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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

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SUMMARY

Hepatic expression of various members of the cytochrome P-450 (CYP) superfamily is suppressed during inflammatory responses. We have shown that the specific expression of P-450 2C11 in male rat liver is suppressed transcriptionally by endotoxin treatment. To investigate the molecular mechanisms underlying this phenomenon, we studied the effects of the inflammatory cytokines interleukin (IL)-1, IL-6, tumor necrosis factor-α (TNF), interferon (IFN)-α, and IFN-γ on the expression of P-450 2C11 and the mRNAs of two typical acute-phase protein genes, α_1-acid glycoprotein (AGP) and fibrinogen, in primary hepatocyte cultures. IL-1, IL-6, TNF, and IFN-α all suppressed P-450 2C11 mRNA, whereas IFN-γ had no effect. IL-1 and TNF were more effective than IL-6 in the suppression of P-450 2C11 mRNA. Whereas IL-1 and IL-6 effects on P-450 2C11 were accompanied by induction of AGP and fibrinogen mRNAs, IFN-α and TNF treatments had no effect on AGP. The suppression of P-450 2C11 and the induction of AGP by IL-1 showed similar time courses. The combination of IL-1 and IL-6 showed additivity in suppression of P-450 2C11, at maximally effective concentrations of cytokines. The effects of IL-1 on P-450 2C11 and AGP expression were blocked by IL-1 receptor antagonist protein. We also studied the effects of IL-1 and IL-6 on the transient expression of chloramphenicol acetyltransferase reporter gene constructs containing 200 or 1287 base pairs of the 5′ flanking region of the *CYP2C11* gene, transfected into primary hepatocytes. The chloramphenicol acetyltransferase activities in cells transfected with the 200-base pair construct were reduced to about 33% and 58% of control levels by treatment with IL-1 or IL-6, respectively, suggesting that sequences important for cytokine down-regulation lie within the proximal promoter region of the *CYP2C11* gene.

P-450s are a superfamily of monooxygenases, many of which are expressed in the liver. The hepatic *CYP* gene products play a crucial role in biotransformation of drugs and toxic chemicals, leading to detoxification or to activation of parent compounds to toxicologically or pharmacologically active species (1). P-450 2C11 is a constitutively expressed *CYP* gene product in male rat liver, where it constitutes a major fraction of total microsomal P-450s (2–5). It is responsible for the monooxygenation of endogenous steroids, as well as a broad range of drug substrates (2, 3, 6).

Inflammation or infection in animals results in increased synthesis and secretion of "acute-phase proteins" and decreased synthesis and secretion of "negative acute-phase proteins" in liver (7, 8), accompanied by impairment of drug metabolism (9) and a decrease in total P-450 content and P-450-associated activities (10). We found that the expression of P-450 2C11 and P-450 2C12 (another major, constitutively expressed CYP gene product in female livers) was suppressed pretranslationally during acute-phase reactions evoked by treatment with bacterial endotoxin or turpentine (11) and that the suppression of P-450 2C11 is primarily

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ABBREVIATIONS: P-450, cytochrome P-450; CYP, cytochrome P-450 gene; IL, interleukin; TNF, tumor necrosis factor-α; IFN, interferon; IL-1ra, interleukin-1 receptor antagonist protein; AGP, α_1-acid glycoprotein; CAT, chloramphenicol acetyltransferase; bp, base pair(s); RSV, Rous sarcoma virus; PBS, phosphate-buffered saline; STAT, signal transducers and activators of transcription.

achieved at the level of transcription (12). *In vivo* studies in our own laboratory and others have shown that IFN inducers (13–17) and IL-1 (18–21) can mimic the down-regulation of *CYP* gene products during infection and inflammation, indicating that the suppression of *CYP* gene products during inflammation and infection is likely mediated by inflammatory cytokines. However, the mechanisms involved are not known.

The effects of cytokines on the expression of other *CYP* genes in cultured cells have been reported (21–26). A recent study with human hepatocytes (25) has shown that IL-1, IL-6, TNF, and, to a lesser extent, IFN-γ suppress P-450s 1A2, 2C, 2E1, and 3A. Studies with rat hepatocytes to date have been limited to suppression of *CYP* gene products after induction; for instance, IL-6 suppressed phenobarbital induction of P-450 2B1 (22), and IL-1 suppressed the induction of P-450 1A1 and P-450 1A2 expression by polycyclic aromatic compounds (23). We have shown that IL-1, but not IL-6, suppresses growth hormone-induced expression of P-450 2C12 (21). Although down-regulation of the low-level constitutive expression of P-450s 1A1, 1A2, and 3A3 by IL-6 in human hepatoma cells has been reported (24, 26), the physiological significance of this is uncertain because of the altered phenotype of hepatoma cells. Therefore, the effects of cytokines on constitutive stable expression of specific *CYP* genes in hepatocyte culture have yet to be investigated.

It has been shown that two male-specific P-450s, 2C11 and 2C13, are constitutively expressed in primary cultured rat hepatocytes on Matrigel in the absence of growth hormone (27). We have used this system to examine the effects of cytokines on the expression of the constitutively expressed P-450 2C11, whose transcriptional regulation by inflammation *in vivo* we have characterized previously (11, 12). We studied the effects of inflammatory cytokines and IFNs on the expression of P-450 2C11, in comparison with their effects on the expression of two typical acute-phase genes, AGP and fibrinogen. To further understand the molecular mechanisms involved, we examined the effects of IL-1 and IL-6 on the transient expression of *CYP2C11* gene promoter fragments linked to the CAT reporter gene. The constitutive and stable expression of the *CYP2C11* gene in cultured hepatocytes was significantly suppressed by IL-1, TNF, and, to a lesser extent, IL-6, accompanied by an induction of AGP gene expression. The transient expression of *CYP2C11* promoter-CAT constructs in cultured hepatocytes was significantly suppressed by IL-1 or IL-6, indicating that *cis* regulatory elements within the *CYP2C11* promoter region are likely involved in mediating these cytokine responses.

Experimental Procedures

Materials. Male Sprague-Dawley rats (200–300 g) from Harlan Sprague Dawley (Indianapolis, IN) were used for hepatocyte isolation. Cell culture medium (Waymouth's MB 752/1), insulin, antibiotics, other cell culture supplies, murine recombinant IL-1 and IL-6, and TNF were purchased from GIBCO-BRL Life Technologies (Bethesda, MD). Murine recombinant IFN-α and IFN-γ were purchased from Boehringer Mannheim (Indianapolis, IN). Recombinant IL-1ra was kindly provided by Dr. D. E. Tracey, the Upjohn Co. (Kalamazoo, MI). The β-fibrinogen cDNA probe was generously provided by Dr. G. M. Fuller of the University of Alabama at Birmingham. Matrigel was prepared by us, according to the method of Ref. 28, from the murine Engelbreth-Holm-Swarm sarcoma generously provided by Dr. H. Kleinman, National Institutes of Health. Monospecific, immunoabsorbed, anti-P-450 2C11 polyclonal antibody was prepared as described by Morgan *et al.* (4), and the *CYP2C11* promoter fragment-CAT constructs were generated by the method of Ström *et al.* (29).

Hepatocyte isolation and culture. Isolation of rat hepatocytes was performed by *in situ* collagenase perfusion (27). The viability of hepatocytes was 70–85% (as determined by trypan blue exclusion) and the yield was 200–400 × 10^6 viable cells/liver. The hepatocytes were plated in Waymouth's medium containing 0.15 μM insulin (27), on 60-mm culture dishes (Falcon; Becton-Dickinson, Lincoln Park, NJ) coated with 0.4 ml of Matrigel (2.0 mg/ml), at a density of 3.5 × 10^6 cells/dish. Medium was changed to remove the dead cells 4 hr after plating and every 48 hr thereafter.

Isolation of total RNA and slot-blot assays. Total hepatocyte RNA was prepared by the acid-phenol extraction method (30). The relative abundances of P-450 2C11, AGP, and fibrinogen mRNAs in total RNA were measured by a slot-blot hybridization assay as described previously (11), using full length cDNAs for P-450 2C11 and fibrinogen and an oligonucleotide complementary to nucleotides 655–684 of AGP mRNA (31) as probes. Bound ^{32}P-labeled probes were detected by autoradiography and quantified by analysis on a Lynx video densitometer (Applied Imaging, Santa Clara, CA). All results were normalized to the poly(A)$^+$ RNA content in the samples, measured by probing slot-blots with an oligo(dT)$_{30}$ probe (18, 32). The amounts of total RNA used were previously determined to be in the linear range for the assay. Using the same hybridization and washing conditions, the P-450 2C11, AGP, and fibrinogen probes each recognized a single mRNA band of appropriate size on Northern blots of total RNA from hepatocytes cultured on Matrigel for 6 days.

Microsomal protein isolation and Western blotting. Microsomes were isolated by differential ultracentrifugation. Briefly, cultured hepatocytes were harvested by suspension in 10 ml of cold PBS (10 mM potassium phosphate buffer, 0.15 M NaCl, pH 7.4), with 5 μM EDTA. After incubation on ice for 20 min, the cells were centrifuged at 500 rpm for 5 min to remove Matrigel. The cell pellet was resuspended in 1 ml of PBS and transferred to a microcentrifuge for pelleting. The pellets were resuspended in 0.5 ml of 0.1 M phosphate buffer, pH 7.0, and sonicated twice for 10 sec using a Kontes Micro-Ultrasonic cell disrupter. They were then centrifuged at 4000 rpm for 5 min and at 13,000 rpm for 13 min in a table-top microcentrifuge. The supernatant was transferred to an ultracentrifuge tube and centrifuged at 100,000 rpm for 15 min in a Beckman TLK tabletop ultracentrifuge with a TLA 100.4 rotor. The microsomal pellet was resuspended in 10 mM Tris acetate buffer, pH 7.4, containing 0.1 mM EDTA and 23% (w/v) glycerol and was stored at −80°. All procedures were performed at 4°. Microsomal protein concentration was determined by the method of Bradford (33).

The amount of P-450 2C11 protein in the microsomes was assayed under conditions of linearity, using an immunoabsorbed polyclonal antibody to P-450 2C11, as described previously (11). The bound second antibody-horseradish peroxidase complex was visualized by staining with 4-chloro-1-naphthol and was quantitated by video densitometry.

Reporter plasmid constructs, transfection of hepatocytes, and reporter gene assays. Plasmids −1287CAT and −200CAT, containing 22 bp of the first exon of *CYP2C11* and the indicated length of 5′ flanking DNA in the pblcat3 vector (29), were used to transfect hepatocytes by electroporation according to the method of Ref. 34, with some modifications. RSV-CAT, a construct containing the RSV promoter linked to the CAT reporter gene (35), was used as a negative control for cytokine responsiveness. Briefly, the freshly isolated hepatocytes were washed twice with cold PBS and then diluted to 12.5 × 10^6 cells/ml with PBS containing 5% fetal bovine serum. Cell suspension (0.8 ml) was added to a 0.4-mm electroporation cuvette, together with 25–50 μg of CAT construct plasmid DNA and 5 μg of luciferase construct plasmid psvLuc (36). After 15 min at 4°, the cuvette was subjected to a pulse of 200 V (960 μF, 200 Ω, 26–35 msec) with a Bio-Rad electroporator. After 10 min at 4°, the

cells were diluted with medium and plated in 60-mm dishes coated with Matrigel. The medium was replaced 4 hr later, to remove dead cells, and every 24 hr thereafter. The electroporation procedures typically resulted in death of 80% of the cells, so that approximately 2×10^6 viable cells were plated in each dish.

Assays for CAT and luciferase activities were performed according to the methods of Refs. 37 and 36, respectively. The CAT activity of each sample was divided by the activity of its luciferase transfection control.

Presentation of results and statistical analyses. The RNA slot blot and protein Western blot assays used are only semiquantitative. Therefore, no attempt has been made to express measurements in any absolute units. Values for each experiment were calculated as a percentage of the mean value (in arbitrary units) for an appropriate control group. One-way analysis of variance and Dunnett's test (or other appropriate *post hoc* test) were used to test for significant differences between the means of different treatment groups. Where unequal variances were detected, the nonparametric Mann-Whitney *U* test was used (as noted in the figure legends). All results are expressed as the mean ± standard error for each group.

Results

P-450 2C11 expression. The expression of P-450 2C11 in primary cultured rat hepatocytes has been found to be reduced rapidly on day 1 of culture, disappearing by day 2 and then gradually reappearing to reach, on day 5, a maximal level that is maintained for several days (27). The mechanism of this phenomenon is not clear, but this stable expression of P-450 2C11 affords the opportunity to examine the effects of cytokines on its constitutive expression in the absence of chemical or hormonal inducers. For this reason, in the present study the hepatocytes were routinely cultured for 5 days to allow the P-450 2C11 gene expression to maximally recover. Evidence for the stable expression of P-450 2C11 mRNA and protein at this point can be seen in Fig. 4. The cells were then treated with various cytokines, to examine the effects of the cytokines on the expression of P-450 2C11, AGP, and fibrinogen mRNAs.

Effects of IL-1, IL-6, and TNF concentrations on P-450 2C11, AGP, and fibrinogen mRNA levels in hepatocytes. Our previous experiments have shown that P-450 2C11 is suppressed during inflammation in adult male rat liver (11, 12). IL-1 and, to a lesser extent, IL-6 can mimic this effect (21). To examine whether these cytokines can act directly on hepatocytes to suppress this gene, we examined the response of P-450 2C11 mRNA to various concentrations of IL-1, IL-6, and TNF in cultured hepatocytes and compared it with the responses of a typical class 1 acute-phase protein, AGP (induced by either IL-1 or IL-6 and synergistically stimulated by both IL-1 and IL-6) (8), and a typical class 2 acute-phase protein, β-fibrinogen (mainly regulated by IL-6) (8).

Figs. 1A and 2 show that IL-1 suppressed the expression of P-450 2C11 mRNA at a concentration as low as 0.5 ng/ml, with maximal suppression occurring at 2 ng/ml. IL-1 reduced the P-450 2C11 mRNA level to approximately 20% of control. Further increases in IL-1 concentrations did not produce more suppression. TNF, which shares many biological activities with IL-1, also suppressed P-450 2C11 significantly at a concentration of 1 ng/ml, with maximal suppression at 5 ng/ml (33% of control) (Fig. 1A). IL-6 also significantly suppressed P-450 2C11 at a concentration of 0.5 ng/ml, with maximal suppression at a concentration of about 5 ng/ml

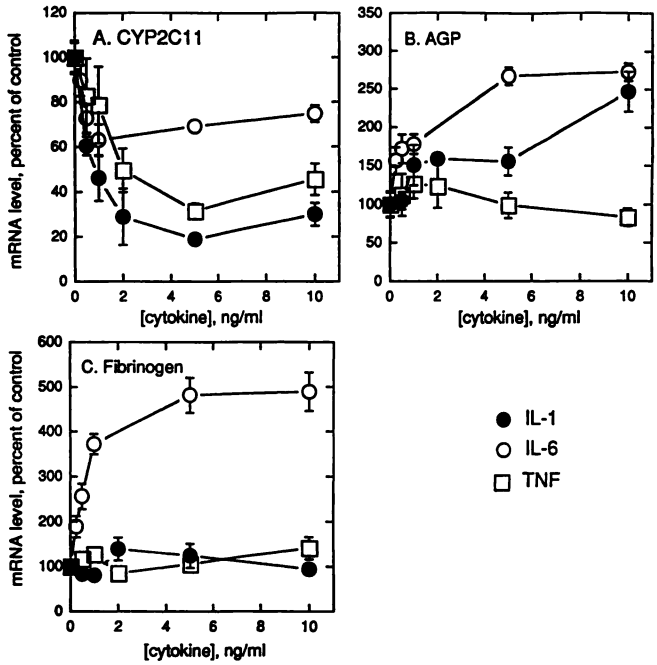

Fig. 1. Concentration dependence of the effects of IL-1, IL-6, and TNF on the expression of P-450 2C11, AGP, and fibrinogen mRNAs. Hepatocytes were cultured on Matrigel for 5 days and then treated for 24 hr with IL-1, IL-6, or TNF at the indicated concentrations. Hepatocytes were harvested and total RNA was isolated from each sample and subjected to RNA slot-blot analysis as described in Experimental Procedures. The P-450 2C11, AGP, and fibrinogen mRNA assays were normalized to the poly(A)$^+$ RNA contents of the samples. The data represent the mean ± standard error of five culture plates for each treatment group and are expressed as percentages of the mean value for untreated (control) cells. The effects of IL-1, IL-6, and TNF on P-450 2C11 mRNA (A), AGP mRNA (B), and fibrinogen mRNA (C) are presented.

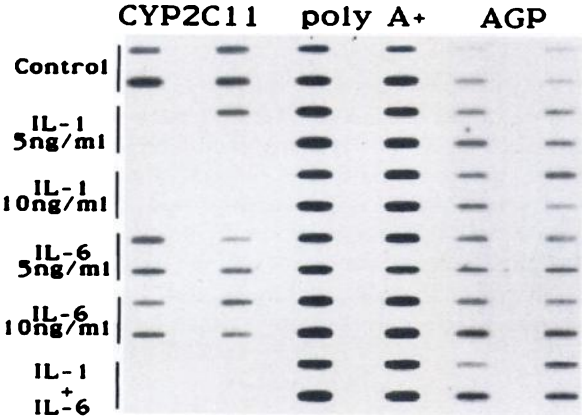

Fig. 2. Representative slot-blot showing the effects of IL-1 and IL-6 on P-450 2C11 and AGP mRNA levels. Hepatocytes cultured as described for Fig. 1 were treated for 24 hr with IL-1, IL-6, or both, at the indicated concentrations. *IL-1 + IL-6*, samples treated with 10 ng/ml levels of each cytokine. Total RNA isolated from each sample was analyzed as in Fig. 1. Four individual culture samples for each treatment group are shown.

(69% of control) (Figs. 1A and 2). Therefore, both IL-1 and TNF were more effective than IL-6 in the suppression of P-450 2C11.

The effects of these cytokines on the expression of AGP are presented in Figs. 1B and 2. IL-1 and IL-6 each induced AGP mRNA to about 250% of control levels. The dose dependen-

cies and magnitudes of these effects were variable in different experiments, although induction by IL-1 and IL-6 could always be observed (Figs. 1– 3). However, TNF had no stimulatory effect on the expression of AGP. The effects of the cytokines on the expression of fibrinogen are shown in Fig. 1C. Neither IL-1 nor TNF had significant effects on the expression of fibrinogen, whereas IL-6 strongly induced fibrinogen mRNA levels, to up to 500% of control, at a concentration of 5 ng/ml.

We have also performed studies to examine the effects of cytokine combinations on P-450 2C11 expression. As seen in Fig. 3, there was a significant additive effect of IL-1 and IL-6 when both cytokines were present at 5 ng/ml. This concentration produced maximal effects of both cytokines when they were tested individually (Fig. 1). There was no significant difference between the effects of IL-1 alone or in combination with IL-6 on P-450 2C11 expression at lower cytokine concentrations. On the other hand, in a separate experiment we found that, at 10 ng/ml, IL-1 plus IL-6 produced a greater effect on AGP expression than did either cytokine alone (IL-1, 132 ± 18%; IL-6, 173 ± 31%; IL-1 plus IL-6, 242 ± 32% of control; $p < 0.05$) (Fig. 2), although no such effect could be detected at the lower cytokine concentrations used in Fig. 3. We found no additive or synergistic effect of IL-1 and TNF on P-450 2C11 expression (data not shown).

Time courses of the suppression of P-450 2C11 mRNA and the induction of AGP mRNA by IL-1. To further characterize the effects of IL-1, we examined the time courses of the responses of P-450 2C11, AGP, and fibrinogen mRNAs to IL-1. As shown in Fig. 4A, significant suppression of P-450 2C11 was first seen at 12 hr after IL-1 addition and reached 35% of control levels by 24 hr. The induction of AGP showed a similar time course (data not shown). A slight induction of fibrinogen mRNA by IL-1 was seen at 24 hr, but not at earlier time points (data not shown). The apparent biphasic behavior of P-450 2C11 mRNA in the first 8 hr after IL-1 treatment (Fig. 4A) has now been observed in two other experiments (data not shown), although it is not statistically significant.

These results indicated that IL-1 suppressed P-450 2C11 mRNA in a dose- and time-dependent manner, and so we next tested whether the suppression of P-450 2C11 mRNA was reflected by a reduction in its apoprotein level. Western blots of P-450 2C11 in microsomal proteins prepared from primary cultured hepatocytes that had been treated with IL-1 are shown in Fig. 5, and the results of quantitative analyses of the Western blot band intensities are presented in Fig. 4. The P-450 2C11 apoprotein level was reduced by about 40% at 24 hr after IL-1 addition, and maximal reduction occurred at about 72 hr, when the apoprotein level was

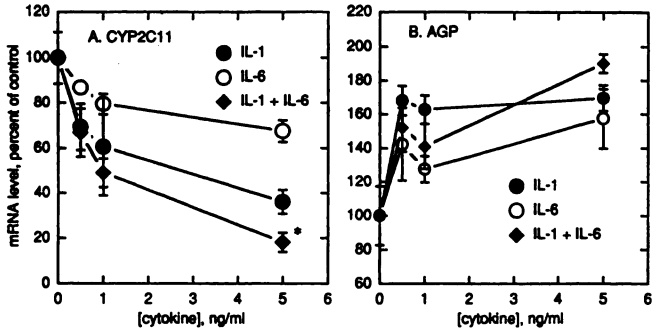

Fig. 3. Additivity of IL-1 and IL-6 in suppression of P-450 2C11 mRNA and induction of AGP mRNA. Hepatocytes were cultured as described for Fig. 1 and were exposed for 24 hr to IL-1, IL-6, or both, at the indicated concentrations. For cells treated with IL-1 and IL-6, each cytokine was present at the concentration indicated (*abcissa*). *, Significantly different from cells treated with IL-1 alone, $p < 0.05$ (Mann-Whitney test).

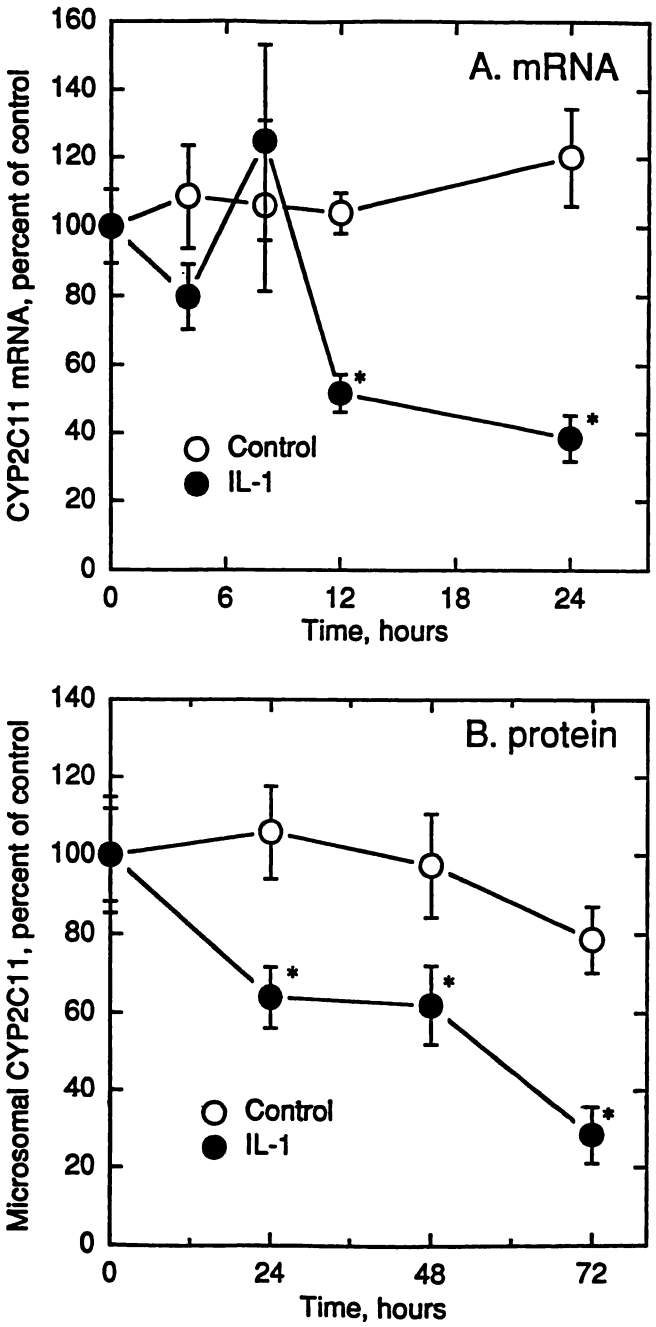

Fig. 4. Time course of the response of P-450 2C11 to IL-1. Hepatocytes were cultured for 5 days as described for Fig. 1. IL-1 at 6 ng/ml was then added with the medium change, and the cells were harvested at the indicated times. A, Total RNA isolated from each sample was subjected to slot-blot assays as described for Fig. 1. Each data point represents the mean ± standard error of five independent samples. B, Microsomal proteins isolated from each sample were subjected to 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose paper, and assayed for P-450 2C11 apoprotein as described in the text. Each data point represents the mean ± standard error of three independent samples. *, Significantly different from time 0 control group mean, $p < 0.05$.

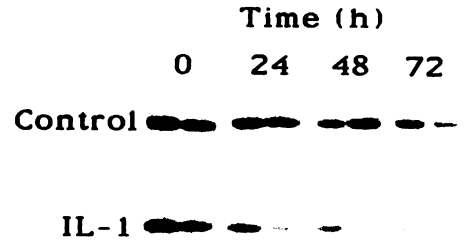

Fig. 5. Western blot showing the reduction in P-450 2C11 apoprotein levels produced by treatment with IL-1. Microsomal samples (5 μg) from control cells or cells treated with 6 ng/ml IL-1 were subjected to Western blot analysis as described in the text. Two samples from each treatment group of the experiment shown in Fig. 4B are shown.

reduced to about 28% of control levels. P-450 2C11 expression in untreated cells was maintained relatively constant during the same period of time. As expected, the reduction of P-450 2C11 apoprotein was delayed with respect to the suppression of its mRNA level (initial experiments showed no significant changes after 12 hr of IL-1 treatment) (data not shown).

IL-1ra blockade of IL-1 suppression of P-450 2C11 and induction of AGP. IL-1ra has been shown to compete with IL-1 for binding to the human type I IL-1 receptor, with K_i values of 17 and 100 nM for IL-1α and IL-1β, respectively (38), and the binding of IL-1ra to IL-1 receptors prevents the biological actions of IL-1 (39). Fig. 6 shows that the suppression of P-450 2C11 mRNA and the induction of AGP mRNA by IL-1β were blocked by IL-1ra at a concentration of 70 nM (1.24 μg/ml).

Effects of IFNs on P-450 2C11 and AGP mRNA expression in hepatocytes. IFN inducers were reported to suppress P-450 2C11 expression *in vivo* (13). However, injection of IFN-γ in rats suppressed P-450 3A2 but not P-450 2C11 (40). Therefore, we tested whether recombinant IFN-α and IFN-γ are capable of suppressing *CYP2C11* gene expression in cultured hepatocytes. IFN-γ alone neither suppressed CYP2C11 expression (Fig. 7) nor induced AGP (data not shown). In contrast, IFN-α significantly suppressed P-450 2C11 mRNA expression in a dose-dependent manner (Fig. 7), without inducing AGP mRNA (data not shown). The combination of IFN-γ and IFN-α had no greater effect than did IFN-α alone.

Effects of IL-1 and IL-6 on the transient expression of *CYP2C11* promoter fragment constructs in transfected hepatocytes. IL-1 and IL-6 down-regulate expression of P-450 2C11 mRNA in cultured hepatocytes (see above), and we have shown that the down-regulation of P-450 2C11 in animals treated with endotoxin occurs at the transcriptional level (12). To determine whether 5′ flanking regions of the *CYP2C11* gene are involved in its transcriptional suppression by cytokines, we transfected the cultured hepatocytes, by electroporation, with *CYP2C11* promoter-CAT constructs containing 22 bp of the first exon and either 1287 or 200 bp of upstream flanking DNA. After 24 hr of culture, the cells were treated with IL-1, IL-6, or IFN-γ for 24 hr, harvested, and assayed for expression of CAT activity.

For untreated cells transfected with the −1287CAT and −200CAT constructs under identical conditions, the CAT activity of the cells transfected with −200CAT was 1.5–2-fold higher than that of cells transfected with −1287CAT (data not shown), reflecting the presence of two negative regulatory elements, at bp −1230 to −1188 and −409 to −368, in the *CYP2C11* promoter (29). Activity in cells transfected with RSV-CAT was about 5-fold higher than that in cells transfected with −1287CAT (data not shown). Fig. 8 shows that IL-1 and IL-6 decreased CAT activity to about 33% and 58%

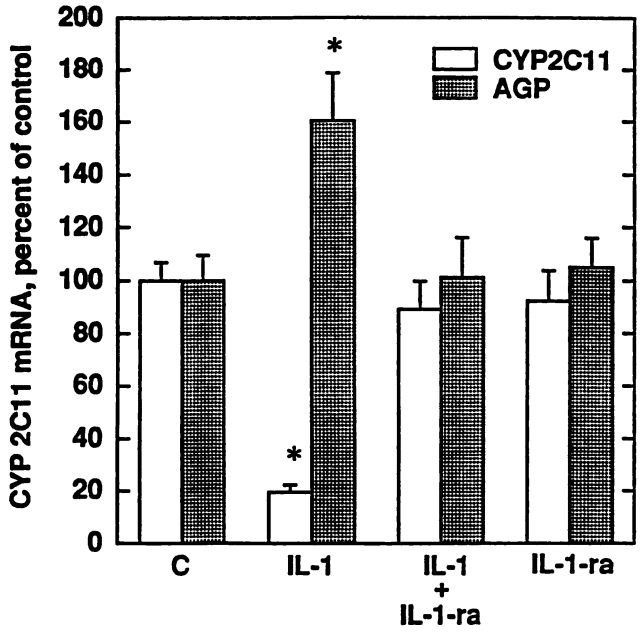

Fig. 6. IL-1ra blockade of the suppression of CYP2C11 mRNA and the induction of AGP mRNA by IL-1. Hepatocytes were cultured as described for Fig. 1 and were treated for 24 hr with 5 ng/ml IL-1, 70 nM (1.24 μg/ml) IL-1ra, or both. Total RNA was isolated and analyzed as described for Fig. 1. Each value represents the mean ± standard error of five independent samples. *, Significantly different from untreated control group mean (C), $p < 0.05$.

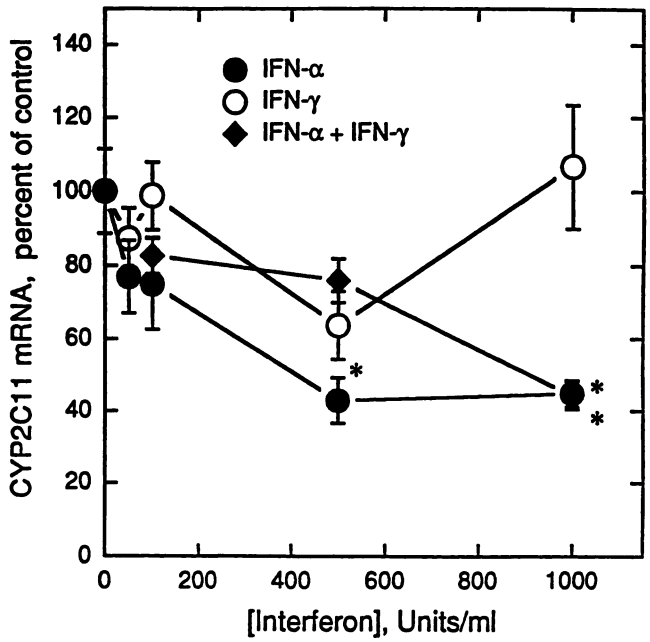

Fig. 7. Effects of IFNs on P-450 2C11 and AGP mRNA levels. Hepatocytes were cultured as described for Fig. 1 and were treated for 24 hr with IFN-α, IFN-γ, or both, at the indicated concentrations. For cells treated with IFN-α and IFN-γ, each IFN was present at the concentration indicated (*abcissa*). *, Significantly different from untreated control group mean, $p < 0.05$.

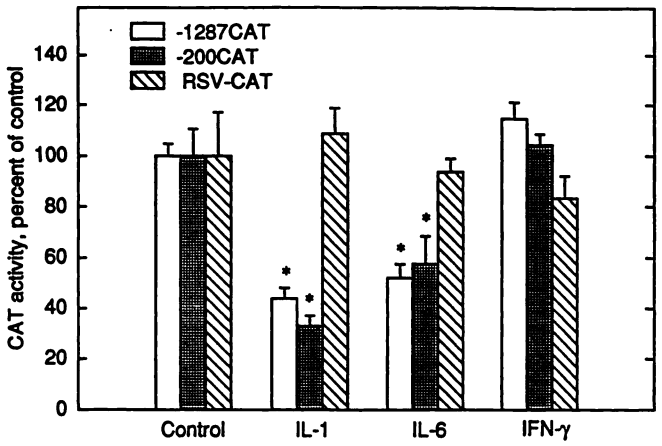

Fig. 8. Effects of IL-1 and IL-6 on the transient expression of *CYP2C11* promoter fragment-CAT constructs in transfected primary hepatocytes. Hepatocytes were transfected with the CYP2C11 constructs −1287CAT (50 μg/plate) or −200CAT (25 μg/plate) or with RSV-CAT (50 μg/plate), as described in Experimental Procedures. All cells were co-transfected with a luciferase vector control. After 24 hr of culture, the cells were treated with IL-1 (5 ng/ml), IL-6 (10 ng/ml), or IFN-γ for 24 hr and then harvested for assay of CAT and luciferase activities. The CAT activity of each sample was normalized to luciferase activity before calculation of the group means. Data were then expressed as percentages of the activity in untreated cells bearing the same plasmid. Differences in the CAT activities of untreated cells bearing different plasmids are given in the text. The data represent the mean ± standard error of three to five samples for each group. *, Significantly different from untreated control group mean, $p < 0.05$.

of control levels, respectively, in cells transfected with the −200CAT construct and that these effects were similar in cells transfected with the −1287CAT construct. Importantly, IFN-γ, which does not suppress expression of the endogenous *CYP2C11* gene, did not affect expression of the CAT reporter plasmids either. Lastly, IL-1 and IL-6 did not affect transcription directed by the RSV promoter (Fig. 8). These results suggest that cytokine down-regulation of P-450 2C11 expression in cultured hepatocytes occurs at the transcriptional level and that sequences important for cytokine down-regulation lie within the proximal 200 bp of the *CYP2C11* promoter and the first 22 bp of the gene.

Discussion

Previous *in vivo* and *in vitro* work has suggested that inflammatory cytokines are involved in the suppression of various P-450s (18–25) that occurs during inflammation or with other immunostimulatory conditions. The present study clearly demonstrates that the expression of P-450 2C11, the major constitutively expressed P-450 in male rat liver, is strongly suppressed in cultured hepatocytes by IL-1 and TNF, as well as by IL-6 and IFN-α. Our results using a *CYP2C11* promoter-CAT construct not only show that the IL-1 and IL-6 effects are achieved at the transcriptional level but also have provided the first demonstration that response elements mediating cytokine down-regulation are present within the 5′ flanking region of a *CYP* gene.

This is the first study to show a rapid (within 12 hr) suppression of a *CYP* gene that is constitutively and stably expressed in primary hepatocyte cultures. Recently, Abdel-Razzak *et al.* (25) reported the suppression of multiple *CYP* genes in human hepatocytes by IL-1, IL-6, TNF, and, to a lesser extent, IFN-γ. However, in their studies, the cells were incubated with cytokines for 72 hr, which may not be appropriate for the acute phase of inflammation and may affect gene expression by different mechanisms. In addition, their work was complicated by the fact that P-450 expression was very low when treatment was initiated and increased in control cultures during the treatment period. In other work, it has been shown that human P-450s 1A1, 1A2, and 3A3 are suppressed by IL-6 in hepatoma cells (24, 26). However, because the phenotype of hepatoma cells is clearly different from that of liver cells, it remains to be seen whether the hepatoma model is relevant to the *in vivo* situation.

In vivo studies showed that IL-1 (18, 21) mimicked bacterial endotoxin-induced inflammation in the suppression of *CYP2C11* and *CYP2C12* genes, whereas IL-6 had no effect on *CYP2C12* and a much smaller effect on *CYP2C11*. These *in vivo* findings correlate very well with the hepatocyte studies presented here. However, the *in vivo* studies did not establish whether the effects of IL-1 are direct or indirect, because IL-1 induces the synthesis of other cytokines, e.g., IL-6, *in vivo*, which in turn may affect the expression of these genes. The notion that the expression of P-450 2C11 mRNA is directly suppressed by IL-1 is supported by the following evidence presented herein. 1) IL-1 was more effective than IL-6 in the suppression of P-450 2C11 mRNA. 2) IL-1 suppressed P-450 2C11 and simultaneously induced AGP, whose expression is regulated by both IL-1 and IL-6, but only slightly induced fibrinogen, which is induced solely by IL-6. If the effect of IL-1 on P-450 2C11 were via stimulation of IL-6 production, then IL-1 should also induce fibrinogen with a similar dose-response relationship. 3) The suppression of P-450 2C11 by IL-1 occurred at an earlier time point than did the slight induction of fibrinogen by IL-1 (8 hr versus 24 hr). 4) IL-6 was more effective than IL-1 in the induction of both AGP and fibrinogen but was less effective than IL-1 in the suppression of P-450 2C11 mRNA.

In addition, the present study also showed that TNF, which has biological activities similar to those of IL-1 in other cells (41), was as effective as IL-1 in suppression of P-450 2C11 mRNA. It is puzzling that TNF did not induce AGP mRNA in these cells, because it has been reported to do so in hepatoma cells (42). However, as expected, TNF also did not induce the mRNA for the IL-6-specific acute-phase gene fibrinogen. Based on our results, it is not possible to say whether IL-1 suppression and TNF suppression of P-450 2C11 share a signal transduction pathway, as they do in other cell types (43, 44).

Poly(dI·dC) induces primarily IFNs of the α/β type. Its injection into rats causes suppression of the constitutive expression of P-450s 2C11 (13), 2C12 (14), and 2E1 (17) and of the inducible expression of P-450s 2E1, 1A1, 1A2 (17), 3A1 (16), and the P-450 4A family (15). In contrast, recombinant rat IFN-γ injections down-regulated P-450 3A2 protein and mRNA without affecting P-450 2C11 (40). The fact that in this study IFN-α, but not IFN-γ, suppressed P-450 2C11 supports the hypothesis that its *in vivo* suppression by the double-stranded RNA is mediated by induction of IFN-α/β. Importantly, we have clearly shown that P-450 2C11 suppression is not merely a consequence of induction of acute-phase genes, because both TNF and IFN-α suppressed the P-450 mRNA in the absence of induction of either AGP or fibrinogen.

The regulation of acute-phase proteins by inflammatory cytokines has been extensively investigated (8, 45). Based on their responses to individual cytokines and combinations thereof, acute-phase proteins are usually divided into two classes. Class I proteins, including AGP, are mainly regulated by IL-1, with synergistic stimulation by IL-6 in the presence of glucocorticoids. Class II proteins, e.g., fibrinogen, are regulated principally by IL-6 and glucocorticoid (8). It was reported that the induction of P-450 1A1 and P-450 1A2 by polycyclic aromatic compounds was greatly reduced in isolated rat hepatocytes by IL-1 but not by IL-6 or TGF-β (23). In the present study, we compared the effects of IL-1 and IL-6 on both classes of acute-phase protein genes and the *CYP2C11* gene. We demonstrated that the induction of mRNA for the class 1 acute-phase protein AGP and the suppression of P-450 2C11 mRNA by IL-1 showed similar time dependencies. Whether P-450 2C11 suppression and AGP induction by these cytokines share similar signaling pathways remains to be determined, but the additivity of IL-1 and IL-6 in P-450 2C11 suppression at high concentrations suggests that they act through different pathways to produce suppression of P-450 2C11.

Cell type-specific IL-1 signal transduction has been described in several cell types (e.g., T cells and fibroblasts) (43, 44, 46). However, IL-1 signal transduction pathways in hepatocytes are still unknown. Nuclear factor-κB is a family of transcription factors that are involved in many of the gene regulatory effects of IL-1 and TNF, including induction of acute-phase proteins (47, 48), and may potentially be involved in IL-1 suppression of P-450 2C11. Similarly, although signal transduction for the IL-6 family of cytokines through the Janus (Jak) kinase/STAT pathway is becoming better understood (49), the role of STAT proteins or other IL-6-regulated transcription factors, such as cAMP response element-binding protein-β and -δ (48, 50), in the down-regulation of P-450 2C11 remains to be determined.

The biological effects of IL-1 are initiated by its binding to IL-1 receptors. Two types of IL-1 receptors have been identified in various cells (51), although the type II receptor is believed not to possess the ability to transduce signals (51). IL-1ra competes with IL-1 for binding to the IL-1 receptor and inhibits the biological effects of IL-1 (38, 39). Our studies demonstrated that the induction of AGP and the suppression of P-450 2C11 by IL-1 are blocked by IL-1ra, indicating that hepatic IL-1 receptors are involved in both of these effects. Sujita *et al.* (52) also reported that the effect of IL-1 in depressing total P-450 levels in hepatocytes occurred within the concentration range for IL-1 binding to its hepatic receptor.

Our studies with the *CYP2C11* promoter fragment-CAT constructs showed that CAT activity was reduced by the treatment of cells with IL-1 or IL-6, although the difference between the IL-1 and IL-6 effects on the promoter was less marked than the difference between their effects on the endogenous genes. These results suggest that the down-regulation of P-450 2C11 by IL-1 or IL-6 is primarily achieved at the level of gene transcription. This is further supported by the fact that both mRNA levels and apoprotein levels for P-450 2C11 were reduced by IL-1 and the reduction of apoprotein levels for P-450 2C11 followed the reduction of its mRNA levels. These results also indicated that *cis* regulatory elements within the *CYP2C11* promoter region are likely involved in mediating these cytokine responses and that these elements lie within the region between bp −200 and bp +22, relative to the transcription start site.

In conclusion, further studies on cytokine signal transduction in hepatocytes are required to understand the mechanism of the acute-phase response and the impairment of drug metabolism during inflammation. A comparison of the regulation of P-450 2C11 and AGP should prove highly useful in this regard. Hepatic P-450s play crucial roles in the metabolism of drugs and toxic chemicals. Because the expression of many hepatic P-450s is suppressed during inflammation and infection, studies on the molecular mechanism underlying this phenomenon are pharmacologically and toxicologically significant.

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