Modulation of Hepatic Cytochrome P450s by *Citrobacter rodentium* Infection in Interleukin-6- and Interferon- γ -Null Mice^[S]

Beatrice A. Nyagode, Choon-Myung Lee, and Edward T. Morgan

Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia

Received June 16, 2010; accepted August 17, 2010

ABSTRACT

After infection with *Citrobacter rodentium*, murine hepatic cytochrome P450 (P450) mRNAs are selectively regulated. Several serum proinflammatory cytokines are elevated, the most abundant being interleukin-6 (IL6). To elucidate the role of cytokines in the regulation of P450s during infection, we orally infected wild-type, IL6(–/–), or interferon- γ (–/–) [IFN γ (–/–)] female C57BL/6J mice with *C. rodentium* and analyzed hepatic P450 expression 7 days later. The majority of P450 mRNAs were equally affected by infection in each genotype, indicating that IL6 and IFN γ are not the primary mediators of P450 down-regulation in this disease model. The down-regulation of CYP3A11 and CYP3A13 and induction of CYP2D9 mRNAs were attenuated in the IL6(–/–) mice, suggesting a role of IL6 in the regulation of only these P450s. Similar evidence im-

plicated IFN γ in the regulation of CYP2D9, CYP2D22, CYP3A11, CYP3A25, and CYP4F18 mRNAs in *C. rodentium* infection and CYP2B9, CYP2D22, and CYP2E1 in the bacterial lipopolysaccharide model of inflammation. This is the first indication of an in vivo role for IFN γ in hepatic P450 regulation in disease states. The deficiency of IL6 or IFN γ affected serum levels of the other cytokines. Moreover, experiments in cultured hepatocytes demonstrated that tumor necrosis factor α (TNF α) is the most potent and efficacious of the cytokines tested in the regulation of murine P450 expression. It is therefore possible that part of the IFN γ (–/–) and IL6(–/–) phenotypes could be attributed to the reduced levels of TNF α and part of the IFN γ (–/–) phenotype could be caused by reduced levels of IL6.

Introduction

In humans and experimental animals, infections or inflammatory stimuli cause changes in the activity and expression levels of cytochrome P450s (P450s) in the liver and other tissues (Morgan, 1997; Renton, 2004). Such changes can have adverse effects because these proteins catalyze the biosynthesis and catabolism of molecules of physiological importance and the metabolic clearance of the majority of clinically used drugs.

Lipopolysaccharide (LPS; endotoxin), a toxic component of the cell wall of Gram-negative bacteria, has been used extensively as a model of inflammation. Its administration to mice down-regulates the expression and/or activity of most P450s examined (Warren et al., 1999, 2001; Siewert et al., 2000;

Li-Masters and Morgan, 2001; Ashino et al., 2004; Goralski et al., 2005; Richardson and Morgan, 2005; Xu et al., 2006; Chaluvadi et al., 2009b). We reported previously that murine hepatic P450 mRNAs are selectively modulated during *Citrobacter rodentium* infection in a pattern that is different from the LPS model (Richardson et al., 2006; Chaluvadi et al., 2009a). *C. rodentium*, a noninvasive rodent pathogen, causes colitis in mice that is similar to that caused by the human pathogen enteropathogenic *Escherichia coli* and is therefore used as a model of its infection (Higgins et al., 1999; Gonçalves et al., 2001). The pattern of regulation of hepatic P450 expression is similar in both C3H/HeOuJ and C57BL/6 strains of mice infected with *C. rodentium*, with most P450 isoforms being down-regulated. CYP4A mRNAs and proteins are the most sensitive to this down-regulation.

To predict the effects of an inflammatory disease state on drug metabolism in humans, it is necessary to understand the mechanisms by which P450s are regulated during inflammation. In the early stages of a response to inflammatory stimuli, different cytokines are released into the

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.
doi:10.1124/jpet.110.171488.

[S] The online version of this article (available at <http://jpet.aspetjournals.org>) contains supplemental material.

ABBREVIATIONS: P450, cytochrome P450; IL, interleukin; IFN, interferon; WT, wild type; CFU, colony-forming units; TNF α , tumor necrosis factor α ; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MIP1 α , macrophage inflammatory protein 1 α ; MPO, myeloperoxidase; NOS, nitric-oxide synthase; FBG, α -fibrinogen; AGP, α 1-acid glycoprotein; AGT, angiotensinogen; RT-PCR, reverse transcription-polymerase chain reaction; LPS, lipopolysaccharide; KC, C-X-C motif ligand 1; IP-10, interferon-inducible protein-10; Ctrl, control; Inf, infected.

systemic circulation. Mice infected with *C. rodentium* exhibit significant elevations in serum levels of several cytokines, including interleukin-6 (IL6) and interferon- γ (IFN γ) (Chaluvadi et al., 2009a) that are capable of modulating P450 expression in cell cultures. This raises the question of whether these cytokines contribute to hepatic P450 regulation during infection with *C. rodentium*.

The suppression of the constitutive expression of different isoforms from CYP1–CYP3 families by IL6 has been documented in hepatocyte cultures of different mammals including pigs (Kleine et al., 2008), rabbits (Kourylko et al., 2006), rats (Williams et al., 1991; Chen et al., 1995), and humans (Abdel-Razzak et al., 1993; Muntané-Relat et al., 1995; Aitken and Morgan, 2007). There is also evidence that IL6 has significant *in vivo* roles in rodents. Ashino et al. (2004) reported that IL6 deficiency blocked the down-regulation of CYP3A11 and CYP2C29 in mice treated with Bacillus Calmette-Guerin vaccine. The turpentine-induced down-regulation of CYP1A2, CYP2A5, and CYP3A11 mRNAs observed in wild-type (WT) mice was completely abolished in IL6-deficient [IL6(–/–)] mice (Siewert et al., 2000). The injection of rats with IL6 caused the suppression of CYP2C11 and CYP2E1 but not CYP3A2 mRNA (Morgan et al., 1994).

IFN γ has immunomodulatory effects on several cell types, including the activation of murine macrophages that have microbicidal activity (Dalton et al., 1993). Compared with WT mice, IFN γ -deficient [IFN γ (–/–)] mice have been reported to be significantly more susceptible to infection by *Mycobacterium bovis* (Dalton et al., 1993), *Listeria monocytogenes* (Huang et al., 1993), and *C. rodentium* (Simmons et al., 2002). The injection of rats with IFN γ resulted in the suppression of CYP3A2 mRNA, whereas the activities associated with CYP2C11 and CYP2A1 were unaffected (Craig et al., 1990). IFN γ has been reported to down-regulate the mRNA and/or activity levels of different P450 isoforms in the hepatocytes of rats (Tapner et al., 1996) and humans (Abdel-Razzak et al., 1993; Donato et al., 1997; Aitken and Morgan, 2007).

The cytokines that contribute to the regulation of hepatic P450s in *C. rodentium* infection are unknown. As noted above, IL6 has been demonstrated to be important for P450 down-regulation in at least two models of sterile inflammation. Although IFN γ can down-regulate human and rodent P450s *in vivo* and in cell culture, the effect of IFN γ gene deletion on P450 regulation has not been tested in any *in vivo* inflammatory disease model. Therefore, the objective of this study was to examine the role of IL6 and IFN γ in mediating the regulation of constitutive P450s of the mouse liver during *C. rodentium* infection and the role of IFN γ in the LPS model of inflammation, by comparing the responses to these agents in WT and cytokine-deficient mice.

Materials and Methods

Unless otherwise specified, all reagents and chemicals were obtained from Sigma-Aldrich (St. Louis, MO).

Mice. Female mice, 3, 5, 7, and 9 weeks old, were purchased from The Jackson Laboratory (Bar Harbor, ME). IL6- and IFN γ -deficient mice were originally generated on mixed C57BL/6 $\times$ 129 background and subsequently back-crossed for more than 10 generations to a C57BL/6J background (Dalton et al., 1993; Kopf et al., 1994). C57BL/6J mice were used as WT controls. The mice were housed in groups of four or six to a cage and were acclimatized to the animal

facility for at least 1 week before the beginning of an experiment. All protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Emory University.

Animal Treatment. In the *C. rodentium* experiments, 9-week-old mice were housed in groups of four to a cage. Each treatment with cytokine-null mice was carried out in two separate experiments for a total of eight animals per treatment group. The experiment involving animals of different age groups was done once with four animals per treatment group. A wild-type strain of *C. rodentium* (51116) was obtained from the American Type Culture Collection (Manassas, VA). After overnight growth in Luria broth, without shaking at 37°C, the bacteria were serially diluted in sterile phosphate-buffered saline, and the nominal concentration was calculated spectrophotometrically. Infected mice were administered an estimated 2×10^8 colony-forming units (CFU) per mouse of *C. rodentium* in 20% sucrose solution instead of drinking water for 24 h. This nominal dose was found to be effective in previous work (Richardson et al., 2006). To minimize differences between infections, all animals under treatment were infected with the same bacterial preparation. Control mice received 20% sucrose in the same time period. The bacterial concentrations given to each cage were determined by retrospective plating on MacConkey agar from the volumes of liquid consumed. Actual doses were between 3 and 7×10^7 bacteria/mouse in the cytokine-null experiment and between 8 and 14×10^7 bacteria/mouse in the age group experiment. Infected mice were housed in a Biosafety level 2 facility to prevent transmission of infection to other mouse colonies. Food consumption and changes in body weight were monitored daily, and animals were sacrificed 7 days after administration of sucrose or bacteria. In the LPS experiment, mice were housed in groups of six to a cage. Mice in the treatment group were given an intraperitoneal injection delivering 1 mg/kg LPS (*E. coli* 0111:B4, ultrapure; Invitrogen, Carlsbad, CA) dissolved in sterile saline, whereas control mice received an intraperitoneal injection of sterile saline alone. Food was withheld from the animals after the injection, and the animals were sacrificed after 24 h.

Tissue Collection. Blood was collected from the animals at sacrifice and allowed to clot for approximately 30 min at room temperature. Serum was separated by centrifugation for 10 min at 7500g and stored at –80°C until analyzed. Liver and spleen were dissected out of the abdominal cavity and rinsed in cold 1.15% potassium chloride then weighed. The liver was then portioned, flash-frozen, and stored at –80°C for subsequent RNA or microsome preparation or kept on ice for the determination of viable bacteria. The colon was removed, washed of fecal matter by using cold 1.15% potassium chloride, sectioned, and kept on ice for the determination of viable bacteria and myeloperoxidase (MPO) analysis.

Determination of Tissue Bacterial Loads. The number of viable bacteria was determined from organ homogenates. Liver and colon were weighed and homogenized at low speed with a tissue-mixer (IKA Works, Inc., Wilmington, NC) in 1 ml of phosphate-buffered saline. Liver homogenate, blood, or serial dilutions of the colon homogenates were plated onto MacConkey's agar on which *C. rodentium* forms small pink colonies. The number of CFU was determined after overnight incubation at 37°C, and the results were reported per gram of tissue or 50 μ l of blood.

Microsome Preparation. Liver homogenates were first centrifuged at 7500g (25 min) to yield a supernatant that was again centrifuged for 35 min at 250,000g. The resulting microsomal pellet were resuspended in 10 mM Tris acetate buffer, pH 7.4, containing 0.1 mM EDTA and 23% glycerol, and stored at –80°C. Protein concentrations were determined with a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Waltham, MA) with bovine serum albumin as the protein standard.

Hepatocyte Isolation and Treatment. Mouse hepatocytes were isolated by a two-step *in situ* collagenase perfusion procedure as described previously (Sewer and Morgan, 1997). After anesthesia with ketamine/xylazine solution, the livers of female WT C57BL/6J mice were perfused via the portal vein with 120 to 150 ml of Krebs-

Ringer-bicarbonate buffer followed by 50 to 70 ml of 0.3 mg/ml collagenase type IV, at a flow rate of 14 to 15 ml/min. Hepatocytes with at least 80% viability were then plated on 12-well collagen-coated plates (BD Biosciences, San Jose, CA) with Williams E medium (Atlanta Biologicals, Norcross, GA) containing 10 mM HEPES, pH 7.4, 150 nM insulin, 50 nM dexamethasone, 10 mg/ml penicillin/streptomycin, and 10% fetal bovine serum. After 4 h, cells were overlaid with fresh Williams E medium containing 0.23 mg/ml Matrigel (BD Biosciences) and allowed to incubate overnight. Cultures were maintained at 37°C in 5% carbon dioxide throughout the experiment. The effects on untreated cells of regular media changes either every day or every other day were compared over a 6-day period to optimize experimental conditions. To investigate the effect of different cytokines on P450 mRNA levels over time, the primary cultures were treated on the third or sixth day with 10 ng/ml each of mouse recombinant IL6 (Sigma-Aldrich), IFN γ (Calbiochem, San Diego, CA), or tumor necrosis factor α (TNF α ; R&D Systems, Minneapolis, MN), and the cells were harvested after 6, 12, and 24 h. The hepatocytes were also treated with 1, 3, 10, or 30 ng/ml of each cytokine for 24 h. All cytokines were reconstituted and stored according to manufacturer specifications as high concentration stocks. They were diluted from stock solutions into media just before treatment.

RNA Extraction, cDNA Synthesis, and Quantitative Reverse Transcription-Polymerase Chain Reaction. Total liver and hepatocyte RNA were prepared by using RNA-Bee isolation reagent (Tel-Test Inc., Friendswood TX), according to the manufacturer's instructions. RNA concentration was determined spectrophotometrically by measuring absorbance at 260 nm, and RNA purity and integrity were confirmed by formaldehyde-agarose gel electrophoresis followed by visualization with ethidium bromide.

To synthesize cDNA, purified total RNA was reverse-transcribed with a SuperScript First-Strand Synthesis System kit (Invitrogen), according to the manufacturer's protocol. Primers were custom-synthesized by MWG Biotech, Inc. (High Point, NC) or Operon Biotechnologies, Inc. (Huntsville, AL) and have been published previously (Richardson and Morgan, 2005; Chaluvadi et al., 2009a). The relative expression of mouse liver mRNAs was measured by real-time RT-PCR using the ABI PRISM 7000 Sequence Detection System and SyBr Green Master Mix reagent (Applied Biosystems, Foster City, CA) as described previously (Richardson and Morgan, 2005). To normalize the intersample variation in quality inherently associated with RNA preparation, the transcription level of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was quantified for all samples. No significant differences were detected in GAPDH expression among treatment groups. The value obtained from each target gene was then normalized by using the GAPDH value to calculate the relative target gene mRNA levels in comparison with the corresponding control groups by the C_t method described by Livak and Schmittgen (2001). The expression level in control samples was arbitrarily set at 1.

Immunoblotting. P450 protein levels in mouse hepatic microsomes were measured by Western blotting and chemiluminescent detection as described previously (Chaluvadi et al., 2009a). P450 antibodies were generously provided by Dr. James Halpert (University of Texas Medical Branch, Galveston, TX; rat CYP2B and CYP3A), Dr. Gordon Gibson (University of Surrey, Guildford, United Kingdom; rat CYP4A), Dr. Magnus Ingelman-Sundberg (Karolinska Institute, Stockholm, Sweden; rat CYP2E1), and Dr. Masahiko Negishi (National Institute of Environmental Health Sciences, Research Triangle Park, NC; mouse CYP2D9). Polyclonal antibodies to rat CYP2B, CYP3A, CYP4A, CYP2D, and CYP2E proteins were diluted 1:5000, whereas CYP2C was diluted 1:20,000. All assays were performed within a linear range. Proteins were detected by using a SuperSignal West Pico chemiluminescent substrate kit (Thermo Fisher Scientific). The intensity of chemiluminescent protein bands were visualized by fluorography on X-ray film and scanned, and the

protein bands were quantified by densitometry using Kodak Molecular Imaging software (Eastman Kodak Co., Rochester, NY).

Cytokine Analysis. Serum samples were assayed for the cytokines IL1 β , IL2, IL6, IFN γ , and TNF α and the chemokines C-X-C motif ligand 1 (KC), C-X-C motif chemokine 10 [interferon-inducible protein-10 (IP-10)], and macrophage inflammatory protein 1 α (MIP1 α) by using a Milliplex mouse cytokine/chemokine kit (Millipore Corporation, Billerica, MA) following the manufacturer's protocol as described previously (Chaluvadi et al., 2009a). Data were analyzed by using MasterPlex software 1.2 (Hitachi Software Engineering America, Ltd., San Francisco, CA), and the concentrations are expressed in pg/ml.

Myeloperoxidase Analysis. Colon segments were weighed then homogenized in a buffer containing 0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer, pH 6.0. The homogenates were then subjected to three freeze-thaw cycles, followed by sonication on ice for 10 s (Microson Ultrasonic cell disruptor; Misonix Inc., Farmingdale, NY). The resulting solution was centrifuged at 13,000g for 15 min at 4°C. Protein concentration in the supernatant was measured with a bicinchoninic acid protein assay kit (Thermo Fisher Scientific) with bovine serum albumin as the protein standard. Aliquots of supernatant containing 10 μ g of protein or MPO standard were added to 200 μ l of substrate (0.167 mg of *o*-dianisidine per ml, 0.0005% hydrogen peroxide in extraction buffer), and the reaction was allowed for 10 min. MPO activity was calculated by comparison with a standard curve generated within a linear range of detection with the authentic enzyme when measured colorimetrically at 450 nm with a Thermomax microplate reader (Molecular Devices, Sunnyvale, CA). MPO activity is expressed as units per milligram of protein. One unit of enzyme activity is defined as the amount that consumes 1 μ mol of H $_2$ O $_2$ /min.

Statistical Analysis. All data are presented as mean values $\pm$ S.E.M. unless otherwise designated. Control and experimental groups were compared either by unpaired Student's *t* test or one-way analysis of variance followed by Dunnett's test. All statistical analysis was done with SPSS software (SPSS Inc., Chicago, IL).

Results

***C. rodentium* Infection of Cytokine-Null Animals.** We reported previously that after *C. rodentium* infection the time course of P450 regulation in mice followed that of colonic inflammation and bacterial colonization, peaking at 7 to 10 days after infection (Chaluvadi et al., 2009a). We therefore carried out these studies 7 days after infection and compared the infected animals (Inf) with untreated control groups (Ctrl), because our previous study found that pair-fed groups were similar to untreated controls. Each experiment with WT, IL6(−/−), or IFN γ (−/−) genotypes was performed in two separate groups of four mice. The results were essentially identical in the duplicate experiments; therefore, they were combined in this article. All animals survived the duration of the *C. rodentium* infection. In the present study, no mice exhibited obvious signs of infection with the exception of slight diarrhea. There was no significant change in body weight (data not shown). The livers of infected mice were 14 to 25% larger and their spleens were 55 to 90% heavier than those of mice not infected with *C. rodentium* (Table 1).

Tissue Bacterial Loads. To measure bacterial loads, blood, liver, and colon homogenates were cultured on MacConkey agar plates. In infected WT, IL6(−/−), and IFN γ (−/−) mice, the bacterial loads in the blood and liver were at least 10⁸ times lower than those in the colon (Table 2). The bacterial load was higher in the blood of WT than cytokine-deficient mice, although not significantly so because of

TABLE 1

Liver and spleen weights of control and infected mice

Values are the mean $\pm$ S.E.M. ($n = 8$). Numbers lacking any asterisks are not significantly different, and all data have been tested down to 0.001. Differences between control and infected mice were determined by t test.

	Body Weight Percentage			
	Liver		Spleen	
	Control	Infected	Control	Infected
WT	4.57 $\pm$ 0.12	5.72 $\pm$ 0.12***	0.377 $\pm$ 0.032	0.718 $\pm$ 0.11**
IL6(-/-)	4.31 $\pm$ 0.11	5.26 $\pm$ 0.15***	0.305 $\pm$ 0.073	0.500 $\pm$ 0.08
IFN γ (-/-)	4.40 $\pm$ 0.11	5.05 $\pm$ 0.12**	0.353 $\pm$ 0.021	0.548 $\pm$ 0.05**

** $P < 0.01$ and *** $P < 0.001$ compared with control.

TABLE 2

Citrobacter rodentium counts in colon, liver, and blood of infected miceValues are the mean CFU $\pm$ S.E.M. ($n = 8$). Numbers lacking any asterisks are not significantly different, and all data have been tested down to 0.001. Differences between wild-type and cytokine-deficient mice were determined by t test.

	Bacterial Counts in Tissue		
	Blood	Liver	Colon ($\times 10^8$)
	CFU		
WT	1508 $\pm$ 1252	465 $\pm$ 62	2886 $\pm$ 1267
IL6(-/-)	13 $\pm$ 5	336 $\pm$ 112	1034 $\pm$ 2894
IFN γ (-/-)	73 $\pm$ 73	210 $\pm$ 47**	5221 $\pm$ 2725

** $P < 0.01$ compared with wild-type controls.

TABLE 3

Neutrophil recruitment as measured by MPO activity in colons of wild-type and cytokine-null mice

Values are the mean $\pm$ S.E.M. ($n = 8$). Numbers lacking any asterisks are not significantly different, and all data have been tested down to 0.001. Differences between wild-type and cytokine-deficient mice were determined by t test.

	Colon MPO Activity	
	Control	Infected
	units/mg protein	
WT	3.48 $\pm$ 0.78	6.54 $\pm$ 0.98*
IL6(-/-)	3.26 $\pm$ 0.67	2.95 $\pm$ 0.60
IFN γ (-/-)	1.94 $\pm$ 0.28	12.1 $\pm$ 2.05**

* $P < 0.05$ and ** $P < 0.01$ compared with control.

the variability in WT data. Although the bacterial loads in the liver and colon were not significantly different between WT and IL6(-/-) mice, bacterial colonization tended to be higher in IFN γ (-/-) colon and was significantly lower in the liver.

Effect of *C. rodentium* Infection on Colon Myeloperoxidase Activity. *C. rodentium* induces a localized inflammatory response in the colon, characterized by infiltration of immune cells such as neutrophils and lymphocytes. To investigate whether the absence of IL6 or IFN γ affected the recruitment of neutrophils to the colon we measured MPO levels in infected colon tissue. MPO activity in the colons of infected WT and IFN γ (-/-) mice were 2- and 6-fold greater, respectively than in uninfected (control) mice, whereas there was no difference between the MPO activity of control and infected IL6(-/-) mice (Table 3).

Effect of *C. rodentium* Infection on Hepatic P450 mRNA and Protein Expression. The regulation of the following P450s was not discernibly different among the WT, IL6(-/-), and IFN γ (-/-) mice: CYP1A2 and CYP2A5 mRNAs were unchanged; CYP2E1, CYP4A10, and CYP4A14 were reduced to 22 to 74% of control levels, and CYP2D9 and CYP4F18 mRNA were induced between 1.8 and 4.4 times their control levels (Fig. 1). Whereas in WT and IL6(-/-)

Fig. 1. Effect of oral *C. rodentium* infection on mRNA expression of hepatic P450s in WT, IL6(-/-), and IFN γ (-/-) mice. Over 24 h, mice were allowed to drink either sucrose solution (Ctrl) or *C. rodentium* in sucrose (Inf) then they were sacrificed after 7 days and livers were harvested for measurement of mRNA by real-time RT-PCR. Values are expressed as relative levels of mRNA expression after normalization to GAPDH, with the sucrose-treated (Ctrl) group set to 1. Values represent mean $\pm$ S.E.M. ($n = 8$), and significant differences are in comparison with control groups. *, $p < 0.05$ by t test.

mice the mRNA levels of CYP2D22 and CYP3A25 were reduced to between 55 and 65% of control levels, mRNA levels of those P450s remained unchanged in IFN γ (-/-) mice. Infection did not change levels of CYP3A13 in WT and IFN γ (-/-) mice, but reduced them to 69% of control in IL6(-/-) mice. The down-regulation of CYP3A11 mRNA observed in WT was blocked in both IL6- and IFN γ -null animals. CYP2B9 was down-regulated in all three strains, but the magnitude of the effect was diminished in the IL6(-/-) and IFN γ (-/-) mice. In uninfected mice, constitutive expres-

Fig. 2. Effect of oral *C. rodentium* infection on expression of hepatic P450 proteins in WT, IL6^{-/-}, and IFN γ ^{-/-} mice. Liver microsomes were prepared from the mice described in Fig. 1, and P450 protein levels were measured by Western blotting. For CYP2B and CYP2D the heavy (lower) band is designated (H), light band is designated (L), and the sum of both is designated (T). Top, Western blots of samples from infected and control liver microsomes. Bottom, quantitative analysis of Western blot data. Values represent mean $\pm$ S.E.M. ($n = 8$), and significant differences are in comparison with control groups. *, $p < 0.05$ by t test.

sion levels of all mRNAs measured were comparable among all three strains (Supplemental Fig. 1).

In all three genotypes, CYP4A protein levels were down-regulated to less than 50% of control levels by infection with *C. rodentium*, whereas protein levels of CYP2E and the upper (lighter) CYP2B band, designated CYP2B(L), were unchanged (Fig. 2). Levels of the lower (heavier) CYP2B band, designated CYP2B(H), were up-regulated in WT and IFN γ ^{-/-} mice but not in IL6^{-/-}. Although there was no overall change in total CYP2B-like protein levels, designated CYP2B(T), of WT and IL6^{-/-} mice, there was an almost 2-fold increase in IFN γ ^{-/-} mice. Infection did not significantly down-regulate CYP2C protein levels in WT mice, in contrast to the down-regulation observed in both cytokine-deficient strains. Total CYP2D proteins and the individual bands CYP2D(H) and CYP2D(L) recognized by the antibody were down-regulated in WT mice but not in IL6^{-/-} or IFN γ ^{-/-} mice. CYP3A proteins were decreased by at least 25% in both WT and IFN γ ^{-/-} mice but not IL6^{-/-} mice.

Effect of *C. rodentium* Infection on Hepatic Cytokine and Acute-Phase Protein mRNA Expression. To determine the contribution of IL6 and IFN γ to changes in levels of hepatic cytokine and acute-phase protein mRNAs during *C. rodentium* infection, their mRNAs were quantified by real-time RT-PCR. Infection did not change IL6 mRNA expression in either WT or IFN γ ^{-/-} mice but induced TNF α and

nitric-oxide synthase (NOS) 2 mRNAs in all three groups (Fig. 3). Compared with WT, the absence of IL6 resulted in the potentiated up-regulation of IL1 β , TNF α , and NOS mRNAs, but abrogated the induction of α -fibrinogen (FBG) and α 1-acid glycoprotein (AGP) mRNAs. In WT mice, infection caused a 36% decrease in angiotensinogen (AGT) mRNAs that was abolished in IFN γ ^{-/-} mice. The absence of IFN γ not only abrogated the up-regulation of IL1 β and FBG mRNAs seen in WT mice but also attenuated the up-regulation of TNF α , AGP, and NOS2 mRNAs.

Effect of *C. rodentium* Infection on Serum Cytokines. In WT mice, *C. rodentium* infection elevated the levels of all serum cytokines measured except for IL1 β and MIP1 α , which remained unchanged in all three groups of mice (Fig. 4). Levels of the chemokine KC (mouse analog of human IL8) were elevated in all three groups of mice from approximately 80 pm/ml in control animals to between 215 and 315 pg/ml in infected animals. Both TNF α and IP-10 were elevated in IL6^{-/-} mice but were either absent (TNF α) or unchanged (IP-10) in IFN γ ^{-/-} mice. The cytokine-deficient mice had very small amounts of basal IL2 that were not significantly increased by infection. Levels of IFN γ were unchanged by infection in IL6^{-/-} mice, but IL6 levels increased from undetectable levels in IFN γ ^{-/-} mice to approximately 4 pg/ml. IL6 was induced more than 200-fold by infection in WT mice.

Effect of Age on Responses to *C. rodentium* Infection. We reported previously the effect of *C. rodentium* on P450 regulation in 7- to 9-week-old mice at 7 days after infection (Chaluvadi et al., 2009a). Although the data trends observed in that article and those presented in this study are in general agreement, we noticed that some responses were less robust in the present study. We therefore investigated the effect of age on P450 regulation at 7 days after infection, comparing infected animals with untreated groups of 4-, 6-, 8-, and 10-week-old mice. With the exception of slight diarrhea, none of the animals exhibited any other obvious signs of infection. We did not observe any significant effect of age on tissue bacterial loads or colon MPO activity (Supplemental Tables 1 and 2), and despite some differences in constitutive expression of mRNAs, the regulation of P450 mRNAs by *C.*

Fig. 3. Effect of oral *C. rodentium* infection on cytokine and acute-phase protein mRNA expression in the liver of WT, IL6^{-/-}, and IFN γ ^{-/-} mice. Total liver RNA from the mice described in Fig. 1 was analyzed by real-time RT-PCR. Values are expressed as relative levels of mRNA expression after normalization to GAPDH with sucrose-treated (Ctrl) group set to 1. Values represent mean $\pm$ S.E.M. ($n = 8$), and significant differences are in comparison with control groups. *, $p < 0.05$ by t test.

Fig. 4. Effect of oral *C. rodentium* infection on serum cytokines of WT, IL6 $^{-/-}$, and IFN $\gamma^{-/-}$ mice. Mice were treated as described in Fig. 1 then sacrificed after 7 days, at which time blood was collected for measurement of serum IL1 β , IL2, IL6, IFN γ , TNF α , KC, IP-10, and MIP1 α . Significant IFN γ immunoreactivity was detected in the IFN $\gamma^{-/-}$ mice, which have a targeted mutation in exon 2 of the gene that introduces a termination codon after the first 30 amino acids of the IFN γ protein. The immunoreactivity is presumably caused by expression of the truncated nonfunctional protein; therefore we omitted these measurements from the graphs. Values represent mean $\pm$ S.E.M. ($n = 8$), and significant differences are in comparison with control groups. *, $p < 0.05$ by t test.

rodentium infection was very similar in all four age groups (Supplemental Figs. 2 and 3).

Effect of LPS Treatment on Hepatic P450 mRNA of C57BL/6J Wild-Type and IFN γ -Deficient Mice. The effect of LPS on hepatic P450 mRNA levels was investigated in WT and IFN $\gamma^{-/-}$ mice 24 h after a 1 mg/kg i.p. injection. In contrast to the selective effects of *C. rodentium*, LPS treatment down-regulated most of the mRNAs studied except CYP2D9, which was induced 3-fold in WT mice and almost by 13-fold in IFN $\gamma^{-/-}$ mice (Fig. 5). CYP3A13, which was not affected in WT mice, was induced 2-fold in IFN $\gamma^{-/-}$ mice by LPS treatment. Most down-regulated mRNAs were affected to the same extent in the two genotypes, but the down-regulation of CYP2B9, CYP2D22, CYP2E1, and perhaps CYP3A41 seemed to be attenuated in the IFN $\gamma^{-/-}$ mice.

Effect of Cytokine Treatment on mRNAs in Mouse Hepatocytes. In preliminary experiments carried out to optimize culture conditions, media changes were done either every day or every other day. A comparison of basal expression over 6 days revealed that there were generally no significant

Fig. 5. Effect of LPS injection on hepatic P450 mRNAs in WT and IFN $\gamma^{-/-}$ mice. Animals were injected intraperitoneally with either saline (Ctrl) or 1 mg/kg LPS, and livers were harvested after 24 h for measurement of mRNA expression by real-time RT-PCR. Values are expressed as relative levels of mRNA expression after normalization to GAPDH with the saline-treated group set to 1. Values represent mean $\pm$ S.E.M. ($n = 6$), and significant differences are in comparison with control groups. *, $p < 0.05$ by t test.

differences in levels of GAPDH, CYP2D22, CYP3A11, and CYP4A10 between the two conditions (data not shown). In both cases, in comparison to freshly plated cells, there was an initial rapid drop in P450 expression levels then a plateau after the second day followed by evidence of further expression decrease by the sixth day.

Three or 6 days after plating, primary cultures were treated with 10 ng/ml each of mouse recombinant IL6, IFN γ , or TNF α , and the cells were harvested after 6, 12, and 24 h. The results from the 3- and 6-day cytokine treatments generally had similar trends (Fig. 6). Hepatocytes treated on the third day with TNF α had more pronounced mRNA level changes than those treated on the sixth day. Although TNF α had some effect on all mRNAs measured on one or both days, IL6 affected only CYP3A25 on hepatocytes treated on the third day and at the 24-h time point of cells treated on the sixth day. IFN γ had no significant effect on almost all mRNAs measured.

To investigate the effect on P450 mRNA of different cytokine concentrations, on the third day after plating, the hepatocytes were treated with 1, 3, 10, or 30 ng/ml of each cytokine for 24 h. The mRNAs for CYP2D9, CYP3A11, CYP3A25, and CYP4A10 were down-regulated after treatment with either IL6, IFN γ , or TNF α , with TNF α being more potent and

Fig. 6. Effect of cytokine treatment on mRNA expression over time in mouse primary hepatocytes. Three or 6 days after plating, primary cultures were treated with 10 ng/ml each of mouse recombinant IL6, IFN γ , or TNF α , and the cells were harvested after 6, 12, and 24 h. After their isolation, mRNAs were measured by real-time RT-PCR. Values are expressed as relative levels of mRNA expression after normalization to GAPDH with the untreated samples harvested at each time point set to 1. Values represent mean $\pm$ S.E.M. ($n = 3$), and significant differences are in comparison with untreated samples at respective time points. *, $p < 0.05$ by t test.

efficacious than IL6 (Fig. 7). IFN γ was also more potent than IL6, causing its maximal effects at the lowest concentration tested, but it was the least efficacious of the three cytokines. Treatment with either IL6 or IFN γ resulted in levels of CYP3A13 and CYP4F18 that were not significantly different from untreated cells, whereas TNF α treatment resulted in the down-regulation of CYP3A13 and up-regulation of CYP4F18 mRNA expression levels. Both IL6 and TNF α down-regulated levels of CYP2D22 but IFN γ did not. Treatment with TNF α resulted in the elevation of both IL6 and IFN γ mRNAs in the hepatocytes, whereas treatment with IL6 did not significantly affect levels of either IFN γ or TNF α , and treatment with IFN γ elevated levels of both IL6 and TNF α (data not shown).

Discussion

To investigate the role of IL6 and IFN γ in mediating the regulation of hepatic P450s during *C. rodentium* infection, we compared the responses of IL6(−/−) and IFN γ (−/−) with those of WT animals. Our results show that the regulation of CYP4A10 and CYP4A14, which are most sensitive to down-regulation in *C. rodentium* infection, and CYP2E1, does not require either IL6 or IFN γ because they were similarly affected in WT and cytokine-null animals. On the other hand, the data indicate the involvement of IL6 in the regulation of CYP2D9, CYP3A11, and CYP3A13 and the involvement of IFN γ in the regulation of CYP2D9, CYP2D22, CYP3A11,

Fig. 7. Effect of different concentrations of cytokine treatment on mRNA expression in mouse primary hepatocytes. Three days after plating, primary cultures were treated with 0, 1, 3, 10, and 30 ng/ml each of mouse recombinant IL6, IFN γ , or TNF α , and the cells were harvested after 24 h. After their isolation, measurement of mRNA was done by real-time RT-PCR. Values are expressed as relative levels of mRNA expression after normalization to GAPDH with the untreated samples harvested at each time point set to 1. Values represent mean $\pm$ S.E.M. ($n = 5$ treated; $n = 8$ control), and significant differences are in comparison with untreated control samples. *, $p < 0.05$ by Dunnett's test.

CYP3A25, and CYP4F18 mRNAs. Down-regulation of CYP2B9 may partially depend on IL6 and IFN γ as well.

The selective effects of IL6 or IFN γ deletion on hepatic P450 regulation cannot be attributed to decreased infectivity or virulence of the bacterium. In fact, IFN γ -null mice are more susceptible and exhibit greater pathology of *C. rodentium* infection (Simmons et al., 2002), whereas IL6-null mice exhibit prolonged bacterial colonization and greater mucosal inflammation and mortality than WT mice (Dann et al., 2008). In this study, bacterial loads in the liver and colon were not significantly different among the genotypes, indicating that colonization was unaffected.

The down-regulation of CYP3A11 mRNA by infection seen in WT mice was abrogated in IL6(-/-) mice (Fig. 1). Siewert et al. (2000) reported that reduction of CYP3A11 mRNA levels during turpentine-induced inflammation was abrogated in IL6(-/-) mice. Thus, IL6 has a role in the in vivo regulation of CYP3A11 in both disease models. The down-regulation of CYP3A13 mRNA occurred only in the IL6(-/-) mice, perhaps indicating that IL6 opposes another signal that tends to induce CYP3A13. These findings suggest that human CYP3A4, which is down-regulated by IL6 in human hepatocytes (Abdel-Razzak et al., 1993; Muntané-Relat et al., 1995; Jover et al., 2002; Aitken and Morgan, 2007), may also be regulated by IL6 in vivo in certain disease states. Trautwein et al. (1992) reported the time-dependent suppression of CYP2D by IL6 in C3H/HeJ mice. Here, the absence of IL6 attenuated the up-regulation of CYP2D9, suggesting that IL6 is important for this inductive response in vivo. Conversely, absence of IL6 potentiated the up-regulation of hepatic IL1 β and TNF α mRNAs (Fig. 3). During inflammation, IL6 is thought to be the most important cytokine in regulating the synthesis of hepatic acute-phase proteins (Castell et al., 1989; Siewert et al., 2000). Here, we demonstrate the involvement of IL6 in the regulation of FBG and AGP mRNAs but not AGT in mice during infection (Fig. 3).

Compared with WT mice, the absence of IFN γ abrogated the down-regulation of CYP2D22, CYP3A11, and CYP3A25 mRNAs and the up-regulation of FBG mRNA, indicating the involvement of IFN γ in their in vivo regulation during *C. rodentium* infection, probably at the transcriptional level (Figs. 1 and 3). The up-regulation of CYP4F18, AGP, and TNF α mRNAs was also attenuated in IFN γ (-/-) mice, suggesting a possible role for IFN γ in these inductive responses as well.

Although we were able to quantify P450 proteins that react with antibodies to rat liver proteins, the inability to identify mouse-specific immunoreactive proteins precludes making close associations between mRNA and protein regulation except in the case of CYP2E1 in which there was no significant effect of infection in either WT or cytokine-null mice. Similar to CYP4A10 and CYP4A14 mRNAs, CYP4A proteins were the most sensitive to down-regulation.

Injection of LPS is a pleiotropic inflammatory stimulus that results in the acute down-regulation of many hepatic P450 mRNAs (Morgan et al., 2008). Siewert et al. (2000) reported that LPS administration down-regulated CYP1A2, CYP2A5, CYP2E1, and CYP3A11 mRNAs in IL6(-/-) mice. Likewise, LPS down-regulated most of the mRNAs studied comparably in both WT and IFN γ (-/-) mice (Fig. 5). Although *C. rodentium* infection did not change mRNA levels of CYP1A2, CYP2A5, and CYP2C29 in WT or IFN γ (-/-) mice, LPS depressed these mRNAs profoundly in both strains. The down-regulation of CYP2D22 and CYP2B9 seemed to be attenuated in IFN γ (-/-) mice, suggesting that IFN γ might have partial roles in the regulation of these two P450s in both the *C. rodentium* and LPS models. Although the induction of CYP2D9 in LPS-treated mice was enhanced by the absence of IFN γ (-/-), suggesting a suppressive effect of IFN γ on this mRNA, CYP2D9 was similarly induced by *C. rodentium* in WT and IFN γ (-/-) mice (Figs. 1 and 5). We have shown previously the induction of CYP3A13 mRNA by LPS in mice of two different genetic backgrounds (Richardson and Morgan, 2005; Chaluvadi et al., 2009b). With C57BL/6 mice,

CYP3A13 mRNA was induced only in IFN γ (-/-) mice, implying the involvement of IFN γ in the LPS modulation of CYP3A13.

The effective and sustained action of proinflammatory cytokines is modulated by synergy or additivity with other cytokines as well as antagonism by opposing cytokines. Thus, as noted by Siewert et al. (2000), when a genetic deletion of a cytokine or its receptor fails to change the regulation of a P450 in response to an inflammatory stimulus it could reflect functional redundancy of cytokines. This could also be true in the *C. rodentium* model. In this study IFN γ (-/-) mice did not have detectable levels of circulating IL6 and TNF α , and IL6(-/-) mice had normal levels of IFN γ but undetectable TNF α levels. Thus, part of the IFN γ (-/-) and IL6(-/-) phenotypes could be attributed to the reduced levels of TNF α ; similarly, part of the IFN γ (-/-) phenotype could be caused by reduced levels of IL6. However, IFN γ has roles in the regulation of CYP2D22, CYP3A25, and CYP4F18 that are independent of IL6, because these P450s are regulated similarly by infection in WT and IL6(-/-) mice. Blunted induction of serum IL2 in both IL6(-/-) and IFN γ (-/-) mice could also contribute to the observed differences in P450 regulation. Given the complex interplay of cytokines that occur during inflammation and infection, our findings emphasize the point that a change in the response of a P450 (or any other gene product) in a cytokine-null animal must be interpreted cautiously because it could reflect an indirect role of that or another cytokine. On the other hand, this is the first report that IFN γ contributes to hepatic P450 regulation in a model of infection and inflammation.

To further explore effects of these cytokines on P450 expression, we used murine primary hepatocyte cultures. The largest changes in P450 expression were seen with TNF α treatment (Fig. 7) that exhibited a concentration-dependent effect on all mRNAs tested. Suppression of P450s by TNF α has been documented in hepatocyte cultures of pigs (Kleine et al., 2008), rats (Chen et al., 1995), and humans (Abdel-Razzak et al., 1993; Aitken and Morgan, 2007; Kleine et al., 2008). In our hands, the effect of IL6 on mouse P450 expression was very similar to that of TNF α , but less pronounced. IL6 treatment of hepatocytes either slightly induced or did not affect CYP3A13 expression (Figs. 6 and 7), whereas the absence of IL6 resulted in the down-regulation of CYP3A13 by infection (Fig. 1). Together, these observations suggest that in vivo IL6 may act to counter a down-regulation by another factor. Unlike IL6 and TNF α , there was no clear concentration-dependent P450 regulation observed with IFN γ treatment. IFN γ was the least effective cytokine at down-regulating P450s and did not have any significant effect on either CYP2D22 or CYP3A13. Although information gleaned from hepatocytes in culture may convey that a given P450 can be affected by a cytokine, interpretations should be made with caution because a particular effect might be different in response to inflammation or infection in vivo. Nevertheless, our hepatocyte experiments suggest that TNF α is the most potent and efficacious of the cytokines tested in the regulation of murine hepatocyte P450 expression, and this may partially explain why the effects of IL6 or IFN γ deficiency that we observed in vivo were limited in scope and/or magnitude.

In conclusion, our in vivo results indicate distinct roles for both IL6 and IFN γ in the regulation of different hepatic P450

mRNAs and proteins during *C. rodentium* infection, whereas other P450s seem to be regulated by other mechanisms. The P450s most sensitive to down-regulation in the *C. rodentium* model, the CYP4As, are unaffected by deficiency of either IL6 or IFN γ . In view of the emerging concept of IL6 as a central mediator of inflammation (Morgan et al., 2008), it is surprising that IL6 gene deletion affected the regulation of so few P450s in the *C. rodentium* model of infection. Our results indicate that IL6 is not the primary or sole mediator of P450 down-regulation in all disease models, and the utility of serum cytokines as biomarkers of hepatic P450 activity will differ with the type of disease and the identity of the P450 enzyme. Experiments are ongoing to explore the effect of the absence of TNF α on P450 regulation in this model of infection. The results presented here and those reported from other studies in mice deficient in different cytokines or cytokine receptors have failed to identify any one cytokine responsible for the effects of LPS (Warren et al., 1999, 2001; Siewert et al., 2000; Ashino et al., 2004). These results strongly suggest possible functional cytokine redundancy in the LPS model.

Acknowledgments

We thank Dr. Ryan D. Kinloch and Mr. Malik J. Raynor for technical assistance and Dr. Dean P. Jones and Mr. Yongliang Liang (Clinical Biomarkers Laboratory, Department of Medicine, Emory University) for equipment to quantify serum cytokines.

References

- Abdel-Razzak Z, Loyer P, Fautrel A, Gautier JC, Corcos L, Turlin B, Beaune P, and Guillouzo A (1993) Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. *Mol Pharmacol* **44**:707–715.
- Aitken AE and Morgan ET (2007) Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. *Drug Metab Dispos* **35**:1687–1693.
- Ashino T, Oguro T, Shioda S, Horai R, Asano M, Sekikawa K, Iwakura Y, Numazawa S, and Yoshida T (2004) Involvement of interleukin-6 and tumor necrosis factor α in CYP3A11 and 2C29 down-regulation by *Bacillus Calmette-Guérin* and lipopolysaccharide in mouse liver. *Drug Metab Dispos* **32**:707–714.
- Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger T, Trullenque R, Fabra R, and Heinrich PC (1989) Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett* **242**:237–239.
- Chaluvadi MR, Kinloch RD, Nyagode BA, Richardson TA, Raynor MJ, Sherman M, Antonovic L, Strobel HW, Dillehay DL, and Morgan ET (2009a) Regulation of hepatic cytochrome P450 expression in mice with intestinal or systemic infections of *Citrobacter rodentium*. *Drug Metab Dispos* **37**:366–374.
- Chaluvadi MR, Nyagode BA, Kinloch RD, and Morgan ET (2009b) TLR4-dependent and -independent regulation of hepatic cytochrome P450 in mice with chemically induced inflammatory bowel disease. *Biochem Pharmacol* **77**:464–471.
- Chen JQ, Ström A, Gustafsson JA, and Morgan ET (1995) Suppression of the constitutive expression of cytochrome P-450 2C11 by cytokines and interferons in primary cultures of rat hepatocytes: comparison with induction of acute-phase genes and demonstration that CYP2C11 promoter sequences are involved in the suppressive response to interleukins 1 and 6. *Mol Pharmacol* **47**:940–947.
- Craig PI, Mehta I, Murray M, McDonald D, Aström A, van der Meide PH, and Farrell GC (1990) Interferon down-regulates the male-specific cytochrome P450IIIA2 in rat liver. *Mol Pharmacol* **38**:313–318.
- Dalton DK, Pitts-Meek S, Keshav S, Figari IS, Bradley A, and Stewart TA (1993) Multiple defects of immune cell function in mice with disrupted interferon- γ genes. *Science* **259**:1739–1742.
- Dann SM, Spehlmann ME, Hammond DC, Iimura M, Hase K, Choi LJ, Hanson E, and Eckmann L (2008) IL-6-dependent mucosal protection prevents establishment of a microbial niche for attaching/effacing lesion-forming enteric bacterial pathogens. *J Immunol* **180**:6816–6826.
- Donato MT, Guillén MI, Jover R, Castell JV, and Gómez-Lechón MJ (1997) Nitric oxide-mediated inhibition of cytochrome P450 by interferon- γ in human hepatocytes. *J Pharmacol Exp Ther* **281**:484–490.
- Gonçalves NS, Ghaem-Maghani M, Monteleone G, Frankel G, Dougan G, Lewis DJ, Simmons CP, and MacDonald TT (2001) Critical role for tumor necrosis factor α in controlling the number of luminal pathogenic bacteria and immunopathology in infectious colitis. *Infect Immun* **69**:6651–6659.
- Goralski KB, Abdulla D, Sinal CJ, Arsenault A, and Renton KW (2005) Toll-like receptor-4 regulation of hepatic Cyp3a11 metabolism in a mouse model of LPS-induced CNS inflammation. *Am J Physiol Gastrointest Liver Physiol* **289**:G434–G443.
- Higgins LM, Frankel G, Douce G, Dougan G, and MacDonald TT (1999) *Citrobacter rodentium* infection in mice elicits a mucosal Th1 cytokine response and lesions similar to those in murine inflammatory bowel disease. *Infect Immun* **67**:3031–3039.
- Huang S, Hendriks W, Althage A, Hemmi S, Bluethmann H, Kamijo R, Vilcek J, Zinkernagel RM, and Aguet M (1993) Immune response in mice that lack the interferon- γ receptor. *Science* **259**:1742–1745.
- Jover R, Bort R, Gómez-Lechón MJ, and Castell JV (2002) Down-regulation of human CYP3A4 by the inflammatory signal interleukin-6: molecular mechanism and transcription factors involved. *Faseb J* **16**:1799–1801.
- Kleine M, Schrem H, Borlak J, and Klempnauer J (2008) Clinical versatility of porcine hepatocytes in the light of interspecies differences in cytochrome P450 regulation and expression. *Xenotransplantation* **15**:208–217.
- Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, Zinkernagel R, Bluethmann H, and Köhler G (1994) Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature* **368**:339–342.
- Kourylko O, Fradette C, Arcand M, and du Souich P (2006) Modulation of CYP1A2 and CYP3A6 catalytic activities by serum from rabbits with a turpentine-induced inflammatory reaction and interleukin 6. *Drug Metab Dispos* **34**:27–35.
- Li-Masters T and Morgan ET (2001) Effects of bacterial lipopolysaccharide on phenobarbital-induced CYP2B expression in mice. *Drug Metab Dispos* **29**:252–257.
- Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2(-\Delta\Delta CT)$ method. *Methods* **25**:402–408.
- Morgan ET (1997) Regulation of cytochromes P450 during inflammation and infection. *Drug Metab Rev* **29**:1129–1188.
- Morgan ET, Goralski KB, Piquette-Miller M, Renton KW, Robertson GR, Chaluvadi MR, Charles KA, Clarke SJ, Kacevska M, Liddle C, et al. (2008) Regulation of drug-metabolizing enzymes and transporters in infection, inflammation, and cancer. *Drug Metab Dispos* **36**:205–216.
- Morgan ET, Thomas KB, Swanson R, Vales T, Hwang J, and Wright K (1994) Selective suppression of cytochrome P-450 gene expression by interleukins 1 and 6 in rat liver. *Biochim Biophys Acta* **1219**:475–483.
- Muntané-Relat J, Ourlin JC, Domergue J, and Maurel P (1995) Differential effects of cytokines on the inducible expression of CYP1A1, CYP1A2, and CYP3A4 in human hepatocytes in primary culture. *Hepatology* **22**:1143–1153.
- Renton KW (2004) Cytochrome P450 regulation and drug biotransformation during inflammation and infection. *Curr Drug Metab* **5**:235–243.
- Richardson TA and Morgan ET (2005) Hepatic cytochrome P450 gene regulation during endotoxin-induced inflammation in nuclear receptor knockout mice. *J Pharmacol Exp Ther* **314**:703–709.
- Richardson TA, Sherman M, Antonovic L, Kardar SS, Strobel HW, Kalman D, and Morgan ET (2006) Hepatic and renal cytochrome p450 gene regulation during *Citrobacter rodentium* infection in wild-type and toll-like receptor 4 mutant mice. *Drug Metab Dispos* **34**:354–360.
- Sewer MB and Morgan ET (1997) Nitric oxide-independent suppression of P450 2C11 expression by interleukin-1 β and endotoxin in primary rat hepatocytes. *Biochem Pharmacol* **54**:729–737.
- Siewert E, Bort R, Kluge R, Heinrich PC, Castell J, and Jover R (2000) Hepatic cytochrome P450 down-regulation during aseptic inflammation in the mouse is interleukin 6 dependent. *Hepatology* **32**:49–55.
- Simmons CP, Gonçalves NS, Ghaem-Maghani M, Bajaj-Elliott M, Clare S, Neves B, Frankel G, Dougan G, and MacDonald TT (2002) Impaired resistance and enhanced pathology during infection with a noninvasive, attaching-effacing enteric bacterial pathogen, *Citrobacter rodentium*, in mice lacking IL-12 or IFN- γ . *J Immunol* **168**:1804–1812.
- Tapner M, Liddle C, Goodwin B, George J, and Farrell GC (1996) Interferon γ down-regulates cytochrome P450 3A genes in primary cultures of well-differentiated rat hepatocytes. *Hepatology* **24**:367–373.
- Trautwein C, Ramadori G, Gerken G, Meyer zum Büschenfelde KH, and Manns M (1992) Regulation of cytochrome P450 IID by acute phase mediators in C3H/HeJ mice. *Biochem Biophys Res Commun* **182**:617–623.
- Warren GW, Poloyac SM, Gary DS, Mattson MP, and Blouin RA (1999) Hepatic cytochrome P-450 expression in tumor necrosis factor- α receptor (p55/p75) knockout mice after endotoxin administration. *J Pharmacol Exp Ther* **288**:945–950.
- Warren GW, van Ess PJ, Watson AM, Mattson MP, and Blouin RA (2001) Cytochrome P450 and antioxidant activity in interleukin-6 knockout mice after induction of the acute-phase response. *J Interferon Cytokine Res* **21**:821–826.
- Williams JF, Bement WJ, Sinclair JF, and Sinclair PR (1991) Effect of interleukin 6 on phenobarbital induction of cytochrome P-450IIB in cultured rat hepatocytes. *Biochem Biophys Res Commun* **178**:1049–1055.
- Xu DX, Wang JP, Sun MF, Chen YH, and Wei W (2006) Lipopolysaccharide down-regulates the expressions of intestinal pregnane X receptor and cytochrome P450 3a11. *Eur J Pharmacol* **536**:162–170.

Address correspondence to. Dr. Edward T. Morgan, Department of Pharmacology, Emory University School of Medicine, 5119 Rollins Research Center, 1510 Clifton Road, Atlanta, GA 30322. E-mail: edward.morgan@emory.edu