic hydrocarbon), phenobarbital (PB, a barbiturate), and oltipraz (OPZ, a synthetic derivative of 1,2-dithiole-3-thione).

EXPERIMENTAL PROCEDURES

Recombinant Cytokines and Chemicals—Human recombinant IL-1, IL-6, TNFα, IFNα, and recombinant mouse IL-2 were purchased from Genzyme (Cambridge, UK). Rat IL-4 was from Immugenex (Los Angeles, CA). Human recombinant IFNγ was kindly provided by Roussel-UCLaf (Romainville, France). Platelet-derived human TGFβ was purchased from British Biotech (Oxon, UK). Culture media and fetal calf serum were obtained from Life Technologies, Inc. (Paisley, UK). Collagenase, MC, PB, bovine serum albumin, and bovine insulin were from Sigma (St. Louis, MO). OPZ was kindly supplied by Dr G. Jolles.
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Poulenc Rorer, Antony, France). All other compounds were readily available commercial products.

Cell Isolation and Culture—Adult hepatocytes were obtained after perfusion of male rat (Sprague-Dawley, 150-200 g) liver with a collagenase solution as described previously (13). Cell viability was estimated by trypan blue exclusion. Total RNA was isolated from rat liver. The amplified cDNAs corresponding to GST mRNA were 32P-labeled by random priming using a Rediprime DNA labeling system (Amersham).

Preparation of GST Proteins from Cytosols and HPLC Analysis—The cytosolic GST fraction was prepared from hepatocytes at 4°C according to the method of Kispert et al. (24). Briefly, cells were homogenized in buffer A (150 mM KCl, 50 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, and 2 mM dithiothreitol in 10 mM potassium phosphate buffer, pH 7.0). Samples were applied directly to a column of hydroxypatite (Amersham, Arlington Heights, IL) for dot blots, dilutions of each RNA sample in 42°C, and transferred onto hybond-N nylon filters (Amersham, Arlington Heights, IL). For dot blots, dilutions of each RNA sample in a rose gel, and transferred onto hybond-N nylon filters (Amersham, Arlington Heights, IL) was then changed daily.

All these recombinant cytokines were known to be effective on CYP expression and/or acute-phase protein production in rat hepatocyte primary cultures (5, 14). The cytokines and the three xenobiotic inducers (PB, MC, and OPZ) were added 24 h after cell seeding and at each daily renewal of the culture medium. MC and OPZ were dissolved in dimethyl sulfoxide before addition to the culture medium at the final concentration of either 5 μM MC or 50 μM OPZ in 0.2% dimethyl sulfoxide (DMSO). PB was dissolved in phosphate-buffered saline (PBS) and added to the culture medium at the final concentration of 3.2 mM. Control cultures received the same concentration of vehicle.

Enzyme Assays—GST activities were determined in cell lysates as described by Habig and Jakoby (15) with adaptation for a centrifugal analyzer (COBAS BIO) using the following substrates: 1-chloro-2,4-dinitrobenzene (CDNB), a substrate for most GST subunits, and 1,2-dichloro-4-nitrobenzene (DCNB), a substrate mainly for rGSTM1. All these activities were related to total cellular or cytosolic proteins determined by the method of Bradford (16) adapted for use on a centrifugal analyzer.

RNA Isolation and Blot Analysis—Hepatocyte monolayers were washed with 0.1 mM PBS. Total RNA was extracted by the method of Chomczynski and Sacchi (17). 10 μg of total RNAs were subjected to electrophoresis in a denaturing 6% (v/v) formaldehyde, 1.2% (w/v) agarose gel, and transferred onto hybond-N nylon filters (Amersham, Arlington Heights, IL). Prehybridization and hybridization were performed according to Church and Gilbert (18). Membranes were washed with 3 × SSC, 0.1% Tween 20 for 30 min and then twice with 1 × SSC, 0.1% SDS for 10 min.

Northern analysis or dot blotting and hybridization with specific cDNA probes for rGSTA2, A3, and A4 (α class), M1 and M2 (μ class), P1 (π class) and T1 (θ class). A strong decrease of rGSTA2 and M1 mRNA levels was observed after 12 h of treatment with IL-1 and maintained at 24 h (17 and 27% of corresponding controls after 48 and 72 h of treatment, respectively (p < 0.01)). The DCNB activity decreased to 22 and 28% of corresponding controls after 48 and 72 h of treatment, respectively (p < 0.01).

Effect of IL-1 on Expression of GST mRNAs—To determine the mechanism involved in the decrease of GST activity by IL-1, we analyzed the expression of several GST subunits in the absence or presence of 100 units/ml IL-1 after 8, 12, and 24 h of treatment. The steady-state mRNA levels were measured by Northern analysis or dot blotting and hybridization with specific cDNA probes for rGSTA2, A3, and A4 (α class), M1 and M2 (μ class), P1 (π class) and T1 (θ class). A strong decrease of rGSTA2 and M1 mRNA levels was observed after 12 h of treatment with IL-1 and maintained at 24 h (17 and 27% of control values at 24 h, p < 0.001) (Fig. 2). No effect of IL-1 on rGSTP1 mRNA was evidenced before 48 h of treatment. The mRNA levels of the other GST subunits were unaffected by this treatment (data not shown).

To determine whether IL-1 acted in a dose-dependent manner, we treated hepatocyte cultures with the following three different concentrations of IL-1: 20, 100, and 300 units/ml (Fig. 3). The dose-response analysis was performed after 24 h of treatment for mRNAs encoding rGSTA2 and M1 and after 48 h of treatment for the mRNA encoding rGSTP1. 24 h after cell seeding, the basal expression of rGSTA2 and M1 mRNAs was decreased in a dose-dependent manner in the presence of IL-1. IL-1 already had an inhibitory effect on rGSTA2 and M1 mRNAs at the lowest concentration (20 units/ml). When compared with corresponding controls, IL-1 at 20 units/ml caused a 4- and 3-fold decrease of rGSTA2 and M1 mRNAs, respectively. In contrast, after 48 h of treatment, the expression of rGSTP1 transcripts was increased 6-fold with 20 units/ml IL-1 and 13-fold with 100 units/ml.

Effect of IL-1 on GST Subunit Composition—Quantitative analysis of individual subunits was made possible by HPLC (Fig. 4). Hepatocytes treated by 100 units/ml IL-1 for 72 h were compared with their control counterparts. As observed for mRNAs, IL-1 caused an important decrease (44%) in rGSTM1 protein while the levels of other subunits were unchanged.

Effect of IL-1 on the Inducible Expression of GST mRNAs and Proteins by MC, OPZ, and PB—To determine the effect of IL-1 on GST mRNA induction by xenobiotics, accumulation levels of
rGSTA2, M1, and P1 mRNA were determined following 24, 48, and 72 h of treatment with MC, OPZ, or PB in the absence or presence of the cytokine. Results from one of four hybridization experiments are shown in Fig. 5A. The three types of inducers increased mRNA levels of rGSTA2 and P1 while only OPZ and PB increased rGSTM1 mRNA levels. Statistical analyses showed significant differences in GST expression in cells treated for 48 h with CDNB and DCNB as substrates. Each value represents the mean ± S.D. of three experiments. **, p < 0.01.

The effect of IL-1 on the inducible expression of GSTs was confirmed by HPLC (Fig. 6). IL-1 blocked induction of rGSTM1 by OPZ and PB at 72 h while that of rGSTP1 was unaffected.

**Nuclear Transcription Assay**—To determine whether changes in steady-state mRNA levels of rGSTA2, M1, and P1 by IL-1 were due to changes in the rate of gene transcription, nuclear run-on assays were performed with nuclei from IL-1-treated hepatocytes. Dot blots representative of the transcriptional activity are shown in Fig. 7. A 12-h exposure of hepatocytes to 100 units/ml IL-1 was uneffective on either GST gene transcription. As expected, albumin gene transcription was reduced with IL-1 treatment. In contrast, rGSTA2 and rGSTP1 gene transcription was increased after 4 h of treatment with MC and OPZ and after 12 h of treatment with PB. As observed for the basal levels of rGSTA2, M1, and P1 gene transcription, concomitant treatment with IL-1 failed to antagonize the transcriptional activation brought about by MC and OPZ after 4 h and by PB after 12 h of treatment (data not shown). Actin gene transcription rate, included as a control, was not affected by these treatments. These observations suggested that the regulation of GST expression by IL-1 could be the consequence of a change in the mRNA stability rather than in the rate of gene transcription.

**Mechanism of the IL-1 Effect**—To demonstrate a post-transcriptional regulation of rGSTM1 mRNA by IL-1, the level of steady-state mRNA levels for rGSTA2 and M1, respectively (p < 0.05) (Fig. 5B). PB induction of rGSTA2 mRNAs was decreased by 36% in the presence of IL-1 (although the effect was not significant throughout the four experiments p < 0.068). PB induction of rGSTM1 was decreased by 41% (p < 0.05). The effect of IL-1 on MC-induced rGSTA2 was not significant but showed a trend toward a decrease (p < 0.078). The cytokine was ineffective on rGSTP1 mRNA induction by the xenobiotics.

The effect of IL-1 on the inducible expression of GSTs was confirmed by HPLC (Fig. 6). IL-1 blocked induction of rGSTM1 by OPZ and PB at 72 h while that of rGSTP1 was unaffected.

Effect of Various Cytokines on GST Enzyme Activities. Hepatocytes were cultured for 48 and 72 h in the absence (C) or presence of cytokines: IL-1 (100 units/ml), IL-2 (50 units/ml), IL-4 (5 ng/ml), IL-6 (50 units/ml), IFNα and γ (50 units/ml), TNFα (50 units/ml), and TGFβ (0.1 ng/ml). GST activities were assayed on cell lysates at 48 and 72 h using CDNB and DCNB as substrates. Each value represents the mean ± S.D. of three experiments. **, p < 0.01.
this mRNA was determined in control and IL-1-treated cells in the presence of α-amanitin (Fig. 8). 48 h after seeding, cells were treated for 28 h with α-amanitin (2 μg/ml) in the absence or presence of 100 units/ml IL-1. Under these conditions, α-amanitin did not appear to be cytotoxic to the cells since no morphological alteration of the cultures was detected by light microscopic examination. At various time intervals, total RNA was prepared and analyzed by Northern blot. The mRNA half-life was determined from the slope of the semilogarithmic plot of mRNA band intensity versus time. The decay of rGSTM1 mRNA level in IL-1-treated cells was considerably faster than that of control cells. In cells exposed to α-amanitin, the rGSTM1 RNA exhibited a half-life superior to 30 h versus 15–20 h in cells treated with both α-amanitin and IL-1. These observations strongly suggest that IL-1 enhances the degradation of this transcript.

**DISCUSSION**

Regulation of GSTs by cytokines has still received limited attention. Only an increase of hGSTA1 and A2 by IL-4 in human hepatocytes (28) and a significant suppression of some GST subunits by IFNα/β in the mouse liver have been reported (29). In the present study, we provide the first evidence that IL-1 can directly affect the expression of some major GST genes in rodent hepatocytes. In addition, we demonstrate that the response of each GST subunit to IL-1 is highly specific within gene families.

IL-1 is known to down-regulate the constitutive expression of several genes such as albumin, lipoprotein lipase, and CYPs but, in most cases, IL-1 stimulates the expression of a variety of other genes including acute-phase ones (30). This cytokine regulates most of the genes by a transcriptional mechanism involving members of the C/EBP family, HNF1, AP1, and NF-kB transcription factors (31–33). However, IL-1 signal transduction pathways in hepatocytes are still unknown. We bring here the first demonstration that the GST regulation by IL-1 is controlled predominantly by a post-transcriptional mechanism. This conclusion is based on comparison of steady-state mRNA levels of rGSTA2, M1, and P1 with the transcription rates of their respective genes. Our results show that, despite variations in mRNA contents in response to IL-1, the transcription rates of these genes remained unchanged. The action of IL-1 on steady-state mRNA levels observed only after 12 h of treatment and the rGSTM1 decrease in experiments using α-amanitin are important arguments favoring the idea that this cytokine primarily acts at the post-transcriptional level on GST mRNA stability. There was no previous report showing that GSTs can be regulated by similar mechanisms except for hGSTP1 mRNA half-life, which was increased by ethacrynic acid in human colon carcinoma cell lines (34). Little is known about how IL-1 might decrease mRNA half-life. One could suggest that IL-1 decreases the stability of rGSTA2 and M1 by altering the activity or cellular content of specific factors involved in their mRNA stability. Such a post-transcriptional mechanism has been demonstrated in synovial fibroblasts for

**FIG. 3. Dose-dependent effect of IL-1 on rGSTA2, M1, and P1 mRNAs.** Hepatocytes were treated with either 20 units/ml IL-1 (IL-1 20), 100 units/ml IL-1 (IL-1 100), or 300 units/ml IL-1 (IL-1 300). Total RNA was prepared and analyzed by Northern blot after 24 h of treatment (rGSTA2, M1, and P1) and after 48 h (rGSTP1). Hybridization of the same filters with an 18 S DNA probe was used as a control. C, untreated cells.

**FIG. 4. GST subunit composition in 72 h of control and IL-1-treated hepatocytes.** GST subunits from untreated (C) or IL-1-treated (IL-1) (100 units/ml) hepatocytes were purified by glutathione-affinity column and separated by HPLC as described under “Experimental Procedures.”
the regulation of collagenase mRNA by IL-1 (35). However, in this case, this cytokine increased the stability of this transcript by reversing the destabilizing effects of sequences located in the 3′-untranslated regions. Further investigations of the 3′-untranslated regions of rGSTA2 and M1 mRNAs should inform on the presence of sequences possibly involved in the regulation of these transcripts by IL-1. Other studies reported a post-transcriptional down-regulation of ferritin and C-reactive protein by IL-1, as a consequence of reduced mRNA translation (36, 37).

In contrast to rGSTA2 and M1, rGSTP1 is strongly up-regulated after a 48-h treatment with IL-1. The rGSTP1 is not expressed in adult rat liver but is de novo expressed in vitro (38). Moreover, rGSTP1 dramatically increases in hepatic pre-neoplastic lesions (39). The increased rGSTP1 levels found in these lesions would increase the resistance of these cells to cytotoxic insults. The reason for the inducibility of liver rGSTP1 in vitro is unknown. However, since in cultured hepatocytes the reexpression of subunit P1 is inhibited by dimethyl sulfoxide (38), this suggests a possible correlation between the expression and IL-1 induction of subunit P1 and its high glutathione peroxidase activity. Moreover, our results indicate that IL-1 does not block the induced level of transcription. In addition, we show that the inhibitory effect of IL-1 appears only after 48 h. To precisely define the effect of IL-1, we performed kinetic studies on cells pretreated for 48 h with the inducers (data not shown). Under these conditions, GST expression has

**Fig. 5. Effect of IL-1 on the inducible expression of GST mRNAs.** A. Hepatocytes were cultured for 24, 48, and 72 h in the absence (C) or presence of IL-1 in combination with the inducers, MC, OPZ, or PB. Total RNA was analyzed by Northern blot using the probes for rGSTA2, rGSTM1, rGSTP1, and 18 S as described under "Experimental Procedures." B, densitometric quantitation of rGSTA2, rGSTM1, and rGSTP1 after 48 h of treatment by MC, PB, and OPZ in the absence or presence of IL-1. Results are the mean of four independent experiments. The mRNA levels are expressed as percent of the value of the corresponding control. The statistical analysis (Student’s test) was performed by comparison of inducer-treated cells with inducer/IL-1-treated cells (*, p < 0.05).
already reached a steady state of induced mRNA levels, but IL-1-triggered inhibition was observed after 12 h of treatment. That the IL-1 suppressive effect on GST-induced mRNA levels follows the same kinetics as on the basal mRNA levels might reflect the fact that IL-1 primarily acts on mRNA accumulation levels rather than on GST transcription. This lag in IL-1 action, in addition to the absence of effect of IL-1 on GST transcription either under basal or induced conditions, strongly supports the
idea that IL-1 primarily acts on GST by a negative post-transcriptional mechanism. Finally by contrast with rGSTA2 and M1, rGSTP1 induction by xenobiotics was not affected by IL-1.

Because the expression of many hepatic CYPs and several GSTs is decreased by some cytokines, studies on the molecular mechanisms underlying this phenomenon in vivo are of importance at both a pharmacological and a toxicological viewpoint. In addition to the production of cytokines during inflammation, some cytokines are being used as immunotherapeutic agents for the treatment of chronic inflammation (viral hepatitis B and C), cancer, and some other diseases (44). Since studies indicate that cytokines reduced hepatic drug metabolizing enzymes, altered therapeutic and/or toxic effects of co-administered agents might be expected. Furthermore, the modulation of carcinoagent-inactivating GST expression by cytokines may bear relevance to the process of chemical carcinogenesis and may contribute to interindividual variations in cancer susceptibility. In recent years, considerable attention has been paid to the development of chemointervention strategies involving induction of GSTs (45). For example, OPZ has been proposed as a good candidate for chemoprotection because of its ability to prevent against aflatoxin B1-induced hepatocarcinogenesis in the rat (9). It has been assumed that aflatoxin B1 is also a human hepatocarcinogen and a powerful cocarcinogen in combination with hepatitis B virus, which is often endemic in communities exposed to aflatoxin B1 (46). If our results are confirmed in cultured human hepatocytes, questions would arise about the therapeutic interest of OPZ in the context of inflammation. Because of the ability of certain cytokines to decrease CYPs and GSTs, our results support the idea that the balance between activation and detoxication of carcinogens and other chemicals can be strongly modified during the process of inflammation.

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