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Repression of Human *GSTA1* by Interleukin-1β Is Mediated by Variant Hepatic Nuclear Factor-1C

Lorraine Ng, Kathleen Nichols, Kelly O'Rourke, Ann Maslen, and Gordon M. Kirby

Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada

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ABSTRACT

Down-regulation of glutathione transferase A1 (*GSTA1*) expression has profound implications in cytoprotection against toxic by-products of lipid peroxidation produced during inflammation. We investigated the role of hepatic nuclear factor 1 (HNF-1) in repression of human *GSTA1* expression by interleukin (IL)-1β in Caco-2 cells. In luciferase reporter assays, overexpression of HNF-1α increased *GSTA1* transcriptional activity via an HNF-1 response element (HRE) in the proximal promoter. In addition, constitutive mRNA levels of GSTA1 and HNF-1α rose concurrently in Caco-2 cells with increasing stage of confluence. IL-1β reduced GSTA1 mRNA levels at all stages of confluence; however, HNF-1α mRNA levels were not altered. IL-1β repressed *GSTA1* transcriptional activity, an effect that was abolished by mutating the HRE. Similar results were observed in HT-29 and HepG2 cells. Overexpression of HNF-1α did not counteract IL-1β-mediated repression of *GSTA1* transcription either in reporter assays or at the mRNA level. Involvement of the transdominant repressor C isoform of variant HNF-1 (vHNF-1C) in *GSTA1* repression was demonstrated, because vHNF-1C overexpression significantly reduced *GSTA1* transcriptional activity. Finally, IL-1β caused concentration-related up-regulation of vHNF-1C mRNA levels and increased binding of vHNF-1C protein to the HRE, whereas HNF-1α-HRE complex formation was reduced. These findings indicate that IL-1β represses GSTA1 transcription via a mechanism involving overexpression of vHNF-1C.

During inflammatory conditions, changes in gene expression can result in secretion of acute phase proteins, production of proinflammatory cytokines, particularly interleukin-1 and -6, and tumor necrosis factor (TNF)-α and impairment of drug metabolism (Stevens et al., 1992; Jung et al., 1995; Moshage, 1997). Proinflammatory cytokines may modify drug metabolism by altering the expression of certain key metabolizing enzymes, most notably cytochrome P450 isoforms (Ghezzi et al., 1986) and glutathione transferases (GSTs) (Voss et al., 1996; Maheo et al., 1997; Whalen et al., 2004). For example, μ and α class GST expression is reduced in rat hepatocytes treated with IL-6, in combination with dexamethasone (Voss et al., 1996) and GSTA2 and GSTM1 expression is reduced by IL-1β (Maheo et al., 1997). In contrast, TNF-α and IL-6 positively regulate GSTA4 in cultured mouse hepatocytes (Desmots et al., 2002). In addition, we have demonstrated that IL-1β decreases *GSTA1* expression in Caco-2 cells (Romero et al., 2002), the regulatory mechanisms of which are the focus of the current study.

Human α class GSTs are composed of five subunits (A1–5) and play a key role in the protection of cells against toxic electrophiles and products of oxidative stress. Control of the rodent GST α promoter occurs via two regulatory elements, the xenobiotic response element and the antioxidant response element, which mediate induction by planar aromatic compounds and phenolic antioxidants, respectively (Rushmore et al., 1990; Daniel, 1993; Whalen and Boyer, 1998). However, evidence to date suggests that transcriptional regulation of human and rodent GSTs differs significantly because the 5′-flanking region of *hGSTA1* and *hGSTA2* genes do not contain the same regulatory elements as found in the rodent homologs. Indeed, we have shown that *GSTA1* is down-regulated by chemical inducers of rodent GSTs via a mechanism involving overexpression of vHNF-1C (Romero et al., 2006)

Several studies have identified a role for HNF-1 in GSTA regulation. For example, reduction of human GSTA2 levels during the acute phase response is mediated by HNF-1 (Whalen et al., 2004) and decreased *GSTA2* expression in human renal cell carcinomas (Klone et al., 1990) is associated with diminished levels of HNF-1 (Clairmont et al., 1994). In the *hGSTA1* gene, an HRE is localized between nucleotides

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ABBREVIATIONS: TNF, tumor necrosis factor; GST, glutathione transferase; IL, interleukin; HNF-1, hepatic nuclear factor 1; vHNF, variant hepatic nuclear factor; HRE, hepatic nuclear factor response element; RT-PCR, reverse transcription-polymerase chain reaction; PCR, polymerase chain reaction; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; EMSA, electrophoretic mobility shift assay.

−187 and −170 upstream to the transcription start site (Rushmore et al., 1990). This site functions as an enhancer element that is able to increase transcriptional activity of *GSTA1* up to 30-fold (Lorper et al., 1996). In addition, increased *GSTA2* transcriptional activity has been observed after HNF-1 protein binding to the *GSTA2* promoter (Clairmont et al., 1994). Although IL-1β-mediated reduction of *GSTA1* expression in Caco-2 cells occurs via the HRE, binding of specific HNF-1 isoforms to the HRE has not been demonstrated (Romero et al., 2002).

The present study was performed to investigate the mechanism by which IL-1β down-regulates *GSTA1* expression and the role of HNF-1 in the associated transcriptional repression. We hypothesized that *GSTA1* down-regulation by IL-1β is a result of differential binding of HNF-1 isoforms to the HRE in the *GSTA1* promoter. Our results indicate that IL-1β up-regulates expression and binding of the repressive vHNF-1C to the HRE. Overexpression of vHNF-1C reduces *GSTA1* transcriptional activity. The results of this study indicate an important repressive role for vHNF-1C in IL-1β-mediated down-regulation of *GSTA1*.

Materials and Methods

Recombinant Cytokines and Chemicals. Human recombinant IL-1β was purchased from R&D Systems (Minneapolis, MN). Antibodies against various human HNF-1 isoforms were obtained from BD Biosciences (Mississauga, ON, Canada). [γ-^{32}P]ATP (3000 mCi/mmol) was purchased from GE Healthcare (Piscataway, NJ). All other compounds were readily available commercially.

Cell Culture and Treatment. Caco-2 and HT-29 cells were cultured under 5% CO_2 at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum, and 100 μg/ml penicillin and streptomycin. For analysis of GSTA1, HNF-1α, and vHNF-1 mRNA levels, cells were treated with 10 ng/ml IL-1β for 24 h, after which total cellular RNA was collected.

RNA Isolation and Comparative Real-Time Reverse Transcription-Polymerase Chain Reaction Analysis. Total RNA was extracted from cells following the TRIzol reagent method (Invitrogen, Burlington, ON, Canada). Messenger RNA analysis was conducted by comparative real-time reverse transcription-polymerase chain reaction (RT-PCR) using a Roche Molecular Biochemicals (Indianapolis, IN) LightCycler instrument and the DNA Master SYBR Green I kit (Molecular Biochemicals). One microgram of RNA was first treated with 1 unit of DNase (RQ1 RNase-free DNase; Promega, Madison, WI). Complementary DNA was synthesized from 1 μg of RNA prepared from untreated and treated Caco-2 cells using 0.1 μg of random primers, 20 units of RNase inhibitor (RNasin; Promega), and 200 units of murine Moloney leukemia virus reverse transcriptase (Invitrogen). PCR was done in a 10-μl volume containing 1 μl of SYBR Green I and 5 μM each primer. The final Mg^+ concentration was 2 mM, and the PCR parameters used were as follows: an initial step at 95°C for 6 min and then 35 cycles of PCR (95°C, 15 s; 70°C, 5 s; and 70°C, 15 s). The following gene-specific primers were used: vHNF-1A/B: sense, 5′-ACGGCCTGGGCTCCAACTTGGTCACT-3′; antisense, 5′-AACCCTTAAACCAGATAAGATCCGT-3′; vHNF-1C: sense, 5′-ACGGCCTGGGCTCCAACTTGGTCACT-3′; antisense, 5′-TTCAACCTCCTCCTGAGACTGAGATCAT-3′; HNF-1α: sense, 5′-GACTTCACGCCACCCATCCT-3′; antisense, 5′-TGGGACAGGTGGGACTGGTT-3′; GSTA1: sense, 5′-AGCCGGGCTGACATTCATCT-3′; antisense, 5′-TGGCCTCCATGACTGCGTTA-3′; and GAPDH: sense, 5′-ACAGTCCATGCCATCACTGCC-3′; antisense, 5′-GCCTGCTTCACCACCTTCTTG-3′.

A standard curve was generated for the absolute quantification of vHNF-1A/B and vHNF-1C mRNA levels by real-time PCR. Complementary DNA for vHNF-1A/B and vHNF-1C was produced by PCR amplification from human genomic DNA using the primers listed above. Ten-fold dilutions of the PCR products were then used to construct the standard curve. To confirm that equal amounts of mRNA were assayed from each sample, data were normalized to GAPDH RNA levels.

Plasmids. The HNF-1α expression plasmid (pHNF-1α) was kindly provided by Marie-Josée Vilarem (Institut National de la Santé et de la Recherche Médicale, Montpellier, France) and vHNF-1C expression plasmid (pvHNF-1C) was obtained from Moshe Yaniv (Institut Pasteur, Paris, France). A *LUC* reporter gene plasmid construct containing 1591 base pairs of the 5′-flanking region of the *GSTA1* promoter was prepared by PCR with the following specific oligonucleotide primers: sense primer, 5′-CAGATTTC-CAAACTCCCCATA-3′ and antisense primer, 5′-TGAATTCCAG-GTCCTAATGTATTT-3′.

PCR was performed using human genomic DNA as a template. The amplified fragment was cloned into the pCR-TOPO cloning vector (Invitrogen) following the manufacturer's instructions and then digested with XhoI and HindIII to facilitate subcloning into the pGL3 luciferase vector (Promega). Plasmid DNA was purified and transformed into competent XL1 blue cells, and plasmid DNA was then harvested and purified using a Plasmid Maxi kit (QIAGEN, Mississauga, ON, Canada). The *GSTA1* promoter sequence was confirmed by direct sequencing, and the resultant plasmid was designated as pGSTA1-LUC.

Site-Directed Mutagenesis. The HRE in the proximal *GSTA1* promoter (GGAACACATTAAC; −182 to −170 base pairs) was mutated in pGSTA1-LUC by site-directed mutagenesis using the QuikChange site-directed mutagenesis kit (QIAGEN). The following oligonucleotide primers were designed to span and mutate the consensus sequence (the mutated positions for the HNF-1 binding site are underlined): sense, 5′-CAACCTTGAAAAGGAACACATCGC-CCAGTTTCTTCTGATAAGCAG-3′ and antisense, 5′-CTGCTTAT-CAGAAGAAACTGGGCGCCUATGTGTTCCTTTTAAGGTTG-3′. The presence of the mutation was confirmed by direct sequencing and the resultant plasmid was designated as pGSTA1ΔHNF-1-LUC.

Transfection and Luciferase Assay. Caco-2 cells were transfected in suspension with a density of 1.5×10^5 cells/well in a 24-well plate. A DNA mixture containing 800 ng/μl −1591 GSTA1-LUC reporter plasmid, 10 ng/μl pRL-TK plasmid (*Renilla reniformis*, luciferase plasmid), and increasing concentrations of the pHNF-1α were transfected using Lipofectamine (Invitrogen) according to the manufacturer's recommendations. After 24 h, medium containing 10 ng/ml IL-1β was added. Twenty-four hours later, cells were harvested and washed twice with phosphate-buffered saline, cell extracts were prepared, and aliquots of the lysates were assayed for luciferase enzyme activities as described by the dual-luciferase reporter assay system (Promega). Luciferase activities were normalized to *R. reniformis* luciferase activity to correct for differences in transfection efficiency. The plasmid pSV40-LUC, in which the *LUC* gene is under the control of a simian virus 40 promoter and enhancer, and the promoterless plasmid pLUC-0 served as positive and negative controls, respectively. All transfection studies were repeated at least three times.

Nuclear Extraction. Nuclear extracts were prepared as described by Dignam et al. (1983). In brief, 10^7 cells were harvested, washed, and resuspended in 200 μl of buffer A (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 2 μg/ml pepstatin, 10 μg/ml aprotinin, 0.5 M DTT, 0.575 mM PMSF, and 2 μg/ml leupeptin). Cell lysis was facilitated by passing the cells repeatedly through a 1 ml syringe fitted with a 25-gauge needle. Nuclei were recovered by centrifugation at 14,000*g* for 3 min at 4°C, resuspended in 60 μl of buffer C (20 mM HEPES, pH 7.9, 25% glycerol, 420 mM KCl, 1.5 mM $MgCl_2$, 2 mM EDTA, 2 μg/ml pepstatin, 10 μg/ml aprotinin, 0.5 M DTT, 0.575 mM PMSF, and 2 μg/ml leupeptin), and incubated on ice for 15 min. The samples were then centrifuged at 14,000*g* for 3 min at 4°C to obtain supernatants containing the nuclear fractions. Nuclear extracts were resuspended in 60 μl of buffer D (20 mM HEPES, pH 7.9,

25% glycerol, 0.2 mM EDTA, 2 μg/ml pepstatin, 10 μg/ml aprotinin, 0.5 M DTT, 0.575 mM PMSF, and 2 μg/ml leupeptin). Extracts were stored at −70°C until further analysis. Protein concentrations of nuclear extracts were determined by the Bradford assay using the Bio-Rad protein assay kit (Bio-Rad, Mississauga, ON, Canada).

Electrophoretic Mobility Shift Assay. Protein binding reactions (20 μl) were carried out in volume using 10 μg of nuclear extract and a Gel Shift assay kit (Promega) according to the manufacturer's instructions. For competition studies, samples were incubated with 100-fold molar excess of unlabeled oligonucleotide. The sequence of the HRE double-stranded oligonucleotide probe used for the gel shift assay was 5′-GAAAAGGAACACATTAACCAGTTT-3′. The DNA-protein complexes were resolved on a 6% acrylamide gel in 0.5× Tris borate-EDTA buffer at 200 V for 19 min. The gel was dried and then imaged by phosphorimaging using a Typhoon 9410 scanner (GE Healthcare). Samples were analyzed in triplicate, and gel shift assays results are presented from one representative experiment.

Streptavidin-Agarose DNA Binding Assay. Binding reactions (100 μl) were carried out using 50 μg of nuclear extract with 5 μg of a biotinylated double-stranded HRE probe (Invitrogen) consisting of the same GSTA1 HRE sequence indicated above and incubated with 200 μl of 50% streptavidin-agarose beads (Novagen, Madison, WI). The samples were incubated at room temperature with mixing for 1 h. Samples were then centrifuged at 5000*g* for 30 s and washed three times with ice-cold phosphate-buffered saline before being resuspended in 50 μl of Western loading dye. Samples were then analyzed by Western blot as described below.

SDS-Polyacrylamide Gel Electrophoresis and Western Blot Analysis. HNF-1α protein bound to HRE probes was identified by Western blot analysis as described previously (Romero et al., 2002). In brief, streptavidin-agarose affinity-purified protein was separated by SDS-polyacrylamide gel electrophoresis on a 12% polyacrylamide gel, transferred to nitrocellulose, and incubated for 1 h with rabbit anti-human HNF-1β polyclonal antibody diluted to 1:200. After incubation with goat anti-rabbit peroxidase secondary antibody (Vector Laboratories, Burlington, ON, Canada), bands on blots were detected by chemiluminescence (ECL Plus; GE Healthcare) and visualized using a Typhoon 9410 scanner (GE Healthcare). HNF-1β protein levels were then determined by densitometry. Three independent experiments were performed, and Western blots are presented from one representative experiment.

Statistical Analysis. Statistically significant differences among groups were identified by one-way analysis of variance. Data were further evaluated by Fisher's least significant difference test if groups were found to be significant. Significance was established at $p < 0.05$.

Results

Overexpression of HNF-1α Increases *GSTA1* Transcriptional Activity via the HRE in the Proximal *GSTA1* Promoter. To investigate the role of HNF-1α in *GSTA1* gene regulation, the effect of HNF-1α overexpression on *GSTA1* transcription was assessed and the involvement of the HRE in the proximal human *GSTA1* promoter was determined. Luciferase reporter assays were conducted on cell extracts from Caco-2 cells cotransfected with increasing concentrations of pHNF-1α and either pGSTA1-LUC or pGSTA1ΔHRE-LUC. Increasing concentrations of pHNF-1α resulted in concentration-related increases in *GSTA1* promoter activity in cells cotransfected with pGSTA1-LUC to a maximum of 3.5-fold ($p < 0.001$) relative to controls (Fig. 1). There was no effect of pHNF-1α overexpression on luciferase activity in cells cotransfected with pGSTA1ΔHRE-LUC; however, basal levels of *GSTA1* transcriptional activity were significantly reduced.

Mutation of the HNF-1 Response Element Abolishes IL-1β-Mediated Repression of *GSTA1* Transcriptional Activity. To confirm previous findings that the repressive effect of IL-1β on *GSTA1* expression is mediated through the HRE (Romero et al., 2002), luciferase activities were assessed in Caco-2 cells transfected with either pGSTA1-LUC or pGSTA1ΔHRE-LUC after treatment with 10 ng/ml IL-1β for 24 h (Fig. 2). IL-1β reduced luciferase activity to 74% in pGSTA1-LUC transfectants ($p < 0.05$); however, there was no change in cells transfected with pGSTA1ΔHRE-LUC.

IL-1β Represses GSTA1, but Not HNF-1α Expression at Various Stages of Confluence. During differentiation, Caco-2 cells acquire a phenotype closely resembling mature enterocytes, including markedly increased GSTA1 expression (Vecchini et al., 1997). To determine the importance of HNF-1α expression in GSTA1 up-regulation during differentiation and whether the mechanism of IL-1β-mediated repression of GSTA1 involves down-regulation of HNF-1α expression, the sensitivity of Caco-2 cells to IL-1β effects at different stages of confluence was examined. Caco-2 cells that were either preconfluent (50% confluent), confluent, or 5 days postconfluent were treated with IL-1β, and mRNA levels of both GSTA1 and HNF-1α were examined (Table 1). GSTA1 mRNA progressively increased to a maximum level in postconfluent cells that was 12-fold greater than that in preconfluent cells ($p < 0.05$). IL-1β treatment caused GSTA1 mRNA levels to decrease by 86, 92, and 96% ($p < 0.05$) in preconfluent, confluent, and postconfluent cells, respectively, compared with control cells at the same stage of confluence. Progressive increases in HNF-1α mRNA were observed during differentiation to maximum levels in postconfluent cells that were 3.5-fold higher ($p < 0.05$) than preconfluent levels. HNF-1α mRNA levels were unaffected by IL-1β at all stages of confluence. HNF-1α protein was also unaffected by IL-1β treatment (data not shown). Similar results were obtained in

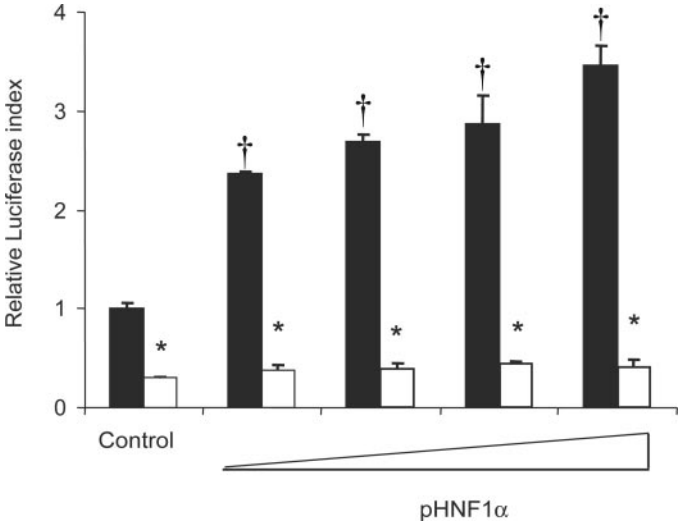

Fig. 1. HNF-1α-mediated increases in *GSTA1* transcriptional activity require a functional HRE. Caco-2 cells were transiently transfected with either pGSTA1-LUC (closed bars) or pGSTA1ΔHNF-1-LUC (open bars) as well as increasing concentrations of pHNF-1α (0.125–1.0 ng/well). Cell extracts were assayed for luciferase activity and normalized to *R. reniformis* luciferase activity. Values indicated by asterisk (*) differed significantly ($p < 0.001$) from cells cotransfected with pGSTA1-LUC and the same concentration of pHNF-1α. Values indicated by dagger (†) differ significantly ($p < 0.001$) from cells transfected with pGSTA1-LUC alone. $n = 3$.

confluent HT-29 and HepG2 cells in which GSTA1 mRNA levels were reduced to 55 and 68% of untreated levels ($p < 0.05$) after IL-1β treatment; however, there was no change in HNF-1α mRNA levels in either cell line.

HNF-1α Overexpression Does Not Attenuate IL-1β-Mediated Repression of *GSTA1* Transcriptional Activity. Because IL-1β does not alter HNF-1α expression, we postulated that IL-1β might interfere with binding of HNF-1α to the HRE. We then tested the possibility that HNF-1α overexpression might attenuate IL-1β-mediated transcriptional repression by increasing the likelihood of HNF-1α-HRE interactions. Cells were transfected with either an empty expression plasmid (pcDNA3.1; control cells) or with pHNF-1α and cultured in the presence or absence of IL-1β. In cells overexpressing HNF-1α, IL-1β reduced reporter activity to levels less than 50% of untreated cells ($p < 0.001$) in all but the highest level of HNF-1α overexpression in which luciferase activity was reduced to only 74% of control levels (Fig. 3A). Likewise, GSTA1 mRNA levels were significantly decreased ($p < 0.05$) after IL-1β treatment, despite overexpression of HNF-1α (Fig. 3B).

IL-1β Does Not Alter Nuclear Protein Binding to the HRE. The results of the reporter assays suggested a potential influence of IL-1β on HNF-1α binding to the HRE in the GSTA1 promoter. This possibility was assessed by EMSA by incubating a radiolabeled HRE probe with nuclear extracts from control and IL-1β-treated Caco-2 cells (Fig. 4). The specificity of complex formation was revealed by the fact that complexes were absent when excess nonradiolabeled probe (specific competitor) was included in the incubates, but complexes were present when incubated with nonradiolabeled activator protein-1 probe (nonspecific competitor). Moreover, complexes did not form between nuclear proteins and a probe with a mutated HNF-1 consensus sequence (ΔHRE). Treatment with IL-1β did not have a significant effect on complex formation with the probe compared with nuclear extracts from untreated control cells. To determine the specific proteins that were bound to the probe, a streptavidin-agarose binding assay was performed (see below).

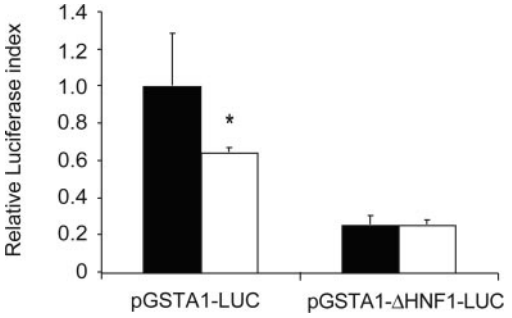

Fig. 2. Site-directed mutagenesis of the HRE abolishes transcriptional repression of *GSTA1* by IL-1β. Caco-2 cells transiently transfected with either pGSTA1-LUC or pGSTA1ΔHNF-1-LUC were either untreated (closed bars) or treated with IL-1β (open bars) for 24 h. Cell lysates were assayed for luciferase activity and normalized to *R. reniformis* luciferase activity. Values indicated by asterisk (*) differ significantly from untreated ($p < 0.05$). $n = 3$.

vHNF-1C Decreases *GSTA1* Transcriptional Activity. To determine whether vHNF-1C has a negative regulatory role on *GSTA1* transcriptional activity, luciferase reporter assays were performed in Caco-2 cells cotransfected with pGSTA1-LUC or pGSTA1ΔHRE-LUC and increasing concentrations of the pvHNF-1C expression plasmid (Fig. 5). There was a significant concentration-related decrease ($p < 0.05$) in *GSTA1* promoter activity in cells cotransfected with pGSTA1-LUC. However, transcriptional activity was not altered by vHNF-1C in cells transfected with pGSTA1ΔHRE-LUC.

IL-1β Increases vHNF-1C mRNA Levels in Caco-2 Cells. Although HNF-1α mRNA levels in Caco-2 cells were not affected by IL-1β, results of reporter assays clearly implicate an HRE-dependent mechanism associated with down-regulation of *GSTA1* by IL-1β. Thus, we hypothesized that IL-1β induces the expression of vHNF-1C subsequently resulting in increased vHNF-1C-HRE complex formation and GSTA1 down-regulation. Messenger RNA levels of vHNF-1C were measured in Caco-2 cells at different stages of confluence exposed to increasing doses of IL-1β (Fig. 6). At doses of 10 and 100 ng/ml, IL-1β significantly increased vHNF-1C mRNA levels in Caco-2 cells at all stages of confluence compared with controls. Likewise, treatment of HepG2 cells with 100 ng/ml IL-1β resulted in a 1.5 ± 0.01-fold increase in GSTA1 transcript levels ($p < 0.01$).

IL-1β Increases Binding of vHNF-1C to the HRE in the Proximal GSTA1 Promoter. To determine whether binding of HNF-1C proteins to the HRE is the mechanism responsible for IL-1β-mediated repression of GSTA1, a DNA binding assay based on the interaction of HNF protein with biotinylated HRE probes (identical to those used in EMSA) was performed. HRE-bound HNF1 proteins were identified by purifying the biotinylated probes on streptavidin-agarose beads followed by immunoblot analysis using polyclonal antibodies to human vHNF1 or HNF-1α. First, we demonstrated that vHNF-1C protein binds to the HRE, by assessing the formation of complexes using nuclear extracts from Caco-2 cells transfected with either pvHNF-1C or an empty

TABLE 1

Effect of IL-1β on GSTA1 and HNF-1α mRNA levels

Caco-2 cells were treated for 24 h with IL-1β and GSTA1 mRNA expression was determined by comparative real-time RT-PCR. Values represent the mean ± S.E. from three independent experiments expressed as -fold differences relative to untreated preconfluent cells.

Cell type	Treatment	Preconfluent		Confluent		Postconfluent	
		GSTA1	HNF-1α	GSTA1	HNF-1α	GSTA1	HNF-1
Caco-2	Control	1.00 ± 0.01	1.00 ± 0.11	2.56 ± 0.30	1.78 ± 0.21†	11.5 ± 2.94†	3.4 ± 0.19†
	IL-1β	0.14 ± 0.01*	0.91 ± 0.05	0.22 ± 0.02*	1.34 ± 0.11	0.50 ± 0.14*	2.89 ± 0.50
HT-29	Control	N.D.	N.D.	1.00 ± 0.10	1.00 ± 0.07	N.D.	N.D.
	IL-1β	N.D.	N.D.	0.55 ± 0.09*	0.93 ± 0.16	N.D.	N.D.
HepG2	Control	N.D.	N.D.	1.00 ± 0.08	1.00 ± 0.08	N.D.	N.D.
	IL-1β	N.D.	N.D.	0.68 ± 0.04*	1.00 ± 0.1	N.D.	N.D.

N.D., not determined.
* Significantly different from untreated cells at the same stage of confluence ($p < 0.05$).
† Significantly different from untreated preconfluent cells ($p < 0.05$).

plasmid (control). Forced expression increases the amount of vHNF-1C bound to the HRE and reduces HNF-1α-HRE complex formation, suggesting that vHNF-1C protein can displace HNF-1α from the HRE (Fig. 7A). The effect of IL-1β on HNF-1α and vHNF-1C binding to HRE was assessed in Caco-2 cells treated with increasing concentrations of IL-1β. IL-1β resulted in increases in vHNF-1C binding to the HRE to a maximum of 2.0-fold higher than control levels (Fig. 7B). In contrast, binding of HNF-1α to the HRE was reduced by increasing concentrations of IL-1β. Similar results were also found in IL-1β-treated HepG2 cells (data not shown).

IL-1β-Mediated Temporal Increases in vHNF-1C Expression Correspond to Enhanced Binding to the HRE. To determine whether temporal increases in vHNF-1C mRNA and protein levels by IL-1β occur concurrently, we conducted time-course experiments in Caco-2 cells (Fig. 8, A and B). Increases in vHNF-1C mRNA and protein levels were observed by 12 h after IL-1β treatment, however, HNF-1α expression remained unchanged. We also performed DNA binding assays to determine whether HNF-1α is displaced from the HRE as vHNF-1C is being recruited. Figure 8C show progressive increases in vHNF-1C-HRE complex formation that are concomitant with reductions in HNF-1α binding.

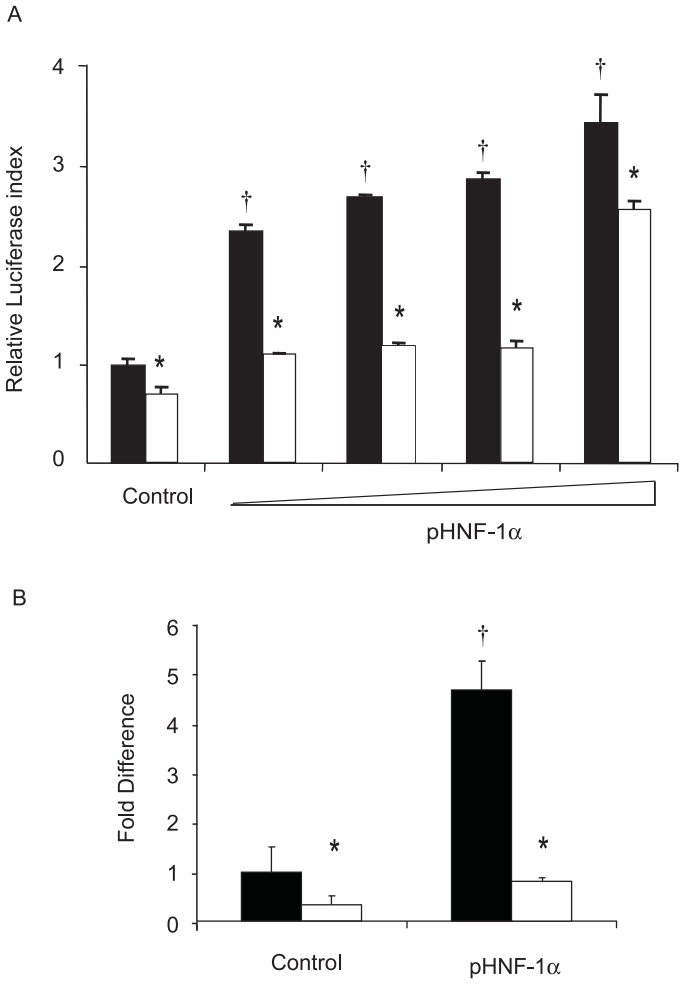

Fig. 3. HNF-1α-mediated increases in *GSTA1* transcription are repressed by IL-1β. A, Caco-2 cells were transiently transfected with the GSTA1-LUC reporter plasmid and increasing concentrations of pHNF-1α (0.125–1.0 ng/well). Cells were then either untreated (closed bars) or treated (open bars) with IL-1β for 24 h. Cell extracts were assayed for luciferase activity, and data were normalized to *R. reniformis* luciferase activity. Values indicated by asterisk (*) differed significantly ($p < 0.05$) from untreated cells cotransfected with pGSTA1-LUC and the same concentration of pHNF-1α. Values indicated by dagger (†) differ significantly ($p < 0.05$) from untreated cells transfected with pGSTA1-LUC alone. $n = 3$. (B) Caco-2 cells were transfected with either an empty expression plasmid (control) or pHNF-1α and were either untreated (closed bars) or treated with IL-1β (open bars) for 24 h. GSTA1 mRNA expression was determined by comparative real-time RT-PCR. Values indicated by asterisk (*) differ significantly from corresponding untreated cells ($p < 0.05$). Values indicated by dagger (†) differ significantly from untreated control cells ($p < 0.05$). $n = 3$.

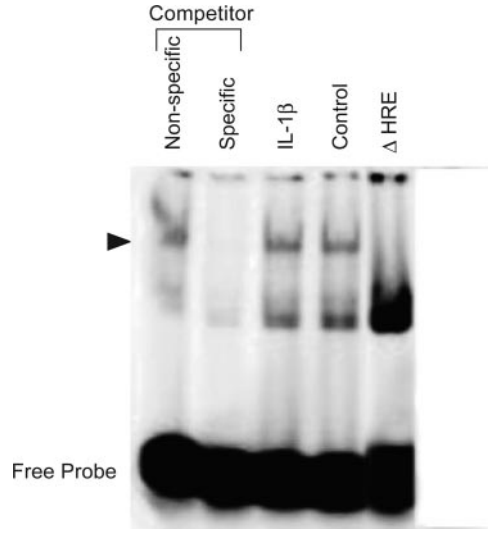

Fig. 4. IL-1β does not alter nuclear protein binding to the HRE. Representative EMSA of nuclear extracts from Caco-2 cells that were either untreated or treated with IL-1β for 24 h. Nuclear extracts were incubated with either ^{32}P-labeled HRE oligonucleotide or ^{32}P-labeled ΔHRE oligonucleotide probe and electrophoresed on a 6% polyacrylamide gel. The arrowhead indicates nuclear protein bound to HRE oligonucleotide probes. For competition assays, a 100-fold molar excess of unlabeled HRE oligonucleotide (specific) or Sp1 oligonucleotide (nonspecific) probe was added to the incubation reaction mixture. $n = 3$.

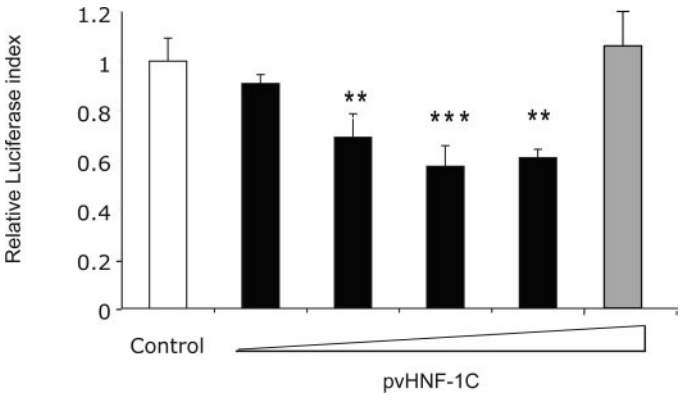

Fig. 5. Overexpression of vHNF-1C represses GSTA1 transcriptional activity. Confluent Caco-2 cells were transiently transfected with pGSTA1-luc (closed bars) or pGSTA1ΔHNF-1-LUC (shaded bar) and cotransfected with either increasing concentrations of pvHNF-1C (1–8 ng/well) or an empty expression plasmid (control; open bar). Twenty-four hours later, cells were harvested, and luciferase activities were measured and corrected for differences in transfection efficiency based on *R. reniformis* activity. Luciferase activities (mean ± S.E.) are expressed as a percentage relative to the 100% untreated control values. Results are the mean of three independent experiments. Significant differences from control values are designated as *, $p < 0.05$) and **, $p < 0.01$.

Discussion

IL-1β regulates numerous enzymes through transcriptional mechanisms involving various transcription factors, including activator protein-1, members of the CCAAT/enhancer-binding protein family, HNF-1, and nuclear factor-κB (Muegge and Durum, 1989). Previous evidence from our laboratory has implicated HNF-1 in IL-1β-mediated down-regulation of *GSTA1* (Romero et al., 2002). HNF-1 is a dimeric transcriptional activator required for basal expression of a wide range of genes, including enzymes involved in drug metabolism and detoxification (Park et al., 2004). For example, transcription of the *UDP-glucuronosyltransferase-1A6*, *-1A8*, *-1A9*, and *-1A10* genes, is activated by HNF-1 binding to their proximal promoters (Auyeung et al., 2003; Gregory et al., 2004). In addition, transcription of the rodent *GSTA2* gene is dependent on promoter binding by HNF-1 (Whalen et al., 2004). The relationship between HNF-1 and *hGSTA1* gene expression has not yet been established.

Several findings from this study indicate a regulatory role for HNF-1α in the control of *GSTA1* transcription. Overexpression of HNF-1α protein increased *GSTA1* transcriptional activity in a concentration-dependent manner in luciferase reporter assays. Transcriptional activation of *GSTA1* by HNF-1α is mediated through an HRE in the proximal promoter, because mutation of this site abrogated the effect. Moreover, constitutive transcriptional *GSTA1* activity is significantly lower when the HRE is mutated. These findings support the results of other studies indicating a transactivator role of HNF-1α in the regulation of α class GSTs. For example, reduced levels of *hGSTA2* are highly correlated with low levels of HNF-1 expression in renal carcinomas (Clairmont et al., 1994; Anastasiadis et al., 1999). Reporter gene assays revealed that transactivation occurs through HNF-1 binding to the *GSTA2* promoter. Although these findings demonstrate a transactivator role for HNF-1α in GST expression, other studies indicate that members of the HNF family mediate transcriptional repression. For example, HNF-1 plays a significant role in the down-regulation of *GSTA2* in rats by IL-6 and dexamethasone (Voss et al., 2002; Whalen et al., 2004). Moreover, reduction of human GSTA2 gene expression by ceramide is mediated by HNF-1 (Park et al., 2004). It has been reported that different isoforms determine whether the regulatory function of HNF-1 is transactivation or repression (Bach and Yaniv, 1993). For example, differential binding of the various isoforms of HNF-1α and vHNF-1 controls transcriptional activation or repression of the intestinal sucrase-isomaltase gene (Wu et al., 1994; Boudreau et al., 2001). A repressive role of vHNF-1 has been demonstrated for the regulation of other genes, including aldolase B and dipeptidyl peptidase IV (Vallet et al., 1995; Erickson et al., 2000). For example, Vallet et al. (1995) demonstrated that vHNF-1 antagonized HNF-1α-dependent transactivation of the aldolase B gene expression in renal proximal tubular cells. Moreover, we have recently shown that down-regulation of *GSTA1* by chemical inducers of ro-

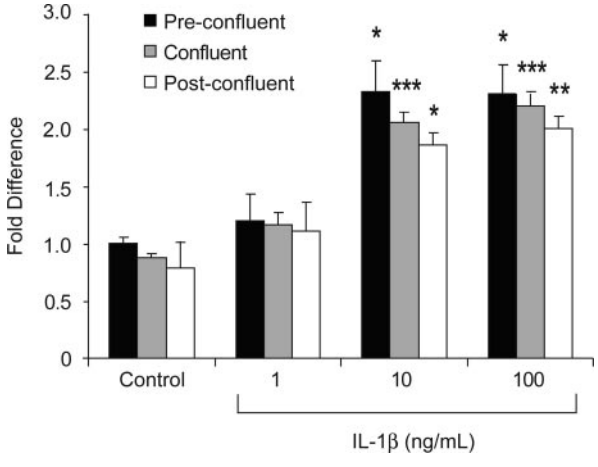

Fig. 6. IL-1β increases vHNF-1C mRNA expression in confluent cells. Caco-2 cells that were preconfluent, confluent, or postconfluent were treated for 24 h with increasing concentrations of IL-1β, and -fold differences in vHNF-1C mRNA expression were assessed by comparative real-time RT-PCR. Values represent the mean ± S.E. from three independent experiments expressed relative to untreated preconfluent cells. Significant differences from untreated cells at the same stage of confluence are designated as *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

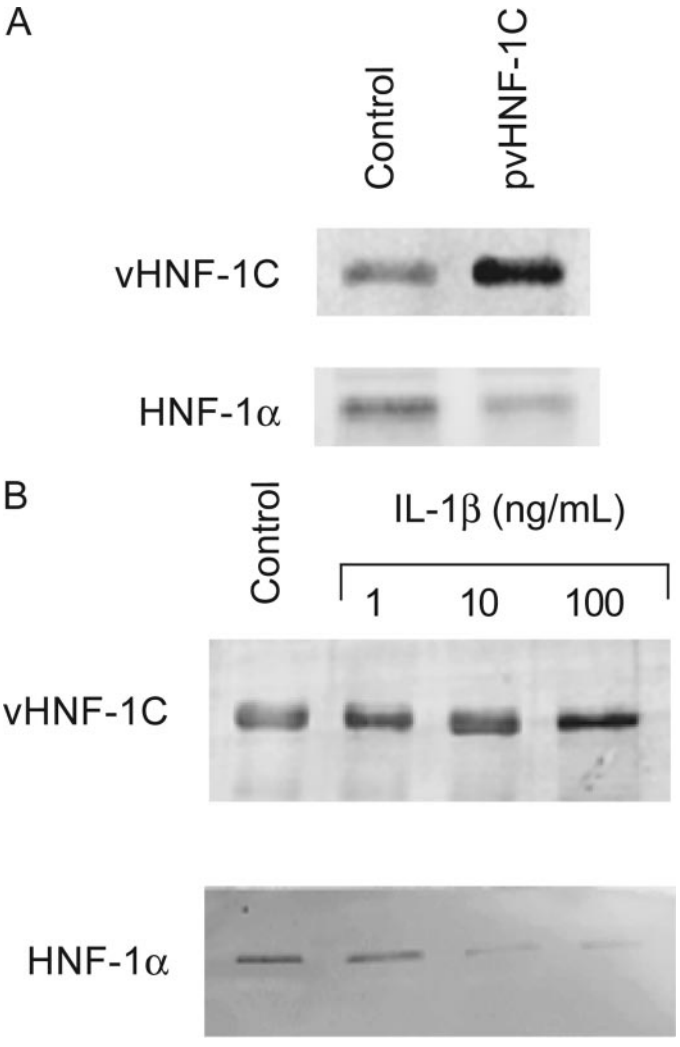

Fig. 7. IL-1β increases binding of vHNF-1C to the HRE. Binding reactions were carried out using nuclear extracts from Caco-2 cells and a biotinylated GSTA1 HRE probe identical to that used for the EMSA (see Fig. 4). The binding reactions were then incubated with streptavidin-agarose, washed, and HNF1 protein bound to the biotinylated probe was collected in 1% SDS, 10 mM DTT, and analyzed by Western blot analysis using polyclonal antibodies to either vHNF-1 or HNF-1α. A, DNA binding reactions were carried out using Caco-2 cells transfected with either an empty expression vector (control) or pvHNF-1C. B, DNA binding reactions were carried out using Caco-2 cells treated with increasing concentrations of IL-1β. Experiments were repeated twice with similar results.

dent GSTs occurs via a mechanism involving enhanced binding vHNF-1C to the HRE in the GSTA1 promoter (Romero et al., 2006). Taken together, these data indicate an important regulatory role for HNF-1 in *GSTA1* gene regulation; however, treatment conditions may determine whether HNF-1 has repressive or transactivating function based on the differential induction and dimerization of the various isoforms of HNF-1α and vHNF-1.

In vitro studies have shown that proinflammatory cytokines decrease *rGSTA* expression in rat hepatocytes in primary culture (Maheo et al., 1997; Whalen et al., 2004) and that *hGSTA* expression is down-regulated by IL-1β in Caco-2 cells (Romero et al., 2002). More recently, it has been shown that IL-1β represses the orphan nuclear constitutive androstane receptor, which in turn is able to repress CAR-mediated up-regulation of *GSTA1* by bilirubin and phenobarbital in human hepatocytes (Assenat et al., 2004). The findings of the current study have elucidated the underlying regulatory mechanisms in IL-1β-mediated repression of *GSTA1* by demonstrating the involvement of the HRE in the proximal GSTA1 promoter because mutation of this site abrogated the repressive effect. Because expression of both *GSTA1* and HNF-1α increases in postconfluent Caco-2 cells, we reasoned that higher levels of HNF-1α might compensate for the repressive effects of IL-1β. However, IL-1β markedly reduced GSTA1 mRNA levels in Caco-2 at all stages of confluence. Because IL-1β did not alter HNF-1α mRNA at any stage of confluence, we concluded that *GSTA1* repression was not a result of HNF-1α down-regulation. Furthermore, overexpression of HNF-1α did not attenuate the repressive effects of IL-1β, indicating that deficiency of HNF-1α was not a factor in the mechanism underlying GSTA1 down-regulation.

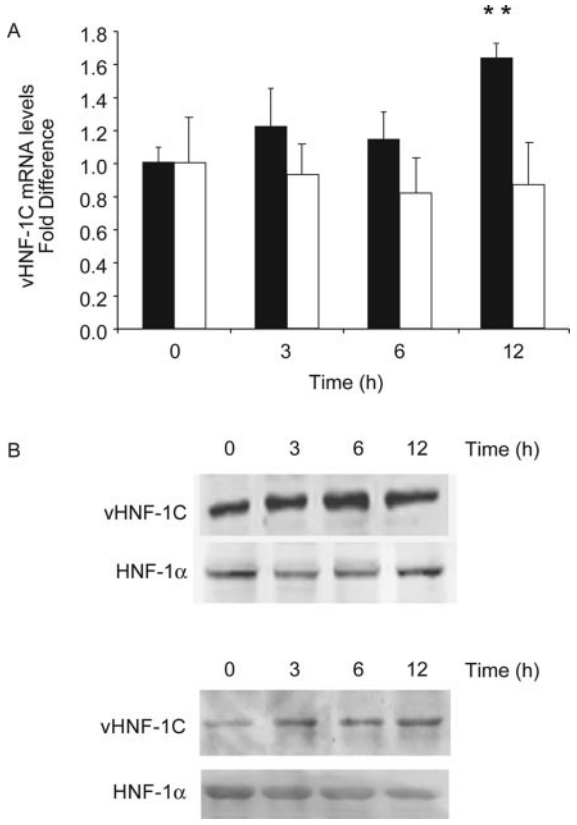

Fig. 8. Temporal increases in vHNF-1C expression by IL-1β correspond to enhanced binding to the HRE. A, Caco-2 cells were treated with IL-1β and vHNF-1C (closed bars) and HNF-1α (open bars) mRNA levels were determined at 3, 6, and 12 h by comparative real-time RT-PCR. Values represent the mean ± S.E. from three independent experiments expressed as -fold differences relative to untreated cells. Values indicated by double asterisk (**) differed significantly from untreated cells at the same stage of confluence ($p < 0.01$). B, temporal changes in vHNF-1C and HNF-1α protein levels were analyzed by Western blot analysis using nuclear extracts isolated from Caco-2 cells at 3, 6, and 12 h after IL-1β treatment. C, binding reactions were carried out by incubation of nuclear extracts with a biotinylated GSTA1 HRE probe, purification on streptavidin-agarose and Western blot analysis using polyclonal antibodies to either vHNF-1 or HNF-1α. Experiments were repeated twice with similar results.

Given that IL-1β had no apparent effect on HNF-1α expression, the extent to which IL-1β influences HNF-1 binding activity was determined by EMSA by examining complex formation between nuclear extracts from IL-1β-treated cells and a radiolabeled probe containing the HNF-1 site. EMSA revealed that IL-1β had no effect on the extent to which complexes formed between nuclear proteins and the HRE, suggesting that either binding of HNF-1α to the HRE had not been altered by treatment or that it had been replaced by another HNF-1 isoform. Indeed, both HNF-1α and vHNF-1 are capable of binding to the HRE; however, the identity of the *trans*-acting factor bound to the HRE probe could not be assessed by conventional EMSA. Although there was no change in the intensity of the shifted band after IL-1β treatment, we hypothesized that the complex consisted of either vHNF-1 monomers or heterodimers with HNF-1α. Distinguishing the specific vHNF-1 isoforms that may be bound to complexes is particularly important in view of the transcriptional repressor function of the vHNF-1C isoform and the transactivator roles played by the A and B isoforms (Bach and Yaniv, 1993). Thus, to test this hypothesis we first verified that overexpression of vHNF-1C has a repressive effect on *GSTA1* transactivation. Moreover, in a previous study we showed that vHNF-1C mRNA levels were high in preconfluent Caco-2 cells (and markedly higher than HNF-1α) but comparatively lower in postconfluent cells (Romero et al., 2006). This finding may partially explain the differences observed in *GSTA1* expression in pre- and postconfluent cells. More importantly, IL-1β caused concentration-dependent increases in vHNF-1C levels in Caco-2 cells at all stages of confluence, suggesting that IL-1β-mediated repression of *GSTA1* may be an indirect consequence of vHNF-1C overexpression. DNA binding assays performed with nuclear extracts from cells in which vHNF-1C is overexpressed demonstrated that the capacity of vHNF-1C to bind to the HRE varies with expression levels. Moreover, vHNF-1C binding was increased and HNF-1α binding was decreased by IL-1β in a concentration-dependent manner. Overall, these data suggest that IL-1β-mediated reductions in *GSTA1* gene expression are a result of increased vHNF-1C expression, enhanced vHNF-1C-HRE complex formation, and associated transcriptional repression of *GSTA1*.

The majority of studies concerning *GSTA* regulation have focused on HNF-1α; therefore, the dynamic relationship between other HNF-1 isoforms, particularly vHNF-1C, and *GSTA1* expression has yet to be fully elucidated. Further studies concerning the repressive role of vHNF-1 may provide a clearer understanding of the mechanisms of IL-1β-mediated down-regulation of *GSTA1* during inflammatory conditions and the compromised cytoprotective capacity of α class GSTs in the inflamed tissues.

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Address correspondence to: Dr. Gordon M. Kirby, Department of Biomedical Sciences, University of Guelph, Guelph, ON, Canada N1G 2W1. E-mail: gkirby@uoguelph.ca