Biochemical Pharmacology, Vol. 56, pp. 1279–1285, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S006-2952(98)00178-6

Comparative Effects of Cytokines on Constitutive and Inducible Expression of the Gene Encoding for the Cytochrome P450 3A6 Isoenzyme in Cultured Rabbit Hepatocytes: Consequences on Progesterone 6β-Hydroxylation

Cécile Calleja, Claudine Eeckhoutte,* Mauro Dacasto,† Gilberte Larrieu,* Jacques Dupuy,* Thierry Pineau* and Pierre Galtier*‡*

*Laboratoire de Pharmacologie-Toxicologie INRA, 180 Chemin de Tournefeuille, BP3, 31931 Toulouse, France; and †Dipartimento di Patologia Animale, Facolta di Medicina Veterinaria, Via Nizza 52, 10126 Torino, Italia

ABSTRACT. Cultured rabbit hepatocytes were used to compare the relative activities of cytokines to inhibit the constitutive or rifampicin (RIF)-induced expression of the cytochrome P450 3A6 gene (*CYP3A6*). Human recombinant cytokines tested were interleukin-1 β (IL-1β) (2 U/mL), interleukin-2 (IL-2) (5,000 U/mL) and interferon-γ (IFN-γ) (50 U/mL). Hepatocytes were cultured in the presence or absence of 25 μM RIF for 24 hr, with or without cytokines alone or in combination. All these cytokines inhibited RIF-induced P4503A6 expression without apparent cellular toxicity. By contrast, only IFN-γ treatment provided a significant decrease (41%) in the constitutive P4503A6 protein level. Moreover, cytokines differed in their ability to repress RIF-dependent transcriptional induction of *CYP3A6*: IL-1β and IL-2 were approximately equipotent, causing an almost 40–50% suppression of *CYP3A6* mRNA and protein levels, whereas IFN-γ exerted repressive effects only on P4503A6-related erythromycin *N*-demethylase activity and inducible protein expression. In fact, although strongly reducing P4503A6 protein content (an approximate 70% decrease), IFN-γ did not exhibit any influence on *CYP3A6* mRNAs with the exception of its association with interleukins. All these results suggest that IL-1β and IL-2 mainly promote a transcriptional repression mechanism, given the absence of effect of these cytokines on the basal P4503A6 level, whereas IFN-γ exerts a post-transcriptional suppressive action on both induced and constitutive P4503A6 expression. Consequently, P4503A6-dependent progesterone 6β-hydroxylase activity also presented a cytokine-specific pattern of inhibition, with a much greater sensitivity than P4503A6 immunoreactive protein to IL-1β and IL-2 + IFN-γ treatments. Thus, this study underlines the significant impact of inflammation on steroid metabolism. BIOCHEM PHARMACOL **56**;10:1279–1285, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. cytochrome P450 3A6; rifampicin; rabbit; hepatocytes; cytokines; progesterone

P450s§ represent a superfamily of enzymes involved in monooxygenase reactions [1]. A large number of them, mainly expressed in liver, are primarily responsible for oxidative degradation of xenobiotics or foreign compounds, such as drugs, environmental pollutants and toxins. But some P450s also catalyze the stereo- or regioselective hydroxylations of various endogenous substrates, such as steroids [2, 3], thus contributing to steroidogenesis pathways [4]. In this respect hepatic P4503As from several animal species including rabbit and human are involved in the 6β-hydroxylation of testosterone and progesterone [5, 6].

It is now recognized that the level and the activity of P450 are markedly affected by inflammation, infection and other conditions that invoke the acute-phase response [7]. In animals, decreased drug-metabolizing activity is seen following the injection of bacteria, viruses, parasites or other inflammatory agents such as turpentine or carrageenan [8, 9]. The broad spectrum of systemic and liver-associated changes occurring during the acute-phase response is apparently mediated by various cytokines released from activated mononuclear cells, including blood monocytes, hepatic Kupffer cells and peritoneal monocytes [10]. Thus, it has been suggested that the decreased P450 activity observed during inflammation could be due to these endogenously released molecules. This hypothesis tends to be confirmed, since numerous reports have already demonstrated that several inflammatory cytokines, notably IL-1β,

* Corresponding author: Dr. Pierre Galtier, INRA, Laboratoire de Pharmacologie-Toxicologie, 180, Chemin de Tournefeuille, BP3, F-31931 Toulouse Cedex, France. Tel. +33 (0) 561 28 51 46; FAX +33 (0) 561 28 53 10; E-mail: pgaltier@toulouse.inra.fr.

§ *Abbreviations*: *CYP3A6*, gene encoding for P4503A6 isoenzyme; END, erythromycin *N*-demethylase; IL-1β, interleukin-1β; IL-2, interleukin-2; IFN-γ, interferon-γ; LPS, bacterial lipopolysaccharide; P450, cytochrome P450; and RIF, rifampicin.

Received 20 October 1997; accepted 22 April 1998.

IL-6 and IFN-γ, the principal mediators of the liver acute-phase response, are major contributors to the decline of hepatic P450 activity and content (for a review, see [11]). In this respect, however, the effects of numerous other cytokines, alone or associated, have not been completely investigated. In particular, although this molecule is widely used in therapeutics, few studies have been concerned with the potential activity of IL-2, the main lymphocyte activator, on P450 expression. Recently, *in vivo* administration of IL-2 was reported to decrease several monooxygenase activities in mouse [12]. Then, we reported that IL-2 was able to decrease induced P450 1A expression in primary cultures of rabbit hepatocytes [13]. By contrast, a low dose of rat IL-2 was shown to slightly increase P450-related activities after *in vivo* administration to rats [14]. Finally, an *in vitro* study demonstrated the presence of the IL-2 receptor in hepatocytes, as already described for IL-1β and IFN-γ [15, 16], and its potential down-regulating capability on the constitutive expression of P450 genes in cultured rat hepatocytes [17]. However, no comprehensive analysis of the *in vitro* effects of IL-2 on the inducible expression of P450 3A has yet been reported, and the potential impact of this interleukin on P450-dependent steroid catabolism remains unknown.

Consequently, we decided to investigate the influence of IL-2, alone or in combination with IFN-γ, on constitutive and RIF-induced P4503A expression and activities, one of the most inducible members of the P450 family in rabbit [18] implicated in hepatic metabolism of progesterone [5]. To this end, we took particular care to use the lowest concentrations of cytokines previously found to regulate *in vitro* CYP expression [17, 19, 20]. The effects of IL-1β, IFN-γ and of their association were also studied, with the aim of comparing the repression pattern of the various cytokine treatments and their consequences on the metabolism of progesterone.

MATERIALS AND METHODS

Materials

Erythromycin, progesterone, DMSO, Waymouth, Ham F12 and William's E culture media, horseradish peroxidase antibodies, collagen, collagenase type IV and BSA were obtained from Sigma. RIF was from Marion Merrell Dow Laboratories. [4-^{14}C]-progesterone (specific activity: 2.0 GBq/mmol) was obtained from DuPont/NEN. [4-^{14}C]-6-β hydroxyprogesterone was kindly provided by Dr. C. Larroque (INSERM U128). Human recombinant IL-1β and IFN-γ were purchased from Boehringer Mannheim and PeproTech EC Ltd, respectively. Human recombinant IL-2 was a generous gift from Dr. Martini (Hoechst Marion Roussel). Antirabbit P450 3A6 IgG and cDNA probes were generous gifts from Dr. P. Maurel (INSERM U128, Montpellier, France) and F. J. Gonzalez (National Institutes of Health). Fetal calf serum (FCS) was from Biochrom KG. The reagents for gel electrophoresis and immunoblotting were supplied by Eurobio. All other chemicals used were of the highest purity commercially available and distilled deionized water was used in all studies.

Hepatocyte Culture and Drug Treatment

Male New Zealand rabbits were obtained from INRA Elevage Lapin. Hepatocytes were isolated and cultured as already described [21]. IL-1β, IL-2 and IFN-γ were dissolved in pure water and distributed in the culture medium without FCS at final concentrations of 2 U/mL, 5,000 U/mL and 50 U/mL, respectively, 72 hr after plating in order to avoid postplating perturbations. Four hours after cytokine input, RIF dissolved in DMSO was added at a final concentration of 25 μM. Treatments lasted for 20 hr, control plates receiving only DMSO (0.1% final volume). Microsomal fractions were classically prepared by differential centrifugation of the sonicate of three pooled cultures [22]. Proteins were determined according to Lowry [23] using BSA as standard.

Biochemical Assays

Cytotoxicity resulting from treatment of hepatocytes was assessed by the titration of lactate dehydrogenase in both cytosolic fraction and culture medium [24]. Total microsomal cytochrome P450 content and the *N*-demethylation of erythromycin were classically determined [25, 26]. As an oxidation product of NO biosynthesis, nitrite was determined spectrophotometrically at 540 nm, based on the diazotization assay, generally referred to as a Griess reaction [27].

Microsomal 6β-Hydroxylation of Progesterone

Microsomes (0.25 mg of protein/mL) were suspended in 1 mL of 50 mM potassium phosphate buffer pH 7.4 containing 0.2 mM unradiolabeled progesterone and 1.6×10^4 Bq [^{14}C]progesterone. Progesterone was added as a 20 mM methanol solution, resulting in a final methanol concentration of 1% which did not interfere with the assay. After a 5-min preincubation at 37°, the reaction was started by the addition of NADPH (1 mM). Incubation was continued for 10 min, whereafter the reaction was terminated by the addition of 2 mL of methylene chloride followed by vigorous shaking. Oxidation products were further extracted with methylene chloride (2 mL twice) and ethyl acetate (2 mL). The combined extracts were dried (Na_2SO_4) and solvent was evaporated at 40° under a stream of nitrogen. The residue, containing progesterone metabolites, was redissolved in 50 μL of methylene chloride and applied onto 0.25 mm thick silica gel TLC plates (Merck F-254, on aluminum sheets). TLC plates were developed twice in cyclohexane/ethyl acetate/ethanol (9: 9: 2) and progesterone metabolites were detected by autoradiography on X-AR films. 6β-Hydroxyprogesterone metabolite was identified by cochromatography with authentic radiolabeled standard. The identity of the other metabolites

TABLE 1. Effect of cytokines on microsomal proteins, total P450 and P4503A-related *N*-demethylase in rabbit hepatocyte cultures

Condition	Induction	Microsomal proteins (mg/mL)	Total P450 (nmol/mg)	END (pmol/min/mg)
Control	—	2.01 ± 0.026	0.61 ± 0.01	1.25 ± 0.03
	RIF	2.22 ± 0.096	0.64 ± 0.03	1.49 ± 0.04†
IL-1β	—	2.63 ± 0.63	0.46 ± 0.1	1.19 ± 0.001
	RIF	2.33 ± 0.055	0.62 ± 0.03	1.35 ± 0.05*
IL-2	—	2.30 ± 0.06	0.53 ± 0.06	1.21 ± 0.02
	RIF	2.21 ± 0.20	0.61 ± 0.004	1.33 ± 0.06*
IFN-γ	—	2.27 ± 0.06	0.50 ± 0.10	1.23 ± 0.05
	RIF	2.61 ± 0.024	0.57 ± 0.005	1.28 ± 0.07*
IL-1β + IFN-γ	—	2.25 ± 0.024	0.55 ± 0.05	1.15 ± 0.001
	RIF	2.25 ± 0.005	0.63 ± 0.001	1.23 ± 0.03*
IL-2 + IFN-γ	—	2.20 ± 0.012	0.55 ± 0.003	1.15 ± 0.06
	RIF	2.34 ± 0.12	0.63 ± 0.03	1.32 ± 0.04*

All values are means ± SD from three different cultures measured in duplicate.
*Significant differences ($P < 0.05$) from RIF-induced control cultures.
†Significant differences ($P < 0.05$) from control cultures receiving only DMSO.

was inferred from their chromatographic properties, as previously described by Larroque et al. [28].

Western Blot and Northern Blot Analysis

Microsomal proteins (5 μg) were resolved on a 10% SDS-PAGE [29], electrophoretically transferred to nitrocellulose sheet and developed by specific IgG as previously described [30]. Staining of the blots was obtained by using a chemoluminescence technology (ECL kit, Amersham). Total RNA was isolated by the classic guanidinium thiocyanate-cesium chloride method [31] and 20 μg were size-fractionated on a 1.2% agarose gel and blotted onto Gene Screen Plus hybridization transfer membrane (DuPont). Hybridization [32] was performed in the presence of appropriate probes (*CYP3A6* and GAPDH as control) radiolabeled with the Ready-to-go kit (Pharmacia Biotech).

Analysis of Data

The relative contents of isoenzyme, mRNA and 6β-hydroxy progesterone were estimated by densitometry of the Western blots, Northern blots and TLC autoradiograms by using an omnimedia XRS 12CX Bioimage Scanner. Band densities of control and treated cultures were compared. Statistical significance was evaluated using ANOVA including a complementary range test in order to compare means. In all the cases, a probability of $P < 0.05$ was considered significant. Certain statistical and linearity tests were performed with Instat 2.02 (Graph PAD Software).

RESULTS

Effects of Cytokines on Cytochrome P450 3A6 Isoenzyme Expression and Activity

As demonstrated by the unchanged values of lactate dehydrogenase leakages (4–5%) and microsomal proteins (Table 1), all cell treatments including RIF induction appeared to be devoid of any cytotoxic effect. In the meantime, significant equal increases in NO release were observed. According to previous studies [33], we then concluded that hepatocytes were clearly responsive to all investigated cytokines under our culture conditions, containing as they did less than 5% of non-parenchymal cell contamination (Fig. 1). No significant variation of total P450 was obtained in the presence of cytokines (Table 1), suggesting a selective cytokine action, if any, on certain P450 isoenzymes. Incubation with RIF led to a weak (1.3-fold) but significant

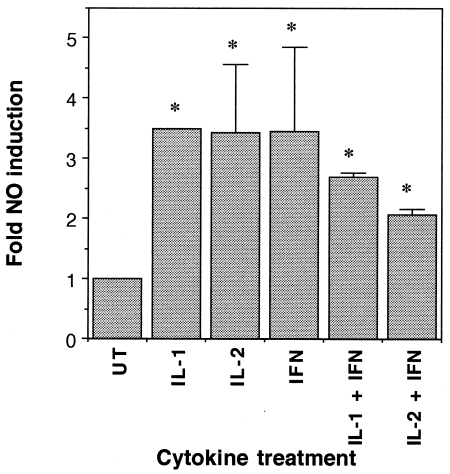

FIG. 1. Induction of NO biosynthesis in rabbit hepatocytes by human recombinant cytokines. Hepatocytes were exposed to the indicated treatments for 24 hr. The final cytokine concentrations were 2 U/mL for IL-1β, 5,000 U/mL for IL-2 and 50 U/mL for IFN-γ, respectively. Nitrite was measured in the culture supernatants as a stable endproduct of NO generation. Results are expressed as induction factors of cytokine-treated samples vs DMSO-treated control (UT). The values represent the means ± SD of three distinct experiments measured in duplicate. *Significant increase due to cytokine, relative to untreated culture ($P < 0.05$)

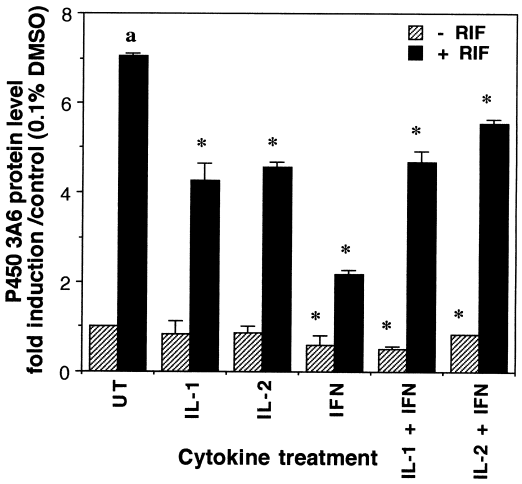

FIG. 2. Effect of cytokines on constitutive and induced P4503A6 protein levels. Treatments and immunoblotting analysis were performed as described in Materials and Methods. Relative P450 protein amounts were determined by densitometry. Data are expressed as induction ratios between induced or uninduced cytokine-treated samples and untreated control (0.1% DMSO). Incubations with RIF are given in the *black columns* whereas *hatched columns* represent incubations without inducer. The values correspond to the means ± SD of three distinct experiments measured in duplicate. [a]Significantly different from untreated control (0.1% DMSO) ($P < 0.05$). *Significantly different from corresponding induced or uninduced control (UT) ($P < 0.05$)

increase in END activity, one which is classically attributed to P4503A6 [34]. In cytokine-treated cells, this RIF induction was slightly but significantly reduced (*ca.* 15–21% decrease) (Table 1). By contrast, END activity of uninduced samples was not impaired, whatever the treatment. Cytokine associations did not provoke any significant additional decrease in END-induced activity.

The relative amount of specific P4503A6 protein was determined by immunoblot analysis. In agreement with previous reports [18], RIF treatment resulted in a significant increase in the *CYP3A6* protein level (about seven-fold over control) (Fig. 2). All cytokine treatments decreased the level of induction of *CYP3A6*. However, IFN-γ was significantly more potent (70% decrease) than IL-1β (40%) and IL-2 (35%). Moreover, only IFN-γ, alone or associated with interleukins, was able to significantly reduce (40–49%) the constitutive *CYP3A6* protein content. Thus, only the induced P4503A6 protein level correlated ($r = 0.96$) with END activity for the different cytokine treatments. Again, no significant additive effect was revealed in the case of cytokine combinations.

The reduction of P4503A6 expression by cytokines was then investigated via Northern blotting analysis (Fig. 3A). As previously reported [18], RIF provided a strong induction of P4503A6 mRNA (about nine-fold over control). Densitometric analysis including normalization to GAPDH mRNAs demonstrated that IL-1β and IL-2 decreased the induced *CYP3A6* mRNAs by 41% and 54%, respectively (Fig. 3B), whereas IFN-γ did not exhibit any inhibitory action on the induced P4503A6 mRNA level. However, in the presence of RIF, IL-1β + IFN-γ and IL-2 + IFN-γ associations resulted in significant potentiated activities (62% and 73%, respectively) in comparison with single interleukin treatments. By contrast, no suppression of constitutive *CYP3A6* mRNAs could be detected, whatever the cytokine treatment. Thus, a significant correlation ($r = 0.93$) between protein content and mRNA level was only observed in the case of IL-1β, IL-2 and IL-1-β + IFN-γ input. No significant variation in GAPDH mRNA was observed (Fig. 3A), indicating that none of these changes were based on unspecific effects of cytokines, such as a decrease in cell viability.

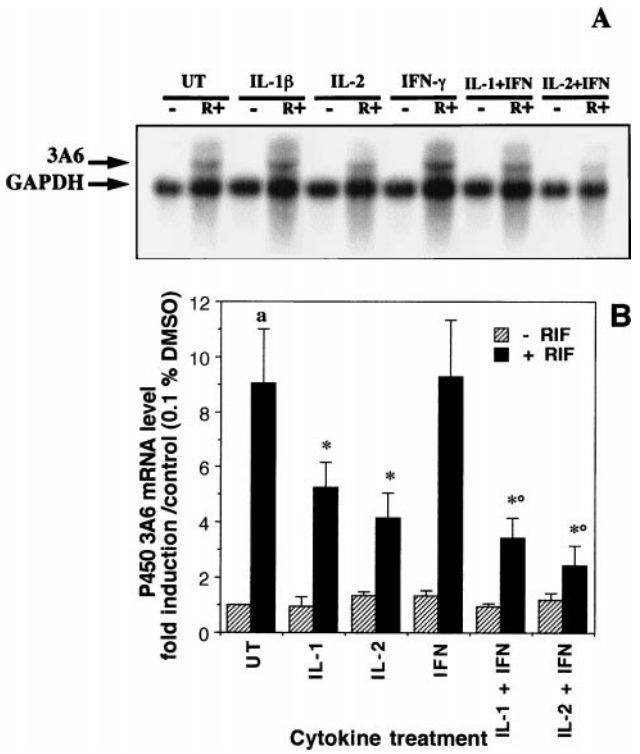

FIG. 3. Effect of cytokines on constitutive and induced *CYP3A6* mRNAs. (A) Rabbit hepatocytes were incubated with indicated treatments for 24 hr, as described in Materials and Methods. Then, total RNAs (20 μg) were subjected to Northern blot analysis. Membranes were hybridized with ^{32}P-labeled cDNA probes, *CYP3A6* and GAPDH as control. UT, untreated cells kept in culture for the same time as treated cells, (-) absence of inducer. Autoradiography representative of two separate experiments. (B) Relative RNA amounts were determined by densitometry and normalized to GAPDH mRNAs. Data obtained are expressed as induction ratios between RIF- and/or cytokine-treated samples and untreated control (0.1% DMSO). Incubations with RIF are given in the *black columns* whereas *hatched columns* represent incubations without inducer. The values correspond to the means ± SD of three distinct experiments measured in duplicate. [a]Significantly different from untreated control (0.1% DMSO) ($P < 0.05$). *Significantly different from corresponding induced or uninduced control (UT) ($P < 0.05$). °Significantly different from single cytokine treatments ($P < 0.05$)

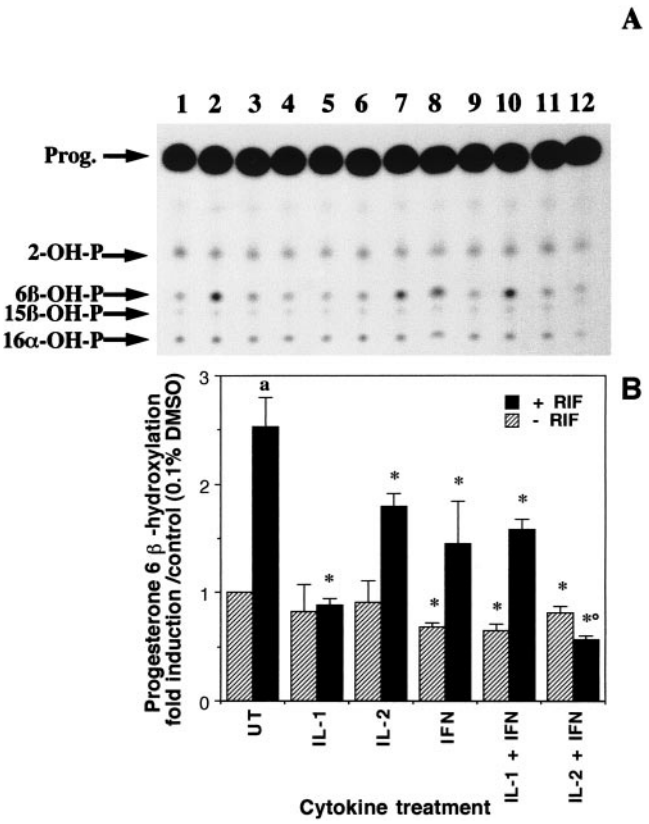

FIG. 4. Effects of cytokines on P4503A6-dependent progesterone 6β-hydroxylation. (A) Shown is an autoradiogram of TLC plate representative of three independent experiments. The five arrows indicate the native substrate progesterone (Prog), its main metabolite, the 6β-hydroxyprogesterone (6β-OH-P) and the minor metabolites, 15β-hydroxyprogesterone (15β-OH-P), 2-hydroxyprogesterone (2-OH-P) and 16α-hydroxyprogesterone (16α-OH-P), respectively. Top numbers correspond to the following treatments: 1. UT (0.1% DMSO); 2. RIF (25 μM); 3. IL-1β (2 U/mL); 4. IL-2 (5,000 U/mL); 5. IFN-γ (50 U/mL); 6. IL-1β + RIF; 7.IL-2 + RIF; 8. IFN-γ + RIF; 9. IL-1β + IFN-γ; 10. IL-1β + IFN-γ + RIF; 11. IL-2 + IFN-γ; and 12. IL-2 + IFN-γ + RIF. (B) Relative amounts of 6β-hydroxyprogestrone were determined by densitometry. Data obtained are expressed as induction ratio between RIF- and/or cytokine-treated samples and untreated control (0.1% DMSO). Incubations with RIF are given in the *black columns* whereas *hatched columns* represent incubations without inducer. The values correspond to the means ± SD of three distinct experiments measured in duplicate. [a]Significantly different from untreated control (0.1% DMSO) ($P < 0.05$). *Significantly different from corresponding induced or uninduced control (UT) ($P < 0.05$). °Significantly different from single cytokine treatments ($P < 0.05$)

CYP3A6-Dependent Metabolism of Progesterone under Cytokine Treatments

As expected, RIF provided a specific and marked increase (approximately 2.6-fold) in the formation of 6β-hydroxyprogesterone (Fig. 4A), the principal metabolite produced after P4503A-dependent progesterone hydroxylation [6]. Densitometric analysis of TLC demonstrated that IL-1β, IL-2, IFN-γ and IL-1β + IFN-γ impaired 6β-hydroxyprogesterone levels by 65%, 29%, 43% and 37.5%, respectively (Fig. 4B). Thus, IL-1β provoked a stronger decrease of the P4503A6-dependent progesterone metabolism than of the corresponding protein. In parallel, IFN-γ+IL-2 association resulted in a significant additive action (*ca.* 78% decrease), in comparison with separate cytokine treatments. By contrast, in agreement with constitutive P4503A6 protein levels ($r = 0.99$), only IFN-γ significantly decreased basal progesterone hydroxylation activity by 20% to 34%. Finally, the formation of the two P4502C-dependent metabolites 2-hydroxyprogesterone and 16α-hydroxyprogesterone [28] remained constant, whatever the treatment. Such a phenomenon suggests a selective cytokine action on P4503A-dependent activity.

DISCUSSION

The results of the present study using rabbit hepatocytes indicate a role for each cytokine in directly reducing constitutive and inducible expression of P4503A6. The data also suggest that cytokines differ in their activity to affect P4503A6 regulation. In fact, despite remaining weakly efficient in regard to END activity, IL-1β and IL-2 were almost equipotent in inhibiting *CYP3A6*-induced expression, including mRNA suppression. By contrast, both were ineffective in reducing P4503A6 basal expression, whereas IFN-γ strongly reduced basal and induced *CYP3A6* protein level and activity without any change in corresponding mRNA. Similarly, the association of IL-2 or IL-1β with IFN-γ provided significant depression of basal 3A6 protein content but not of uninduced P4503A6 messengers. By contrast, treatment with these cytokine combinations led to an increased effect on induced *CYP3A6* mRNA in comparison with single interleukin treatments. We also noticed that the degree of IL-mediated reduction in *CYP3A6* mRNA was more pronounced than the diminution of the respective protein levels; however, this phenomenon did not seem to be cytokine-specific and probably reflected the short duration of the experiments (24 hr), which did not allow us to reach the maximal protein decrease, considering the induced P4503A protein turnover (about 30 hr) [35]. We also observed that, under the same experimental conditions, these cytokines exhibited exactly the same behavior in regard to P4501A1-induced expression [13]. Moreover, like *CYP1A1*, *CYP3A6*-induced expression is known to be mainly under transcriptional control and the half-life of uninduced P4503A6 mRNA is *ca.* 22 hr [18]. Thus, taken together and in agreement with our previous study [13], all these observations suggest that IL-2 acts at a transcriptional level probably by decreasing the *CYP3A6* transcription rate. In fact, increased mRNA degradation does not seem to be implicated in IL-2-mediated regulation of P4503A6 expression, since such a mechanism would also provide a significant reduction in basal mRNA. By contrast, due to distinct transcriptional activation pathways, the reduction in the induced transcription rate could have no impact on basal P4503A6 messenger expression. For the same reasons, a decrease in

the transcription rate is probably also involved in the repressive action of IL-1β, as has already been described for this cytokine [20]. However, in the presence of IL-1β, the higher reduction in progesterone hydroxylase activity by comparison to corresponding protein content could be due to a possible functional inhibition of P450 catalysis. In fact, IL-1β could provoke increased heme loss, since it has been shown to induce heme oxygenase, which is responsible for P450 heme degradation [36]. But, contrary to our observations, such a phenomenon should also lead to a significant decrease in total P450 content. Concerning IFN-γ suppressive activity, a post-transcriptional mechanism is probably involved, since this cytokine does not have any effect on the *CYP3A6* mRNA level. The same observations and conclusions were made for the effects of IFN-γ on P4501A1-induced expression [13]. Two compatible pathways are usually proposed: i) enzyme degradation via its well-known induction of xanthine oxidase, which subsequently generates the free radicals that destroy P450 apoprotein; or ii) reduction in P450 mRNA translation [37, 38]. However, in regard to the effects of cytokine associations, the almost complete absence of cumulative effects observed in the case of induced progesterone metabolism, END activity and P4503A6 protein level suggests that IL- and interferon-promoted mechanisms are not complementary at the protein level. Such a deduction allows us to exclude the possibility of IFN-γ-mediated protein degradation, since it would not be incompatible with a reduced transcription rate. Moreover, this degradative mechanism would not account for the fact that IFN-γ does not have any significant effect on total P450 content. By contrast, both cytokine combinations provide a significantly potentiated suppression of induced *CYP3A6* mRNA, indicating that IFN-γ is able to increase IL-mediated transcription rate reduction. Thus, in this situation, a weak IFN-γ-mediated decrease in P450 mRNA level, either via a degradative pathway or via the same mechanism as interleukins, could be implicated. However that may be, IFN-γ, in agreement with previous reports [39, 13], seems to act mainly at the post-transcriptional level, probably by inhibiting P450 mRNA translation. In this respect, the cytokine-specific inhibition pattern of P4503A6-mediated progesterone 6β-hydroxylation is consistent with the putative distinct cytokine pathways, despite the unexplained greater decrease in P4503A6 protein expression in comparison with the loss of progesterone hydroxylase activity in the presence of IFN-γ. Thus, through their inhibitory activities on P4503A6 expression, all cytokines tested tend to impair progesterone metabolism. As P4503A are also involved in testosterone, androstenedione and cortisol hydroxylation, such *in vivo* effects could have significant consequences on animal and human reproductive functions, such as reduced fertility or the disruption of gestation.

In other respects, the common capability of cytokines to induce NO synthesis does not account for the differential effects observed, suggesting that under our experimental conditions, NO release does not play an important role, if any, in the repression of P4503A expression. This result is inconsistent with previous reports describing this small reactive molecule as a potent mediator of the decrease in P450-dependent metabolism caused by immunostimulants [40]. This difference could be related to the low cytokine concentrations used in our study as compared to those classically used in other *in vitro* investigations [41].

In conclusion, we have demonstrated that progesterone-metabolizing *CYP3A6* expression is differentially modulated by cytokines. Thus, this phenomenon might have relevance to the steroidogenesis pathway and subsequent hormonal regulation. Moreover, since P4503A also play a predominant role in the metabolism of drugs and xenobiotics in man [6], cytokine effects should also contribute to interindividual variations in drug susceptibility through the degree of inflammation. In parallel, an increasing number of clinical trials include therapeutical use of IL-2 and IFN-γ for the treatment of pathologic conditions such as viral hepatitis and cancer [42], whereas clinical investigations indicate that patients administered these cytokines have reduced hepatic monooxygenase activity [43]. Thus, in order to prevent the expected altered therapeutic and/or toxic effects of co-administered agents, further studies are necessary to identify the cytokine-specific mechanism of repression.

We are indebted to Dr. Martini (Hoechst Marion Roussel, France) for providing the IL-2 used in this study. The authors thank Drs. P. Maurel and F. J. Gonzalez for providing P450 antibodies and cDNA probes and Drs L. Brett–Bennis and C. Larroque for their excellent assistance. This work was supported in part by European Union contract AIR 2 CT 93.0860.

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