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Cytokines Down-regulate Expression of Major Cytochrome P-450 Enzymes in Adult Human Hepatocytes in Primary Culture

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SUMMARY

Cytokines are thought to cause the depression of cytochrome P-450 (CYP)-associated drug metabolism in humans during inflammation and infection. We have examined the role of five cytokines, i.e., interleukin-1β, interleukin-4, interleukin-6, tumor necrosis factor-α, and interferon-γ, on the expression of CYP1A2, CYP2C, CYP2E1, CYP3A, and epoxide hydrolase in primary human hepatocyte cultures. Steady state P-450 and epoxide hydrolase mRNA levels, as well as ethoxyresorufin-*O*-deethylase and nifedipine oxidation activities, which are mainly supported by CYP1A1/1A2 and CYP3A, respectively, were measured. Interleukin-1β, interleukin-6, and tumor necrosis factor-α were found to be the most potent depressors of P-450 enzymes. After 3 days of treatment, both mRNA levels and enzyme activities were depressed, typically by at least 40%, whatever the cytokine and the enzyme considered. Interferon-γ also suppressed CYP1A2 and CYP2E1 mRNA levels and ethoxyresorufin-*O*-deethylase activity but had no effect on CYP3A and epoxide hydrolase mRNAs. In addition, interleukin-4 had the opposite effect, compared with other cytokines, on CYP2E1 mRNA, which was increased up to 5-fold; ethoxyresorufin-*O*-deethylase and nifedipine oxidation activities were not significantly affected. These results provide the first demonstration that various cytokines act directly on human hepatocytes to affect expression of major P-450 genes and that a wide range of responses can be observed among the enzymes for a given cytokine, suggesting that different regulatory mechanisms may be involved.

The response of organisms to inflammation and infection is characterized by a number of changes, which, in the liver, include increases in secretion of some plasma proteins referred to as acute-phase proteins, alterations of intermediate metabolism, and impairment of drug metabolism and pharmacokinetics (1–3). The first demonstration of altered drug metabolism during infectious diseases came from observations showing delayed theophylline elimination in patients with influenza (2). Many other studies have since reported depression of drug biotransformation and elimination during inflammatory reactions and infectious diseases. Further, the reduction in drug biotransformation capacity was shown to be paralleled by a decrease in total P-450 content and P-450-associated enzyme activities (4, 5). The majority of observations have been made in animals exposed to immunostimulatory agents such as ILs, TNF-α, and IFNs (5–8).

IFNs and IL-1 are the main factors suspected to be responsible for mediating suppression of P-450 enzymes *in vivo* (4, 6, 9). However, several other cytokines and hormones, either released or administered during the acute-phase response, could account for this inhibition. A direct effect of cytokines on hepatic drug metabolism has been suggested from studies on animal liver cell preparations. Isolated rodent hepatocytes exposed to IL-1β showed a decrease in P-450 content and in some P-450 enzymes (10). Whether different P-450 families are similarly affected by different cytokines is still unclear. Moreover, because changes in the pattern of plasma proteins synthesized by the liver during the acute-phase response vary from one species to another (11, 12) and because major variations may exist in the rates and routes of drug metabolism in animals and humans (13, 14), the effects of cytokines observed in rodent cells cannot be directly extrapolated to humans. In addition, rodent hepatocytes in pure culture quickly lose P-450 expression after 1 or 2 days and never recover from the initial drop. The present study was conducted to determine whether several cytokines, used at concentrations known to affect plasma protein patterns, could modulate expression of different P-450

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ABBREVIATIONS: P-450 or CYP, cytochrome P-450; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; EROD, ethoxyresorufin-*O*-deethylase; CRP, C-reactive protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; 3-MC, 3-methylcholanthrene; SDS, sodium dodecyl sulfate; TBS, Tris-buffered saline; BSA, bovine serum albumin.

enzymes in primary human hepatocyte cultures. This model system represents a unique tool to study the direct effects of cytokines on drug biotransformation in humans. The effects of IL-1β, IL-4, IL-6, TNF-α, and IFN-γ on CYP1A2, CYP2C, CYP2E1, and CYP3A, as well as epoxide hydrolase, were investigated. We present the first evidence that the major inflammatory cytokines, i.e., IL-1β, IL-6, TNF-α, and, to a lesser extent, IFN-γ, suppress all these enzymes, which represent the most abundant monooxygenases in human liver. In contrast, IL-4 has a unique positive effect on CYP2E1 mRNA and apoprotein.

Materials and Methods

Recombinant cytokines and chemicals. All cytokines were human recombinant molecules. IL-1β (5×10^8 units/mg of protein) (referred to as IL-1 in Figs. 2, 3, 4, and 6), IL-6 (10^7 units/mg of protein), TNF-α (2×10^7 units/mg of protein), and IL-4 (10^8 units/mg of protein) were provided by Genzyme (Cambridge, UK). IFN-γ (2.5×10^8 units/mg) was kindly provided by Roussel-Uclaf (Romainville, France). 7-Ethoxyresorufin and nifedipine were from Sigma (St. Louis, MO).

Cell isolation and culture. Human liver samples were obtained from 13 adult donors (seven men and six women, ranging from 44 to 65 years of age) and were designated HL1 to HL13. Tissue samples were either from livers that could not be used for transplantation or from liver fragments resected from primary or secondary tumors. All experimental procedures were done in compliance with French laws and regulations and were approved by the National Ethics Committee. Hepatocytes were isolated by the two-step collagenase perfusion method (15). Cell viability was estimated by trypan blue exclusion and was found to range between 70 and 85%. The cells were seeded at a density of 10^7/80-cm^2 flask in 10 ml of a standard medium consisting of 75% minimum essential medium and 25% medium 199, buffered with 0.22% sodium bicarbonate and supplemented with 10 μg/ml bovine insulin, 0.2% BSA, and 10% fetal calf serum. Cytokines were added 15–24 hr after seeding, at the first medium renewal. The medium was deprived of serum and supplemented with 1 μM hydrocortisone hemisuccinate. The cultures were fed every day thereafter with this medium. IL-1β, IL-4, IL-6, TNF-α, and IFN-γ were added to achieve final concentrations of 100, 150, 50, 50, and 50 units/ml of medium, respectively. At such concentrations, none of the cytokines induced morphological alterations throughout the incubation period. These concentrations were previously selected for studying the effects of the respective cytokines on expression of acute-phase proteins in both rat and human hepatocytes in primary culture (16–18).

Isolation of RNA and blot analysis. Total RNA was prepared from hepatocyte monolayers by the guanidinium thiocyanate-cesium chloride method of Chirgwin *et al.* (19), as modified by Raymondjean *et al.* (20). Cells were homogenized in 0.1 M sodium acetate buffer, pH 5.5, containing 5 M guanidinium thiocyanate, 1 mM EDTA, 68 mM lauroyl sarcosyl, and 0.7 M β-mercaptoethanol and were centrifuged at $12,000 \times g$ for 15 min. Supernatants were centrifuged on a 5.7 M cesium chloride cushion at $85,000 \times g$ for 22 hr. RNA pellets were dissolved in 10 mM Tris·HCl buffer, pH 7.4, containing 1 mM EDTA and 34 mM lauroyl sarcosyl, precipitated by addition of ethanol to 70% (final concentration), washed with 100% ethanol, and dried. RNAs were dissolved in sterile water and stored at −80°. RNA quality was assessed by ethidium bromide staining of agarose gels.

Aliquots of RNA were subjected to electrophoresis in a denaturing 6% (w/v) formaldehyde-1.2% (w/v) agarose gel and were transferred onto Hybond-N nylon filters (Amersham, Arlington Heights, IL). After prehybridization at 65° in 450 mM sodium chloride, 44 mM trisodium citrate, 0.2% (w/v) polyvinylpyrrolidone, 0.2% (w/v) Ficoll, 0.1% (w/v) SDS, 0.2% (w/v) BSA, 20 μg/ml polyadenylic acid, 10 μg/ml salmon sperm DNA, filters were hybridized with the appropriate ^{32}P-labeled cDNA probes. The final posthybridization wash was in 15 mM trisodium citrate, 150 mM sodium chloride, 0.1% (w/v) SDS, at 65°. Dried filters were autoradiographed at −80°. Hybridization signals were quantified by densitometry (Hoefer Scientific Instruments, San Francisco, CA). Individual blots were dehybridized and rehybridized two or three times. Results are expressed as percentages of the control untreated hepatocytes. Human epoxide hydrolase cDNA was amplified by the polymerase chain reaction from plasmid *pheh*32 (21). Human CYP2E1 and CYP1A2 coding sequences were cloned after polymerase chain reaction amplification of total liver cDNA (22). Human CYP2C (23, 24) and CYP3A (25) cDNAs and rat GAPDH (26) cDNA have been described previously. Human CRP cDNA was from the American Type Culture Collection (Rockville, MD) and human albumin cDNA was a generous gift of A. Dugaiczyk.

Western blotting. Cells were harvested and homogenized in phosphate-buffered saline, pH 7.0. Samples were separated on 10% polyacrylamide gels, in the presence of 0.2% SDS, and proteins were transferred onto nitrocellulose filters with a Transblot cell (Bio-Rad) for 4 hr. The filters were then saturated with 3% BSA in TBS (65 mM Tris, 150 mM NaCl), pH 7.4, for 2 hr at 20°. The filters were then incubated for 16 hr, at 4°, with a polyclonal antibody against human CYP2E1 apoprotein (a gift of Dr. C. S. Yang, Rutgers University, Piscataway, NJ). After three washes in TBS with 0.1% Nonidet P-40, the filters were treated for 2 hr at 20° with 1 μCi of ^{125}I-Protein A in TBS with 3% BSA. After three washes in TBS with 0.1% Nonidet P-40, the filters were exposed to Hyperfilm MP (Amersham).

Monooxygenase assays. P-450 enzyme activities were measured in living cultured hepatocytes immediately after treatment. The EROD activity was measured essentially according to the methods of Burke and Mayer (27) and Lubet *et al.* (28). Reaction rates were determined under linear conditions with regard to incubation time and protein concentration. Oxidation of nifedipine to the pyridine derivative 3,5-dimethoxycarbonyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine, an activity that is mainly supported by CYP3A4, was measured in cultures fed minimum essential medium without phenol red and containing nifedipine at a concentration of 2×10^{-4} M. Product production was measured in the supernatant by high performance liquid chromatography, according to the method of Guengerich *et al.* (29).

Statistical analysis. Intraindividual variations in enzyme activities prompted us to express the results using the mean of five to nine repeated measurements of EROD activity and two or three repeated measurements of nifedipine oxidation activity in human hepatocyte cultures from seven donors, referred to as HL5 to HL13, after exposure to every cytokine. Interindividual variations were taken into account through a nonparametric two-way analysis of variance (Friedman's test). When significant differences were observed, each culture condition (plus cytokine) was compared with control (minus cytokine) using the Wilcoxon matched-pairs signed-ranks test. Significance was set at a limit of $<5\%$. The limited number of data in the case of treatment with IFN-γ did not permit statistical analysis.

Results

Expression of P-450 mRNAs as a function of time in culture. When placed in culture, human hepatocytes exhibited an initial decrease in P-450 mRNA levels, followed by an increase after 24 or 48 hr (Fig. 1), as was previously reported (30). In particular, CYP1A2 transcripts were detectable only later than 48 hr after plating. At the time points studied, CYP2C and CYP3A mRNA levels were maximal about 96 hr after plating. CYP2E1 mRNA level became substantial only after 96 hr. Epoxide hydrolase mRNA remained stable at 24 and 48 hr but increased dramatically at 96 hr (Fig. 1). Previous studies from our laboratory showed that the levels of CYP2C and CYP3A mRNAs were highest at 96 hr and remained relatively stable until at least 144 hr (30). Therefore, the

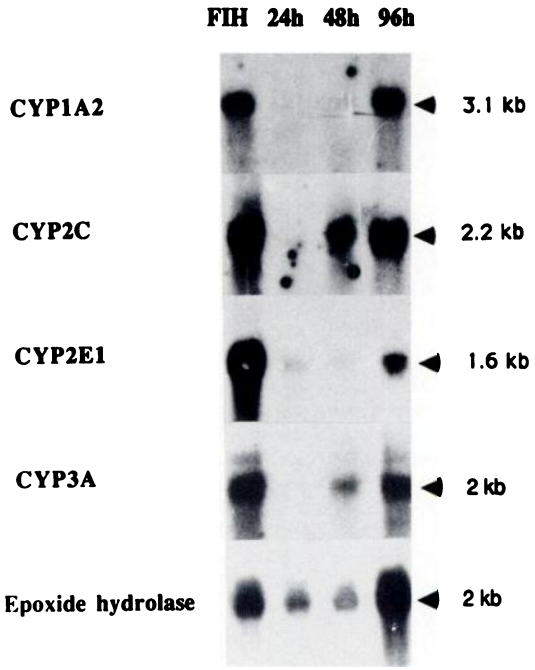

Fig. 1. P-450 and epoxide hydrolase mRNA expression in human hepatocytes as a function of time in culture. Total RNA (20 μg/lane) was subjected to Northern blot analysis at the indicated times after plating. Filters were hybridized with ^{32}P-labeled cDNA probes and autoradiographed. Freshly isolated hepatocytes (*FIH*) were analyzed immediately after dissociation of the liver. Similar data were obtained for cell populations from other donors. mRNA sizes are indicated in kilobases.

amount of mRNA present at 96 hr in control cultures can be considered as that characteristic of the steady state.

Expression of CRP and albumin mRNAs after exposure to cytokines. To demonstrate the responsiveness of hepatocytes to cytokines at the concentrations used, we analyzed expression of CRP and albumin mRNAs. CRP was used as a positive control of the effect of the cytokines, whereas the albumin mRNA level was a negative control under the same conditions. Fig. 2A shows the expression profiles of CRP, albumin, and GAPDH mRNAs in the absence of cytokines. The CRP mRNA level underwent a 3-fold increase 24 hr after cell dissociation and seeding and decreased thereafter to become undetectable at 96 hr, similarly to previous results (31). The albumin mRNA level was diminished to reach a minimum at 48 hr after seeding and then augmented to a level comparable to that of freshly isolated hepatocytes. GAPDH mRNA remained stable throughout the experiment.

CRP message, which was analyzed after 24 hr of treatment with the cytokines, was either increased (IL-1β and IL-6) or decreased to the level of freshly isolated hepatocytes (IL-4) (Fig. 2B). IFN-γ and TNF-α had no significant effect on this message (Fig. 2B). Albumin message, which was analyzed after 72 hr of treatment with the cytokines, was systematically decreased (Fig. 2C). Similar effects of IL-1β, IL-6, and TNF-α on CRP and albumin mRNAs have been observed by others (16–18, 31). The negative effect of IL-4 on CRP and albumin mRNAs has been recently observed in our laboratory.[1]

Depresssion of P-450s and epoxide hydrolase by IL-1β, IL-6, and TNF-α. The number of viable hepatocytes obtained did not permit analysis of both P-450 transcripts and enzyme activities in the same samples.

When cytokines were added for only 24 hr, between 24 and 48 hr of culture, we observed only minor effects on P-450 expression (data not shown). However, 72 hr of treatment produced larger effects. Therefore, the following analysis was carried out after 72 hr of treatment with the cytokines. A representative autoradiogram is presented in Fig. 3. Although IL-1β had similar effects on CYP2E1 and CYP3A mRNAs, a clear difference was observed in the case of IL-4, which produced an increase in the CYP2E1 mRNA level, whereas it had no effect on CYP3A mRNA. The GAPDH mRNA level was not affected by the cytokines.

We then analyzed IL-1β, IL-6, and TNF-α, which are the major cytokines responsible for the increase of acute-phase proteins during inflammation. Exposure to IL-1β induced a decrease in all mRNA species of 60–93% in all cultures analyzed, with the exception of CYP2C, which was not affected in HL1 (Fig. 4). In several experiments, mRNA levels were lower at 72 hr, compared with 24 hr of treatment. Thus, after 72 hr of IL-1β treatment, the CYP3A mRNA level, which decreased by 93%, was actually below that found after 24 or 48 hr of culture in the absence of any cytokine (data not shown). This observation suggests that the effects of cytokines cannot be interpreted solely by delayed or slower recovery of CYP expression. IL-6 induced drops in CYP2E1 and CYP3A mRNA levels ranging between 30 and 70% in all three cultures. CYP2C and CYP1A2 mRNA levels were decreased (25–60%) by IL-6 treatment in only two of three cultures. TNF-α induced systematic decreases, of 30–80%, in all mRNAs in all cultures.

Enzyme activity assays, as stated previously, were performed with hepatocytes from different donors than those used for mRNA analysis. EROD and nifedipine oxidation are mainly supported by CYP1A/1A2 and CYP3A, respectively. Among the various cell preparations, control EROD activity was similar (Table 1), whereas basal nifedipine oxidation activity showed up to a 7-fold variation (Table 2).

Significant differences in both EROD and nifedipine oxidation activities were observed among the different culture conditions [$p < 0.0005$ (Fig. 5A) and $p < 0.003$ (Fig. 5B), respectively; Friedman's test]. When compared with controls, a significant decrease was demonstrated for IL-1β ($p < 0.05$, Wilcoxon's test), reaching 12–89% of basal levels for EROD and 30–90% for nifedipine oxidation activity. In the case of TNF-α, EROD and nifedipine oxidation activities were decreased by 32–85% and 24–90%, respectively ($p < 0.05$, Wilcoxon's test). Although not reaching statistical significance, IL-6 induced decreases of EROD and nifedipine oxidation activities of 18–89% in five of seven cultures, and 33–78% in five of seven cultures, respectively.

Down-regulation of CYP1A2 and CYP2E1 by IFN-γ. Although it acts as an inflammatory mediator, IFN-γ affects a much smaller spectrum of liver proteins during the acute-phase response than do IL-1β, IL-6, and TNF-α (11). At a concentration of 50 units/ml, IFN-γ had no effect on mRNA levels after a single 24-hr addition to hepatocytes, regardless of the P-450 analyzed (data not shown). After 3 days of treatment, CYP2C, CYP3A, and epoxide hydrolase mRNAs were still unchanged, compared with controls (Fig. 4). In contrast, in two of three cultures a marked reduction in CYP1A2 and CYP2E1 mRNA levels was observed.

[1] P. Loyer, G. Ilyin, Z. Abel-Razzak, J. Banchereau, J. F. Denzier, J. P. Campion, C. Guguen-Guillouzo, and A. Guillouzo, unpublished observations.

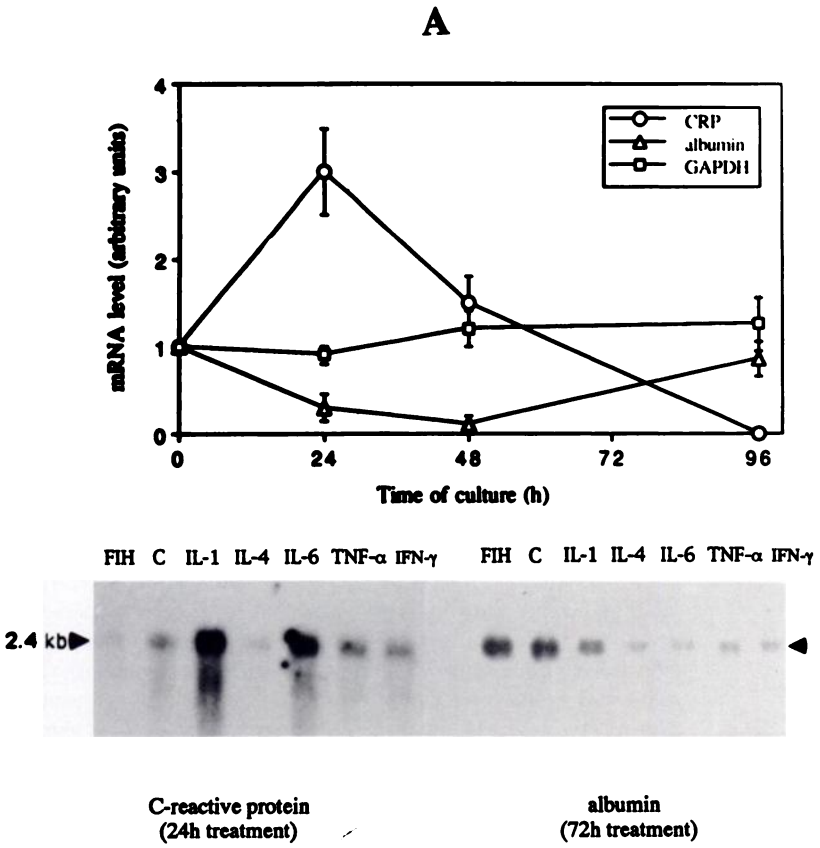

Fig. 2. A, Expression of CRP, albumin, and GAPDH mRNAs. Relative RNA amounts were determined by densitometry. Values represent the mean ± standard error of three experiments, corresponding to three cell populations. For each experiment, mRNA levels in freshly isolated hepatocytes (*FIH*) were used as 100% control values. B and C, Effects of cytokines on CRP and albumin mRNAs. The cytokines were used for 24 hr (B) or 72 hr (C) at the following concentrations: IL-1β, 100 units/ml; IL-4, 150 units/ml; IL-6, 50 units/ml; TNF-α, 50 units/ml; and IFN-γ, 50 units/ml.

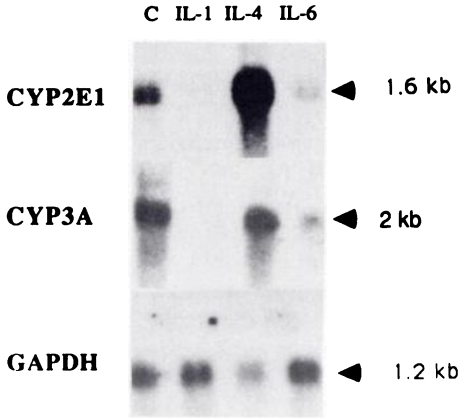

Fig. 3. Effects of IL-1β, IL-4, and IL-6 on CYP2E1, CYP3A, and GAPDH mRNAs. The cytokines were used for 72 hr at the following concentrations: IL-1β, 100 units/ml; IL-4, 150 units/ml; and IL-6, 50 units/ml.

IFN-γ caused a decrease in EROD activity of 29–53% in six cultures; only three cultures are reported in Table 1. For the two cases studied, 21% and 55% decreases in nifedipine oxidation were observed (Table 2). Similar effects of IFN-γ were observed when the cells were exposed to 500 units/ml IFN-γ (data not shown). No statistical analysis was performed in the case of IFN-γ, because the number of independent cultures was too small.

Differential effect of IL-4 on P-450s. After exposure to IL-4, the CYP1A2 mRNA level was decreased by 70% in two of three cultures. The CYP2C mRNA level was also decreased by 50% but in only one of three cultures (Fig. 4). CYP3A and epoxide hydrolase mRNA levels were not affected (Figs. 3 and 4). Strikingly, CYP2E1 mRNA was increased by IL-4 in all three cell populations, by 2.5–5-fold (Fig. 4). This positive effect was already detectable in cells treated with IL-4 for only 24 hr (data not shown). Western blot analysis conducted with proteins from HL4 and HL5 cells showed that CYP2E1 apoprotein was also induced by IL-4 (Fig. 6).

EROD activity was not significantly affected, with the exception of HL5, for which a 49% decrease was observed (Table 1). Nifedipine oxidation activity was either unchanged (HL6, HL7, HL10, and HL13), decreased by 50% (HL9), or increased to 160% (HL11) (Table 2). The effect of IL-4 on P-450 enzyme activities was not statistically significant. However, when directly compared with cultures treated with IL-1, IL-6, or TNF-α, the values for IL-4-treated cultures were statistically different.

Discussion

Inflammation and infectious diseases, which sometimes require administration of drugs over a prolonged period of time, are characterized by the production of cytokines and the alteration of different functions, such as drug metabolism capacity, particularly in the liver. However, the effects on drug-metabolizing enzymes caused by direct exposure to cytokines remain

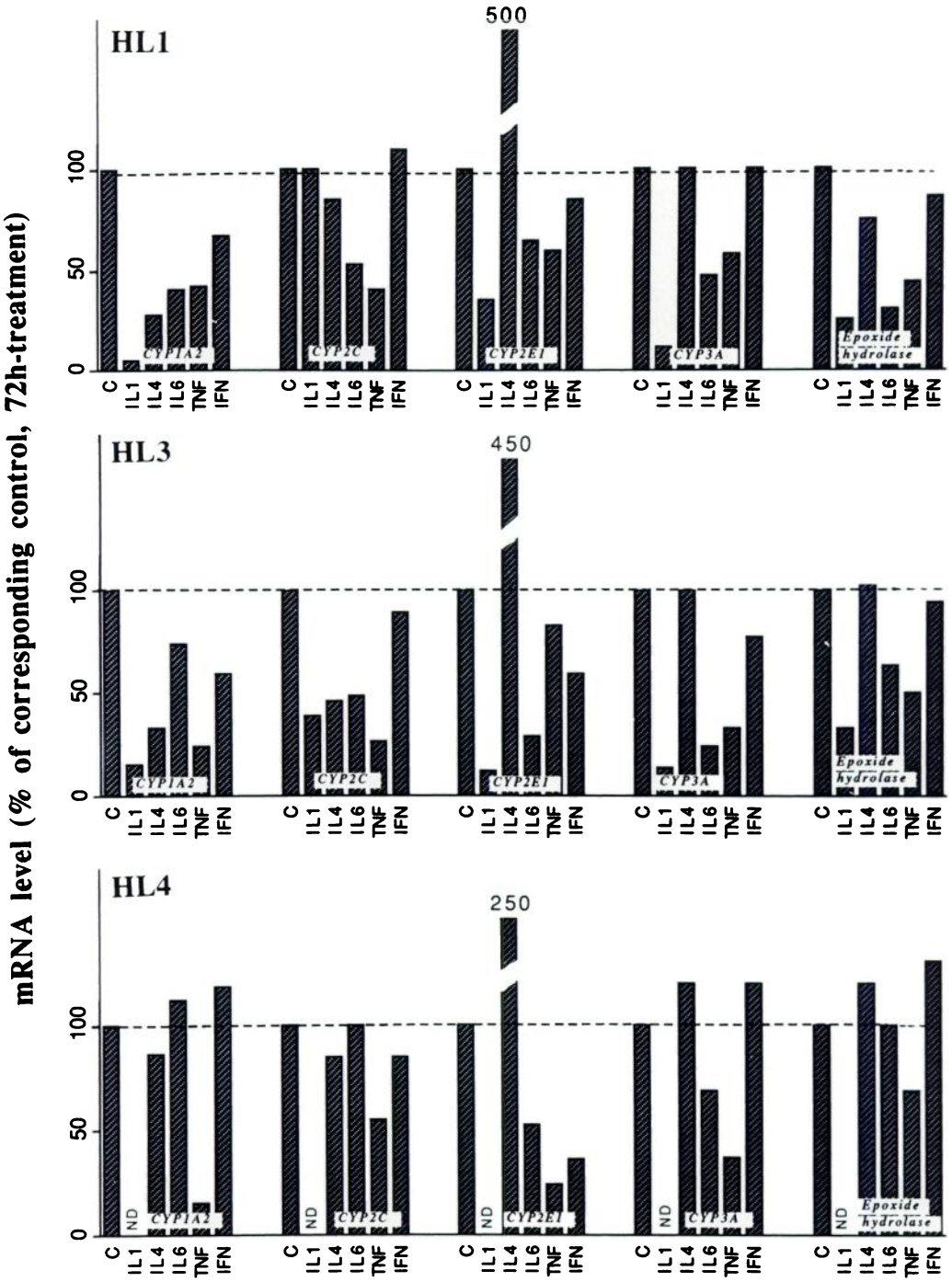

Fig. 4. Effects of IL-1β, IL-4, IL-6, TNF-α, and IFN-γ on P-450 and epoxide hydrolase mRNAs. Cytokine treatments (72 hr) were as described in the legend to Fig. 2. Relative RNA amounts were determined by densitometry. *IL1*, IL-1β; *TNF*, TNF-α; *IFN*, IFN-γ; *C*, control untreated hepatocytes.

largely unknown, especially in humans. Despite some limitations, such as the difficulty of obtaining liver samples, human hepatocytes in primary culture represent an excellent experimental model for studying the effect of cytokines on detoxification enzymes. Various factors, related to the donor and/or organ dissociation conditions, can greatly affect the functional state of freshly isolated and short term cultured parenchymal cells. In addition, marked interindividual variations in different functions, particularly drug-metabolizing enzymes, are common in the human population.

CYP gene expression in human hepatocytes exhibits a behavior that is different from that found for rodent hepatocytes cultivated under the same conditions. Indeed, whereas rodent CYP shows a rapid and irreversible drop, human CYP does recover. Therefore, regulatory mechanisms involved in the regulation of constitutive CYP gene expression in primary culture are likely to be different for rodents and humans. Despite these differences, typical CYP inducers, such as polycyclic aromatic hydrocarbons and macrolide antibiotics, are able to evoke the same inductive effects on orthologous genes from rodents and humans. Consequently, it appears that either model cell system is amenable to the study of CYP gene regulation by various effectors. However, the possibility cannot be ruled out that CYP inducers, although acting in a "conventional fashion," may be influencing distinct regulatory elements, either by delaying or antagonizing the drop in CYP expression in rodent

TABLE 1

Effects of cytokines on EROD activity in primary human hepatocyte cultures

Cytokine treatments (72 hr) were as described in the legend to Fig. 2. EROD activity was determined as described in Materials and Methods. Each value represents the mean ± standard error of at least five samples from the same cell population. Control refers to cells kept in culture for the same time as the cytokine-treated cells.

Condition	EROD activity						
	HL5	HL6	HL7	HL8	HL9	HL10	HL11
	pmol/mg of protein/min						
Control	1.83 ± 0.22	1.63 ± 0.50	1.50 ± 0.16	1.21 ± 0.16	0.92 ± 0.27	1.50 ± 0.22	1.95 ± 0.15
IL-1β	0.20 ± 0.09	1.10 ± 0.51	0.45 ± 0.01	0.46 ± 0.20	0.80 ± 0.19	0.53 ± 0.23	1.50 ± 0.23
IL-4	0.94 ± 0.20	2.60 ± 0.80	1.42 ± 0.20	1.81 ± 0.43	1.79 ± 0.64	1.69 ± 0.46	2.52 ± 0.62
IL-6	0.21 ± 0.15	1.04 ± 0.29	0.69 ± 0.14	0.43 ± 0.15	1.33 ± 0.69	1.24 ± 0.12	2.33 ± 0.47
TNF-α	0.56 ± 0.45	0.85 ± 0.05	0.26 ± 0.15	0.49 ± 0.13	0.50 ± 0.11	0.58 ± 0.23	1.33 ± 0.21
IFN-γ	ND[a]	ND	ND	0.85 ± 0.18	ND	0.92 ± 0.12	0.92 ± 0.12

[a] ND, not determined.

TABLE 2

Effects of cytokines on nifedipine oxidation activity in primary human hepatocyte cultures

Cytokine treatments (72 hr) were as described in the legend to Fig. 2. Nifedipine oxidation activity was determined as described in Materials and Methods. Control refers to cells kept in culture for the same time as the cytokine-treated cells. Each value represents the mean ± standard error of two or three samples from the same cell population.

Condition	Nifedipine oxidation activity						
	HL6	HL7	HL9	HL10	HL11	HL12	HL13
	fmol/mg of protein/min						
Control	4.43 ± 1.33	0.99 ± 0.24	0.62 ± 0.07	0.88 ± 0.17	1.17 ± 0.02	2.57 ± 0.19	2.33 ± 0.24
IL-1β	2.07 ± 0.28	0.38 ± 0.02	0.57 ± 0.12	0.62 ± 0.19	0.11 ± 0.01	2.36 ± 0.20	0.48 ± 0.02
IL-4	4.45 ± 2.35	0.95 ± 0.26	0.29 ± 0.14	0.72 ± 0.26	1.90 ± 0.76	2.24 ± 0.52	1.02 ± 0.36
IL-6	2.14 ± 0.54	0.66 ± 0.10	0.38 ± 0.02	0.78 ± 0.09	0.63 ± 0.04	2.40 ± 0.50	0.55 ± 0.38
TNF-α	2.17 ± 1.07	0.76 ± 0.02	0.16 ± 0.05	0.49 ± 0.21	0.24 ± 0.03	1.16 ± 0.69	0.21 ± 0.12
IFN-γ	ND[a]	ND	ND	ND	0.93 ± 0.15	ND	1.05 ± 0.10

[a] ND, not determined.

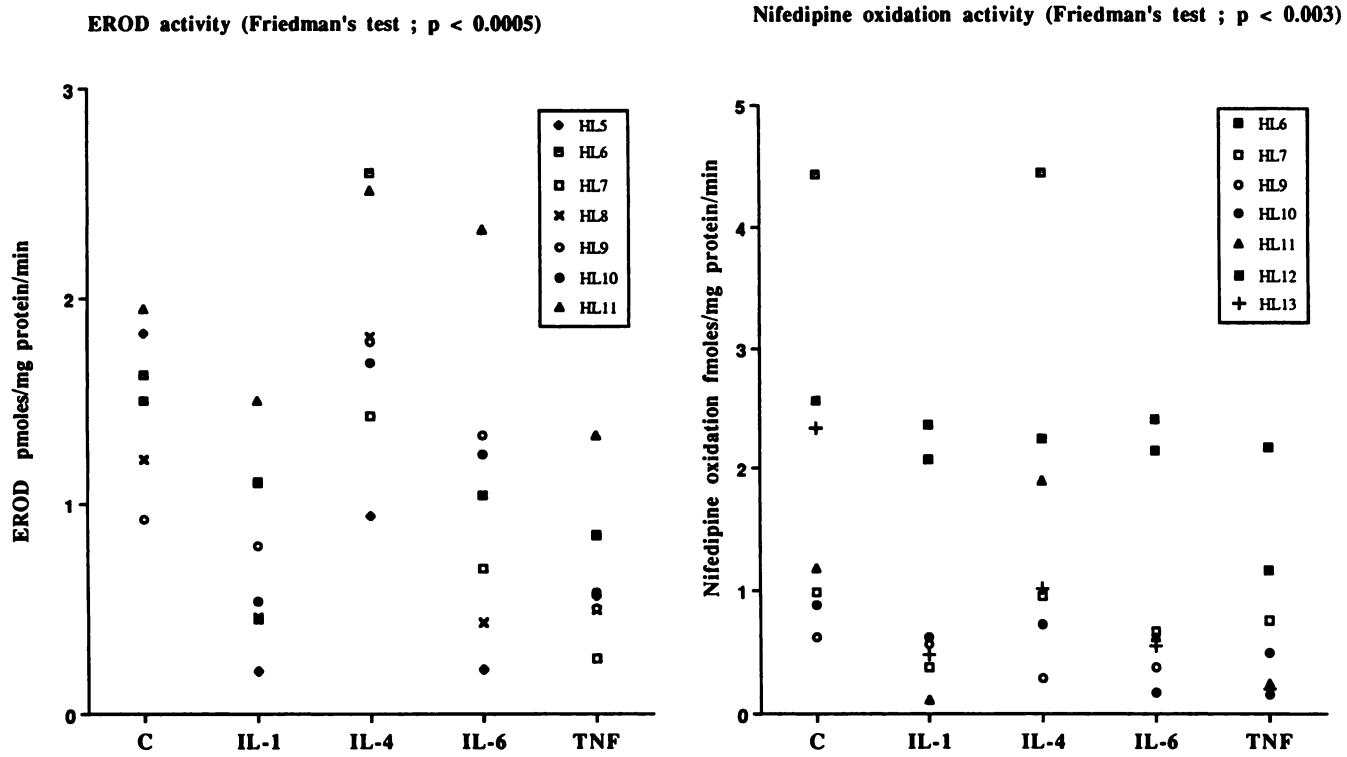

Fig. 5. Graphic representation of variance analysis (Friedman's test) of the effect of cytokines on EROD activity (A) and nifedipine oxidation activity (B). *C*, Untreated cultures; *IL-1*, IL-1β; *TNF*, TNF-α. Each *symbol* refers to cells from one individual. The *p* value shows that the mean (interindividual) values differ significantly between all treatment conditions (see Materials and Methods).

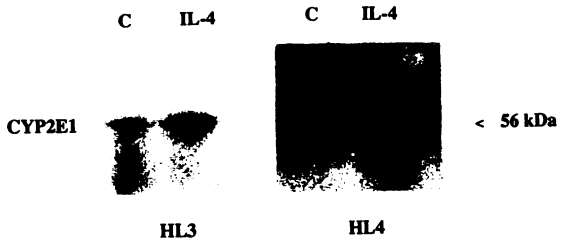

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Fig. 6. Effect of IL-4 on CYP2E1 apoprotein in two independent cultures. Cells were incubated for 72 hr in the presence of IL-4 at a concentration of 150 units/ml. *C*, Untreated cultures.

cells or by accelerating the recovery period in human hepatocytes. Consequently, to interpret results on the regulation of CYP expression in primary hepatocytes, this must be taken into consideration.

CYP mRNA levels drop rapidly in culture, slowly recover (with the time needed to reach maximal levels being about 96 hr in control cultures), and remain relatively stable until at least 144 hr, in the case of CYP2C and CYP3A mRNAs (30). Because cytokines were added between 24 and 96 hr of culture, can our results be interpreted only by the influence of cytokines on P450 recovery? To validate human hepatocytes in primary culture as an alternative model for toxicology studies, this question needs to be addressed with great care. Two lines of argument, both indirect and direct, can be put forward to support our results. Firstly, differences were observed, between cytokines, from one enzyme to another and from one cell population to another. In addition, when enzyme activities in control and treated cultures were compared, statistical significance was demonstrated. Secondly, our results agree with previous data obtained *in vitro*, particularly with cultured rat hepatocytes (although the behavior of mRNAs in control cultures is different, as discussed above). They are also in agreement with those obtained with hepatic and nonhepatic cell lines that stably express CYP genes and, therefore, do not go through any kind of recovery period (32, 33). Thirdly, with one cytokine, i.e., IL-4, a specific increase in CYP2E1 was observed, whereas the other cytokines mainly down-regulated human CYP. This specific effect of IL-4 is not due to a difference in the pattern of expression of CYP2E1, because, in the absence of any cytokine, the mRNA shows a biphasic profile of expression (initial drop, followed by recovery) similar to that of other CYP mRNAs, the accumulation of which was not increased by exposure to IL-4. Fourthly, neither the GAPDH gene nor acute-phase protein genes behaved as did CYP genes in response to cytokines. In addition, the effect of cytokines on CRP and albumin mRNAs evidenced in this study was similar to that observed by others (17, 31).

Two more direct arguments that strongly reinforce the idea that the cytokine effect is not primarily an effect on the recovery period can be made. Firstly, after 24 hr of cytokine treatment, no substantial effect was observed. In several cases, mRNA levels attained after an additional 48 hr of treatment were, in fact, below those found after 24 hr of treatment. This suggests that the effects we observed cannot be interpreted solely by delayed or slower CYP recovery. On the contrary, they suggest that control steady state levels cannot be reached in the presence of the cytokines (as exemplified in the case of CYP3A). Secondly, classical CYP inducers, such as polycyclic aromatic hydrocarbons, barbiturates, and glucocorticoids, have been shown to induce CYP gene expression in primary hepatocyte cultures. These effects were observed regardless of the modifications in CYP expression that resulted from *in vitro* culture. The fact that the inducers provoked results qualitatively similar to those seen *in vivo* was interpreted as good evidence that hepatocytes in primary culture represented a valuable model for the study of CYP gene regulation. For several individuals in our study, human P-450IA1/2 (as measured by EROD activity) was low or undetectable after 24 or 48 hr of culture. Treatment of human hepatocytes with 3-MC led to maximum induction within 24 hr, and CYP1A1/2 activity then remained relatively stable.[2] Addition of IL-1β or IFN-γ after 48 hr of 3-MC treatment resulted, 24 hr later, in a significant decrease in EROD activity. Again, this indicates that, in our culture system, cytokines can down-regulate CYP expression, in this case by antagonizing induction. The same observation has recently been made with rodent hepatocytes (34). The fact that 3-MC behaves typically in culture indicates that the negative effects of IL-1β and IFN-γ are not merely artifactual.

CYP1A2, CYP2C, CYP2E1, CYP3A, and epoxide hydrolase mRNA levels were determined. In addition, we analyzed EROD and nifedipine oxidation activities, which are mainly supported by CYP1A1/1A2 and CYP3A, respectively. Although P-450 enzyme activity assays and mRNA analyses were performed on different cell populations, the effects of the various cytokines on either mRNA or enzyme activity levels were qualitatively similar. IL-1β, IL-6, and TNF-α caused a decrease of all mRNAs and P-450 enzyme activities. Similarly, IFN-γ induced a decrease in CYP1A2 mRNA, as well as EROD activity, and CYP2E1 mRNA, although it did not affect CYP2C, CYP3A, or epoxide hydrolase mRNAs.

IL-1α or -β and IFNs are suspected to have a major effect in altering drug metabolism during inflammation and infection (4, 6, 9). Our observations provide the first evidence that these cytokines are capable of modulating different human P-450s via a direct action on hepatocytes and indicate that, in addition to IL-1, IL-6 and TNF-α also markedly affect the process. The results of *in vivo* studies conducted in animals showed that IL-1α (35), IL-1β (6, 36), IL-6 (35, 36), and TNF-α (6, 36) depressed individual P-450s or total P-450 content. In rat hepatocytes in primary culture, total P-450 amount was diminished by IL-1α treatment (10). Human hepatoma cells treated with IL-6 also exhibited marked decreases in the level of CYP1A1/1A2 and CYP3A mRNAs (32). The presence of IL-6-responsive elements in 5′ flanking regions of the CYP1A1/1A2 genes (37, 38) might suggest a possible role of IL-6 in the transcriptional regulation of these genes. The role of IFNs in depressing P-450 content and P-450-associated enzyme activities is well established (39, 40). Administration of IFNs or IFN inducers to animals has been shown to result in a decrease of several P-450s (4, 7, 40–43). Our results support these observations, because CYP1A2 and CYP2E1 were depressed by IFN-γ. The mechanism by which exposure of hepatocytes to IFN leads to

[2] Abdel-Razzak, L. Corcos, and A. Guillouzo, unpublished observations.

decreases in P-450 and epoxide hydrolase mRNA levels is unknown.

IL-4 (also called B cell-stimulating factor-1), after binding to specific receptors present on the surface of responsive cells (44, 45), exerts various biological effects, such as inhibition of TNF-α-, IL-1β-, and IL-6-mediated stimulation of hepatic lipogenesis (46). Our findings clearly indicate that this cytokine by itself can modulate hepatic functions and that, compared with other cytokines, it has some original effects on P-450s. Indeed, a duality of IL-4-induced effects was observed. On one hand, IL-4 suppressed CYP1A2 and CYP2C transcripts and, on the other hand, it markedly increased CYP2E1 mRNA level. This increase was paralleled by an increase of the apoprotein amount. IL-4 was shown to elicit effects distinct from those provoked by other cytokines during inflammation, which led to its classification as an anti-inflammatory mediator (46–48). Therefore, its opposite effects on P-450s, compared with those produced by IL-1β, IL-6, and TNF-α, may be mechanistically related to its action on serum proteins.

The ability of cytokines to modulate both phase I (P-450s) and phase II (epoxide hydrolase) detoxification enzymes might influence the susceptibility of the individual to various chemicals. To our knowledge, the results reported here provide the first demonstration of statistically significant effects of cytokines on the regulation of CYP expression in the human population. Although these observations can be used directly as an indication of general effects of cytokines in humans, it must be taken into account (i) that major differences in drug metabolism exist among individuals (see, for example, the large interindividual variability in nifedipine oxidation in control cultures), (ii) that the degree of inflammation or viral infection can be quite variable and result in large differences in the production of cytokines, and (iii) that cytokines can also modulate expression of other detoxifying enzymes. Indeed, glutathione *S*-transferases, which have protective effects against reactive molecular species mostly generated by P-450-dependent metabolism, have been reported to be increased after administration of IFN-α to mice (7). Thus, the quantitation of phase I and phase II detoxifying enzyme activities in cultured hepatocytes exposed to cytokines deserves further investigation. Because recombinant cytokines have increasing therapeutic applications, it is of critical importance to determine the potential drug interactions that could result from their administration to patients. Therefore, elucidation of the mechanisms by which cytokines modulate drug-metabolizing enzymes in hepatocytes is an important area for investigation.

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References

1. Andus, T., J. Bauer, and W. Gerok. Effects of cytokines on the liver. *Hepatology* **13**:364–375 (1991).
2. Chang, K. C., B. A. Lauer, T. D. Bell, and H. Chai. Altered theophylline pharmacokinetics during acute respiratory viral illness. *Lancet* **1**:1132–1133 (1978).
3. Renton, K. W. Relationships between the enzymes of detoxication and host defense mechanisms, in *Biological Basis of Detoxication* (J. Caldwell and W. B. Jacoby, eds.). Academic Press, New York, 307–324 (1983).
4. Renton, K. W., and L. C. Knickle. Regulation of hepatic cytochrome P-450 during infectious disease. *Can. J. Physiol. Pharmacol.* **68**:777–781 (1990).
5. Mannering, G. J., and L. B. Deloria. The pharmacology and toxicology of interferons: an overview. *Annu. Rev. Pharmacol.* **26**:455–515 (1986).
6. Pous, C., J. P. Giroud, C. Damais, D. Raichvarg, and L. Chauvelot-Moachon. Effect of recombinant human interleukin-1β and tumor necrosis factor-α on liver cytochrome P-450 and serum α1-acid glycoprotein concentrations in the rat. *Drug Metab. Dispos.* **18**:467–470 (1990).
7. Ansher, S. S., R. K. Puri, W. C. Thompson, and W. H. Habig. The effect of interleukin-2 and α-interferon administration on hepatic drug metabolism in mice. *Cancer Res.* **52**:262–266 (1992).
8. Skai, H., T. Okamoto, R. Yamamoto, R. K. Sindhu, and Y. Kikkawa. Suppressive effect of interleukin-1 on pulmonary cytochrome P-450 and superoxide anion production. *Biochem. Biophys. Res. Commun.* **185**:1083–1090 (1992).
9. Bartini, R., M. Bianchi, P. Villa, and P. Ghezzi. Dexamethasone modulation of *in vivo* effects of endotoxin, tumor necrosis factor and interleukin-1 on liver cytochrome P-450, plasma fibrinogen and serum iron. *J. Leukocyte Biol.* **3**:254–262 (1989).
10. Sujita, K., F. Okuno, Y. Tanaka, Y. Hirano, Y. Inamoto, S. Eto, and M. Arai. Effect of interleukin-1 on the levels of cytochrome P-450 involving interleukin-1 receptor on the isolated hepatocytes of rat. *Biochem. Biophys. Res. Commun.* **168**:1217–1222 (1990).
11. Baumann, H. Hepatic acute phase reaction *in vivo* and *in vitro*. *In Vitro Cell. Dev. Biol.* **25**:115–126 (1989).
12. Heinrich, P. C., J. V. Castell, and T. Andus. Interleukin-6 and the acute phase response. *Biochem. J.* **265**:621–636 (1990).
13. Guengerich, F. P. Human cytochrome P-450 enzymes. *Life Sci.* **50**:1471–1478 (1992).
14. Wrighton, S. A., and J. C. Stevens. The human hepatic cytochrome P-450 involved in drug metabolism. *Crit. Rev. Toxicol.* **22**:1–21 (1992).
15. Guguen-Guillouzo, C., J. P. Campion, P. Brissot, D. Glaise, B. Launois, M. Bourel, and A. Guillouzo. High yield preparation of isolated human adult hepatocytes by enzymatic perfusion of the liver. *Cell. Biol. Int. Rep.* **6**:625–628 (1982).
16. Andus, T., T. Geiger, T. Hiranon, T. Kishimoto, and P. C. Heinrich. Action of recombinant human interleukin-6, interleukin-1β and tumor necrosis factor-α on the mRNA induction of acute-phase proteins. *Eur. J. Immunol.* **18**:739–746 (1988).
17. Castell, J. V., M. J. Gomez-Lechon, M. David, T. Hirano, T. Kishimoto, and P. C. Heinrich. Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase proteins in human hepatocytes. *FEBS Lett.* **232**:347–350 (1988).
18. Busso, N., C. Chesné, F. Delers, F. Morel, and A. Guillouzo. Transforming growth factor-β (TGF-β) inhibits albumin synthesis in normal human hepatocytes and in hepatoma $HepG_2$ cells. *Biochem. Biophys. Res. Commun.* **171**:647–654 (1990).
19. Chirgwin, J. M., R. J. Przybyla, R. J. Macdonald, and W. J. Rutter. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**:5294–5299 (1979).
20. Raymondjean, M., B. Kneip, and G. Schapira. Preparation and characterization of mRNAs from rat heart muscle. *Biochimie* **65**:65–70 (1983).
21. Skoda, R. C., A. Demierre, O. W. McBride, F. J. Gonzalez, and U. A. Meyer. Human microsomal xenobiotic epoxide hydrolase: complementary DNA sequence, complementary DNA-directed expression in COS-1 cells, and chromosomal localization. *J. Biol. Chem.* **263**:1549–1554 (1988).
22. Urban, P., C. Cullin, and D. Pompon. Maximizing the expression of mammalian cytochrome P-450 monooxygenase activities in yeast cells. *Biochimie* **72**:463–472 (1990).
23. Shimada, T., K. S. Misono, and F. P. Guengerich. Human liver microsomal cytochrome P-450 mephenytoin 4-hydroxylase, a prototype of genetic polymorphism in oxidative drug metabolism. *J. Biol. Chem.* **261**:909–921 (1986).
24. Umbenhauer, D., M. V. Martin, L. S. Lloyd, and F. P. Guengerich. Cloning and sequence determination of a complementary DNA related to human liver microsomal cytochrome P-450 *S*-mephenytoin 4-hydroxylase. *Biochemistry* **26**:1094–1099 (1987).
25. Beaune, P. H., D. R. Umbenhauer, R. W. Bork, R. S. Loyd, and F. P. Guengerich. Isolation and sequence determination of a cDNA clone related to human cytochrome P-450 nifedipine oxidase. *Proc. Natl. Acad. Sci. USA* **83**:8064–8068 (1986).
26. Fort, P., L. Marty, M. Piechaczyck, S. E. Sabroty, C. Dani, P. Jeanteur, and J. M. Blanchard. Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. *Nucleic Acids Res.* **13**:1431–1443 (1985).
27. Burke, M. D., and R. T. Mayer. Differential effects of phenobarbitone and 3-methylcholanthrene induction on the hepatic microsomal metabolism and cytochrome P-450-binding of phenoxazone and a homologous series of its *N*-alkyl ethers (alkoxyresorufin). *Chem. Biol. Interact.* **45**:243–258 (1983).
28. Lubet, R. A., R. T. Mayer, J. W. Cameron, R. W. Nims, M. D. Burke, T. Wolff, and F. P. Guengerich. Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) P-450 by phenobarbital and other xenobiotics in the rat. *Arch. Biochem. Biophys.* **238**:43–48 (1985).
29. Guengerich, F. P., M. V. Martin, P. H. Beaune, P. Kremers, T. Wolff, and D. J. Waxman. Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic

polymorphism in oxidation drug metabolism. *J. Biol. Chem.* **261**:5051–5060 (1986).
30. Morel, F., P. H. Beaune, D. Ratanasavanh, J. P. Flinois, C. S. Yang, F. P. Guengerich, and A. Guillouzo. Expression of cytochrome P-450 enzymes in cultured human hepatocytes. *Eur. J. Biochem.* **191**:437–444 (1990).
31. Moshage, H. J., H. M. J. Roelofs, J. F. Van Pelt, B. P. C. Hazenberg, M. A. Van Leeuwmen, P. C. Limburg, L. A. Aarden, and S. H. Yap. The effect of interleukin-1, interleukin-6 and its relationship on the synthesis of serum amyloid A- and C-reactive protein in primary cultures of adult human hepatocytes. *Biochem. Biophys. Res. Commun.* **155**:112–117 (1988).
32. Fukuda, Y., N. Ishida, T. Noguchi, A. Kappas, and S. Sassa. Interleukin-6 down-regulates the expression of transcripts encoding cytochrome P-450IA1, IA2 and IIIA3 in human hepatoma cells. *Biochem. Biophys. Res. Commun.* **184**:960–965 (1992).
33. Stainly, L. A., J. Carmichael, and C. R. Wolf. Cytochrome P-450 induction in human lung tumor-derived cell lines: characterization and effects of inflammatory mediators. *Eur. J. Biochem.* **208**:521–529 (1992).
34. Barker, C. W., J. B. Fagan, and D. S. Pasco. Interleukin-1β suppresses the induction of P-450 1A1 and P-450 1A2 in isolated hepatocytes. *J. Biol. Chem.* **267**:8050–8055 (1992).
35. Wright, K., and E. T. Morgan. Regulation of cytochrome P-450IIC12 expression by interleukin-1α, interleukin-6, and dexamethasone. *Mol. Pharmacol.* **39**:468–474 (1991).
36. Trautwein, C., G. Ramadori, G. Gerken, K. H. Meyer Zum Buschenfelde, and M. Manns. Regulation of cytochrome P-450IID by acute phase mediators in C3H/HeJ mice. *Biochem. Biophys. Res. Commun.* **182**:617–623 (1992).
37. Kawajiri, K., J. Watanabe, O. Gotoh, Y. Tagashira, K. Sogawa, and Y. Fujii-Kuryama. Structure and drug inducibility of the human cytochrome P-450c gene. *Eur. J. Biochem.* **159**:219–225 (1986).
38. Ikeya, K., A. K. Jaiswal, R. Owens, J. E. Jones, D. W. Nebert, and S. Kimura. Human cytochrome CYP1A2: sequence, gene structure, comparison with the mouse and rat orthologous genes, and differences in liver 1A2 mRNA expression. *Mol. Endocrinol.* **3**:1399–1408 (1989).
39. Singh, G., K. W. Renton, and N. Stebbing. Homogeneous interferon from *E. coli* depresses hepatic cytochrome P-450 and drug biotransformation. *Biochem. Biophys. Res. Commun.* **106**:1256–1261 (1982).
40. Craig, P. I., I. Mehta, M. Murray, D. McDonald, A. Astrom, P. H. Van Der Meide, and G. C. Farrel. Interferon down-regulates the male-specific cytochrome P-450IIIA2 in rat liver. *Mol. Pharmacol.* **38**:313–318 (1990).
41. Morgan, E. T., and C. A. Norman. Pretranslational suppression of cytochrome P-450h (IIC11) gene expression in rat liver after administration of interferon inducers. *Drug Metab. Dispos.* **18**:649–653 (1990).
42. Craig, P. I., M. Murray, and G. C. Farrel. Interferon suppresses the isoform-specific activities of hepatic cytochrome P-450 in female rats. *Biochem. Pharmacol.* **43**:908–910 (1992).
43. Skaï, H., T. Okamoto, and Y. Kikkawa. Suppression of hepatic drug metabolism by the interferon inducer polyriboinosinic acid-polyribocitidylic acid. *J. Pharmacol. Exp. Ther.* **263**:381–386 (1992).
44. Beckmann, M. P., D. Cosman, W. Fanslow, C. R. Maliszewski, and S. D. Lyman. The interleukin-4 receptor: structure, function and signal transduction. *Chem. Immunol.* **51**:107–134 (1992).
45. Miyajima, A., T. Kitamura, N. Hrada, T. Yokota, and K. Arai. Cytokine receptors and signal transduction. *Annu. Rev. Immunol.* **10**:295–331 (1992).
46. Grunfeld, C., M. Soued, S. Adi, A. H. Moser, W. Fiers, C. A. Dinarello, and K. R. Feingold. Interleukin-4 inhibits stimulation of hepatic lipogenesis by tumor necrosis factor, interleukin-1 and interleukin-6 but not interferon-α. *Cancer Res.* **51**:2803–2807 (1991).
47. Jansen, J. H., W. E. Fibbe, G. J. H. M. Wientjens, J. Van Damme, J. E. Landegent, R. Willemze, and J. C. Kluin-Nelemans. Interleukin-4 down-regulates the expression of CD14 and the production of interleukin-6 in acute myeloid leukemia cells. *Lymphokine Cytokine Res.* **10**:457–461 (1991).
48. Gautam, S. C., N. F. Chikkala, and T. A. Hamilton. Anti-inflammatory action of interleukin-4: negative regulation of contact sensitivity to trinitrochlorobenzene. *J. Immunol.* **148**:1411–1415 (1992).

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