Biochemical Pharmacology, Vol. 44, No. 1, pp. 137–148, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

EFFECTS OF INTERLEUKIN-6 ON CYTOCHROME P450-DEPENDENT MIXED-FUNCTION OXIDASES IN THE RAT

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(*Received* 22 *November* 1991; *accepted* 16 *March* 1992)

Abstract—Intravenous treatment of male rats with recombinant human interleukin-6 (rhIL6) at 50, 100 and 200 μg/kg (corresponding to 4, 8 and 16 × 10^4 U/animal, respectively) reduced the activities of hepatic microsomal cytochrome P450-dependent monoxygenases to varying degrees. Ethylmorphine-*N*-demethylase activity fell to 53% of control values, an effect similar to that induced by 2.5 mg/kg *Escherichia coli* lipopolysaccharide (LPS). Ethoxycoumarin-*O*-deethylase activity was also sensitive to inhibition, whereas IL6 had little effect on the activities of other P450-dependent enzymes, including ethoxyresorufin-*O*-deethylase. Pentoxyresorufin dealkylase activity, which is representative of the cytochrome P450 IIB 1/2 subfamily, was unaffected by IL6 whereas LPS reduced it to 33.7% of control values. Another hepatocyte-related parameter, serum concentration of α1-acid glycoprotein (AGP), was increased by up to 3.5-fold over baseline by IL6 and 10-fold by LPS. Recombinant human interleukin-1β (rhIL1β) (10 μg/kg, corresponding to 5 × 10^4 U/rat) and recombinant human tumor necrosis factor α (rhTNF) (150 μg/kg corresponding to 24 × 10^4 U/rat) were both as potent as LPS (2.5 mg/kg) in increasing serum AGP levels and reducing hepatic microsomal monoxygenase activities. IL6 did not potentiate the effects of rhIL1β. Hepatic microsomal glucuronyltransferase activities were little affected by LPS and unaffected by rhIL6. Finally, rhIL6 was more potent after i.p. injection than after i.v. or s.c. injection. These results suggest that the effects of LPS, TNF and IL1 on the mixed-function oxidase system *in vivo* may be due partly to an induction of IL6 *in vivo*. The different sensitivities of the enzymes to IL6 but not to IL1 or TNF may be due to the involvement of two distinct mechanisms.

Cytokines are polypeptide mediators produced by various cell types. They act on a multitude of targets via specific cell membrane receptors and their biological activities have been characterized in a variety of physiological and pathological processes. The three main cytokines known to be involved in triggering host defense against infections and tissue injury are interleukin-1 (IL1‡) [1, 2], tumor necrosis factor α (TNF) [3] and interleukin-6 [IL6] [4]. Although each cytokine has specific properties, their activities overlap. TNF induces IL1 production [5], both TNF and IL1 can induce IL6 production [6] and all three cytokines are synthesized and secreted by monocytes–macrophages in response to lipopolysaccharide (LPS), a bacterial cell wall component [7, 8]. LPS- and cytokine-induced IL6 production has been observed both *in vitro* and *in vivo* in experimental and clinical studies [9–11].

The systemic host response to tissue damage is characterized by a series of events including fever, muscle protein breakdown, increased hepatic synthesis of acute-phase proteins and an increase in their plasma concentrations. This acute-phase response is mediated, at least in part, by the three cytokines cited above [12–15]. Another hepatocyte function modified during the acute-phase response is cytochrome P450 (P450)-dependent drug metabolism of the liver and other tissues. The activities of these enzymes are reduced within 24 hr of insult of traumatic, infectious or immune origin [16–19]. LPS injection induces similar enzyme depression in animals [20, 21] and these effects are reproduced by the administration of human recombinant IL1β (rhIL1β) and human recombinant TNF (rhTNF) to mice [22–26] and rats [27]. IL1 has also been shown to be active on cultured hepatocytes [22, 28], suggesting that it could be the common denominator in the effect of various agents *in vivo* and that it may be the mediator involved in the depression of drug-metabolizing enzymes following infection and tissue damage.

Given that IL1 and TNF induce IL6 *in vivo*, that the rat liver possesses specific IL6 receptors [29] and that the main biological property of IL6 during the acute-phase response is to regulate protein synthesis by hepatocytes, we studied the effect of rhIL6 treatment on microsomal enzyme activities in the rat liver. Since hepatic P450 is a superfamily of enzymes [30], we studied the various P450-dependent activities. Ghezzi and coworkers [22, 23, 25, 26] have reported that IL1β and TNF administration to mice has a depressive effect on ethoxycoumarin-*O*-deethylase (ECOD) activity, which is representative

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‡ Abbreviations: IL1, interleukin-1; IL6, interleukin-6; rh, recombinant human; TNF, tumor necrosis factor α; LPS, lipopolysaccharide; P450, cytochrome P450; AGP, α1-acid glycoprotein; ECOD, ethoxycoumarin-*O*-deethylase; EROD, ethoxyresorufin-*O*-deethylase; PROD, pentoxyresorufin-*O*-dealkylase; PNP, *p*-nitrophenol.

of both the P450 IA and IIB subfamilies. In addition to ECOD activity, we determined the effects of cytokines and LPS on microsomal enzyme activities representative of the P450 IA and IIB subfamilies and, less specifically, the P450 IIE and III A subfamilies. The effects of IL6 on serum levels of an acute-phase protein, α1-acid glycoprotein (AGP), were also studied as an indicator of hepatocyte function [31]. AGP was chosen because it is the main binding protein for basic drugs in plasma [32].

MATERIALS AND METHODS

Materials. rhIL6 (sp. act. 4×10^6 U/mg protein) was kindly provided by the Genetics Institute (Dr Capalucci, Cambridge, MA, U.S.A.). One unit is defined as the amount that induces half-maximal [^{3}H]thymidine incorporation into B9 cells [kindly provided by Pr Aarden (Netherlands)]. Endotoxin content (assayed in the limulus test) was less than 2.7 ng/mg protein. rhIL1β (sp. act. 25×10^6 U/mg protein) was a kind gift from Roussel Uclaf Laboratories (Romainville, France). One unit is defined as the amount inducing half-maximal proliferation of mouse thymocytes in the presence of a sub-optimal concentration of phytohemagglutinin A. The IL1β solution contained less than 0.7 ng endotoxin/mg protein. rhTNF (sp. act. 8.1×10^6 U/mg protein) was a kind gift from Knoll AG/BASF (Ludwigshafen, Germany). One unit of TNF is defined as the amount inducing half-maximal cytotoxicity against L 929 cells. The endotoxin content was less than 0.14 ng/mg TNF.

LPS from *Escherichia coli* (O55 : B5) was purchased from Difco (Detroit, MI, U.S.A.). LPS was diluted in sterile apyrogenic 0.9% NaCl and the cytokines were diluted in sterile, apyrogenic 0.9% NaCl containing 1% heat-decomplemented fetal calf serum.

The same batch of fetal calf serum was used after testing on human monocytes to ensure that it did not induce cytokine (TNF, IL1, IL6) production by itself. Experiments were also performed in which rats received i.p. injections of normal saline or i.v. injections of normal saline containing 1% fetal calf serum. Resulting microsomal P450 concentrations were 1.27 ± 0.10 and 1.28 ± 0.05 nmol/mg protein, respectively (four rats per group). ECOD activity was 2.37 ± 0.07 and 2.38 ± 0.10 nmol/min/mg protein, respectively, while serum AGP concentrations were low and identical in both groups (0.18 ± 0.02, 0.18 ± 0.01 g/L).

The doses of cytokines administered in these experiments corresponded to those used frequently by other researchers. The dose of LPS used (2.5 mg/kg) corresponds to the maximal effective non-lethal dose in Fischer rats.

All the chemicals used for the determination of monooxygenase activities were from the Sigma Chemical Co. (St Louis, MO, U.S.A.), except for ethylmorphine chlorhydrate which was kindly provided by Sanofi, Francopia (Paris, France).

Experimental protocol. LPS or cytokine solution (5 mL/kg) was injected by the i.p. or i.v. routes, respectively, into male 9–11-week-old Fischer 344 rats weighing 200–250 g (CNRS, Villejuif, France). Control rats received the solvent alone. The rats were killed by decapitation 24 hr later, unless otherwise stated. Trunk blood was collected and serum samples were kept at −70° until the determination of AGP concentrations by means of rocket immunoelectrophoresis with a specific rabbit anti-rat AGP antiserum [33]. Livers were rapidly excised, weighed and homogenized. Microsomes were prepared by double ultracentrifugation according to the method of Cresteil *et al.* [34]. Total microsomal concentrations of cytochrome P450 were determined on the same day using the method of Omura and Sato [35]. Remaining microsome preparations were aliquoted and kept at −80° for less than 1 month before assaying enzyme activities. ECOD activity, representative of both P450 IA (inducible by arylhydrocarbures) and P450 IIB (inducible by phenobarbital) subfamilies, was determined according to the method of Ullrich and Weber [36]. The P450 IA1 subfamily was explored more specifically by determining ethoxyresorufin-*O*-deethylase (EROD) activity, while the P450 IIB subfamily was explored in terms of pentoxyresorufin-O-dealkylase (PROD) activity, both according to the method of Burke and Mayer [37]. Aniline hydroxylase activity, which is partly representative of the P450 IIE subfamily (inducible by ethanol) and ethylmorphine-*N*-demethylase activity which is representative of P450 III (inducible by steroids) together with the IIB and IIC subfamilies were also determined [38, 39].

Immunoblotting. Proteins were separated using 12.5% SDS–PAGE according to Laemmli [40]; western blots were performed according to Burnette [41], with 20 μg of protein/lane. P450 IIIA was detected using a monoclonal antibody KO3 [42] given by Dr P. Kremers (University of Liege, Belgium).

P450 IA1 was detected with a polyclonal antibody, prepared with purified P450 IA1, as described previously [43]. This antibody cross-reacted with P450 IA1, and also with P450 IA2, so the identity of the band was checked with microsomes from rats induced with 3-methylcholanthrene (not shown). P450 IIB1/2 was detected with a polyclonal antibody prepared with purified P450 IIB1, as described previously [43]. This antibody cross-reacted with both P450 IIB1 and IIB2, but these proteins were not separated by 12.5% SDS–PAGE. The identity of the band corresponding to P450 IIB1/2 was checked with microsomes from rats induced with phenobarbital (not shown).

The protein concentration of hepatic microsomes was measured using the method of Lowry *et al.* [44], with bovine serum albumin as standard.

Results are expressed as means ± SEM (four to eight rats per group). Data were analysed using the Student's *t*-test.

RESULTS

Under our experimental conditions, no major signs of toxicity were observed in the animals following cytokine injection. In addition, the macroscopic aspect of the liver was normal and neither liver weight nor the protein concentrations

Table 1. Liver weight and protein content of hepatic microsomes of rats after injection of cytokines and LPS

Cytokine administered		Liver weight (g/100 g body weight)	Hepatic microsomal proteins (mg/g liver)
IL6	Control	2.84 ± 0.08	13.98 ± 1.13
(50 μg/kg i.v.)	Treated	2.78 ± 0.03*	12.97 ± 0.45*
IL6	Control	2.82 ± 0.08	17.52 ± 1.53
(100 μg/kg i.v.)	Treated	2.98 ± 0.03*	16.41 ± 0.64*
IL6	Control	2.81 ± 0.04	20.42 ± 2.23
(200 μg/kg i.v.)	Treated	2.95 ± 0.03*	20.18 ± 1.71*
IL1	Control	2.82 ± 0.08	17.52 ± 1.53
(10 μg/kg i.v.)	Treated	3.26 ± 0.08*	15.98 ± 0.77*
IL1 (10 μg/kg i.v.) +	Control	2.82 ± 0.08	17.52 ± 1.53
IL6 (100 μg/kg i.v.)	Treated	3.22 ± 0.05*	15.14 ± 0.54*
TNF	Control	3.48 ± 0.04	18.36 ± 0.35
(150 μg/kg i.v.)	Treated	3.39 ± 0.04*	18.64 ± 0.42*
LPS	Control	3.24 ± 0.07	23.02 ± 0.42
(2.5 mg/kg i.p.)	Treated	3.35 ± 0.06*	21.38 ± 0.87*

Each group contained four animals.
Data are the means ± SEM.
Statistical analysis was done by Student's *t*-test, *not statistically different from control group.

of hepatic microsome preparations were modified relative to untreated controls (Table 1).

In a previous study in which various doses of rhIL1β and rhTNF were administered to the same rat strain, we found that the injection of 1 μg rhIL1β/rat (= 5 μg/kg) or 6 μg rhTNF/rat (= 30 μg/kg) reduced hepatic microsomal P450 concentrations to 65 and 70% of control values, respectively [27]. Higher doses (10 μg rhIL1β/kg and 150 μg rhTNF/kg) only slightly accentuated the decrease in P450 concentrations (58 and 62% of control values), effects of the same order as those induced by 2.5 mg LPS/kg (data not shown). We therefore used these higher doses of IL1 and TNF considering them to be near the maximal effective doses toward P450.

Effects of cytokines and LPS on serum concentrations of AGP (Fig. 1)

The low concentration of AGP in the control group (0.21 ± 0.02 g/L) indicated an absence of inflammation. The administration of LPS led to a 10-fold increase in AGP levels (2.12 ± 0.07 g/L) and the effects of IL1β and TNF were of the same order. By contrast, the rise in AGP levels after the injection of 50, 100 or 200 μg rhIL6/kg was only moderate (2–3.5-fold), confirming that IL6 alone has a low capacity to induce this acute-phase protein [12]. As synergistic and additive interactions between IL1 and TNF or IL1 and IL6 have been described [45, 46], we investigated the effect of rhIL6 (100 μg/kg) and rhIL1 (10 μg/kg) in association on AGP levels. Serum levels of AGP were not higher than after the administration of rhIL1 alone.

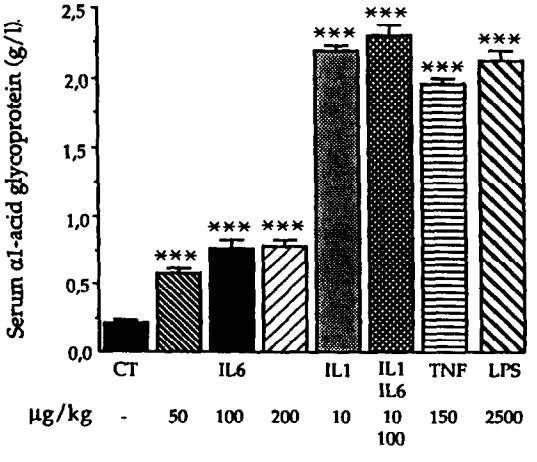

Fig. 1. Effects of cytokines and LPS on serum concentrations of AGP. Results are the means ± SEM (each group contained four animals). ***Significantly different ($P < 0.001$) from control group (CT). Concentrations of AGP in LPS control group were 0.21 ± 0.02 g/L and 0.22 ± 0.02, and 0.23 ± 0.02 g/L in cytokine control groups.

Capacity of IL6 to reduce microsomal P450 and drug-metabolizing enzyme activities

Table 2 shows the effect of rhIL6 (tested at three doses) on P450-dependent monoxygenase activities. ECOD activity was reduced in a dose-dependent manner to 61.8% of control values following the injection of 200 μg IL6/kg (corresponding to 160,000 U/rat) and to 43.8% following 2.5 mg LPS/kg. Ethylmorphine-*N*-demethylase activity was also inhibited by IL6 to 52% of control values versus 53% with LPS. Aniline hydroxylase activity was reduced to 69.9% of control values by IL6 and to 44.6% by LPS. EROD activity was decreased to only 79.0% of control values by IL6, compared with 51.8% by LPS. The most striking difference in sensitivity concerned PROD activity which was unaffected by IL6 but reduced to 33.7% of control values by LPS. Overall, hepatic microsomal

Table 2. Effect of LPS and varying doses of rhIL6 on P450 concentrations and related enzyme activities in hepatic microsomes

	LPS (2.5 mg/kg)		IL6					
			50 μg/kg		100 μg/kg		200 μg/kg	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
P450 (nmol/mg protein)	1.38 ± 0.07	0.84 ± 0.06‡	1.12 ± 0.05	0.96 ± 0.06§	1.03 ± 0.02	0.83 ± 0.06*	1.16 ± 0.02	0.83 ± 0.08†
ECOD (nmol/min/mg protein)	2.10 ± 0.11	0.92 ± 0.11‡	3.40 ± 0.24	2.87 ± 0.13§	2.57 ± 0.06	1.91 ± 0.10†	3.43 ± 0.18	2.12 ± 0.09‡
Ethylmorphine demethylase (nmol/min/mg protein)	19.86 ± 2.05	10.84 ± 1.31‡	25.56 ± 1.13	21.91 ± 2.65§	28.70 ± 1.56	23.70 ± 3.07*	30.93 ± 1.19	16.53 ± 1.24‡
ERCD (pmol/min/mg protein)	30.17 ± 1.83	15.63 ± 0.79‡	31.39 ± 3.45	28.93 ± 0.89§	28.09 ± 1.18	26.67 ± 1.22§	32.61 ± 1.62	24.77 ± 1.25†
PROD (nmol/min/mg protein)	22.13 ± 1.47	7.45 ± 0.06‡	ND	ND	22.19 ± 3.00	21.07 ± 2.26§	29.51 ± 4.88	28.58 ± 4.66§
Aniline hydroxylase (nmol/min/mg protein)	4.13 ± 0.42	1.84 ± 0.23‡	6.40 ± 0.53	5.61 ± 0.31§	4.53 ± 0.45	3.84 ± 0.58§	5.35 ± 0.27	3.74 ± 0.40‡

Each group contained four animals.
Data are the means ± SEM.
Statistical analysis was done by Student's *t*-test (* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$, § not statistically different from control group).
ND, not determined.

Table 3. Effect of rhTNF and rhIL1β alone or associated with recombinant IL6 on P450 concentrations and related enzyme activities in hepatic microsomes

	Control	TNF (150 μg/kg)	Control	IL1 (10 μg/kg)	IL1 (10 μg/kg) + IL6 (100 μg/kg)
P450 (nmol/mg protein)	1.35 ± 0.06	0.88 ± 0.02†	1.03 ± 0.02	0.70 ± 0.03†	0.59 ± 0.03†
ECOD (nmol/min/mg protein)	2.24 ± 0.04	1.23 ± 0.07†	2.57 ± 0.03	1.38 ± 0.04†	1.24 ± 0.05†
Ethylmorphine demethylase (nmol/min/mg protein)	25.77 ± 0.92	12.69 ± 0.99†	28.70 ± 0.90	13.44 ± 0.56†	13.35 ± 0.88†
EROD (pmol/min/mg protein)	30.13 ± 0.51	16.21 ± 0.58†	28.09 ± 0.80	16.65 ± 1.23†	14.83 ± 1.41†
PROD (nmol/min/mg protein)	19.67 ± 0.41	9.24 ± 0.26†	22.19 ± 1.50	6.62 ± 0.22*	5.83 ± 0.41*
Aniline hydroxylase (nmol/min/mg protein)	5.32 ± 0.19	2.59 ± 0.08†	4.53 ± 0.23	2.31 ± 0.11†	2.16 ± 0.20†

Each group contained four animals.
Data are the means ± SEM.
Statistical analysis was done by Student's *t*-test (* $P < 0.01$, † $P < 0.001$).

concentrations of P450 were decreased to 71.5% of control values by IL6 and 60.9% by LPS.

As shown in Table 3, rhIL1β (10 μg/kg, corresponding to 50,000 U/rat) and rhTNF (150 μg/kg, corresponding to 240,000 U/rat) alone were as potent as LPS (Table 2) in inhibiting the various enzyme activities. There was no statistical difference between the effect of IL1β alone and in association with IL6 (Table 3).

The effects of the three cytokines and LPS on ECOD and PROD activities are expressed in Fig. 2A and B, respectively, as percentages of control values. UDP-glucuronyltransferase activities were evaluated on the same microsomal preparations using four different substrates (naphthol, PNP, menthol and testosterone) (Fig. 3). None were modified significantly by 100 or 200 μg rhIL6/kg, while LPS administration led to a fall to 77–83% of control values ($P < 0.05$). By contrast with monooxygenase activities, the inhibition of glucuronyltransferase by rhIL1β did not reach statistical significance (Fig. 3).

Effects of LPS and cytokines on the expression of specific P450 isozymes

Western blots of apoprotein IA1 showed that IL6 alone had no apparent effect on this protein, whereas its levels were decreased by IL1 and, to a lesser extent, by LPS and TNF. Co-treatment by IL1 and IL6 depressed P450 IA1 to the same extent as by IL1 alone (Fig. 4A). Western blots of apoprotein IIB 1/2 showed that IL6 had no effect whereas IL1, LPS and TNF depressed its activity, as did co-treatment with IL1 and IL6 (Fig. 4B). Western blots of apoprotein IIIA 1/2 showed that it was only inhibited by 200 μg IL6/kg; the 100 μg/kg dose had no apparent effect. IL1, IL1/IL6 co-treatment and LPS all depressed the levels of the apoprotein, whereas TNF had no effect (Fig. 4C).

Time course of the decrease in P450 induced by rhIL6

In the preceding experiments the animals were

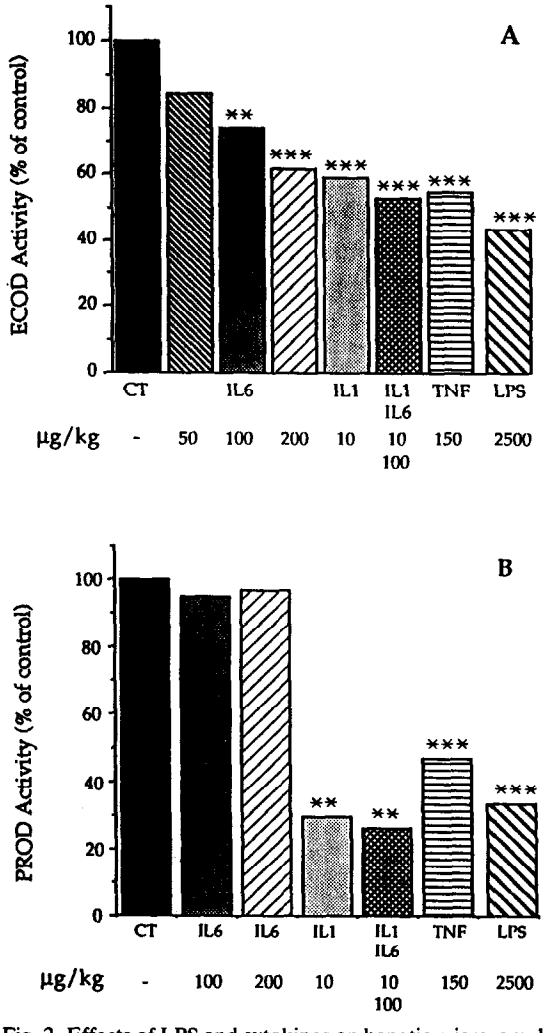

Fig. 2. Effects of LPS and cytokines on hepatic microsomal ECOD (A) and PROD (B) activities. Results are expressed as % of control values (CT). **$P < 0.01$; ***$P < 0.001$.

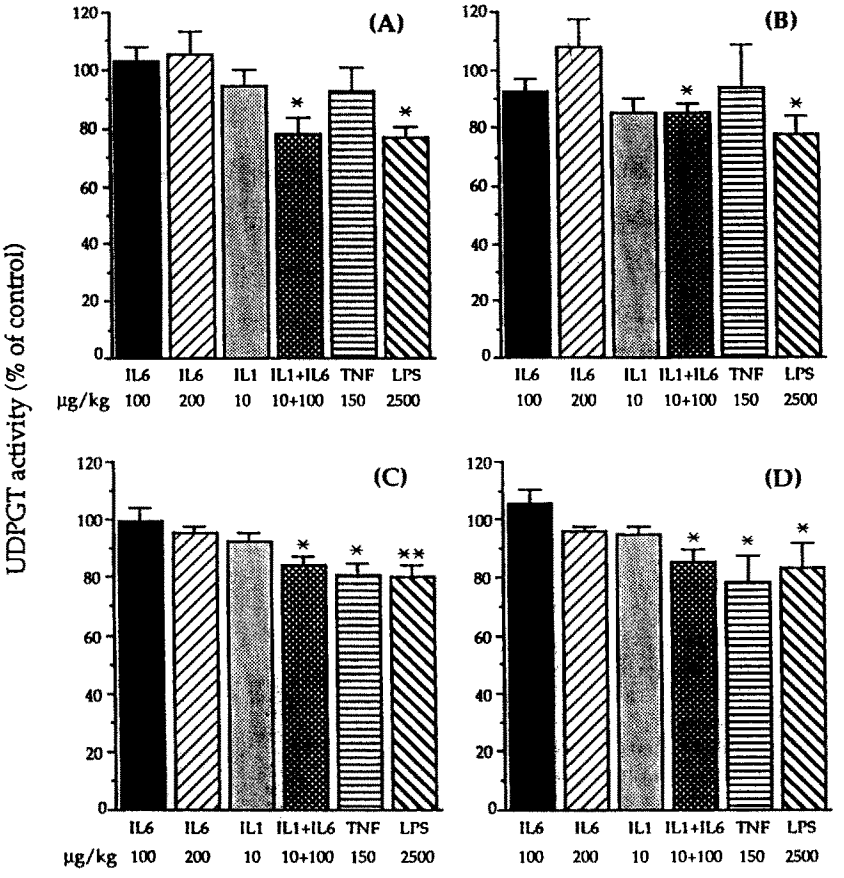

Fig. 3. Effects of LPS and cytokines on hepatic microsomal UDP-glucuronyltransferase (UDPGT) activities using four substrates: (A) naphthol, (B) PNP, (C) menthol, (D) testosterone. Results are the means ± SEM (N = 4). *P < 0.05; **P < 0.01. Mean UDPGT activities in controls were the following: naphthol, 56.46 ± 3.9 nmol/min/mg protein; PNP, 37.83 ± 2.51 nmol/min/mg protein; menthol, 16.35 ± 0.54 nmol/min/mg protein; testosterone, 11.66 ± 0.48 nmol/min/mg protein.

killed 24 hr after LPS or cytokine injection, a time at which the effect of rhIL1β, rhTNF and LPS is maximal [23, 25]. To determine whether or not this was also the case for IL6, groups of rats were killed 8, 24 and 48 hr after IL6 injection. The decrease in P450 concentrations was the same at 8 and 24 hr after administration and values began to return to normal at 48 hr (Fig. 5).

Effects of rhIL6 on P450 levels and drug-metabolizing enzyme activities according to the route of administration

To determine whether the effects of IL1 and TNF on P450-dependent enzymes are partly due to the induction of IL6 *in vivo*, IL6 was administered by the i.v. route which is generally the most potent for a pharmacological agent acting acutely on a highly vascularized organ. As shown in Table 4, the hierarchy of potency of 100 μg rhIL6/kg was s.c. < i.v. < i.p. No such effect was observed with IL1β (5 μg/kg), which reduced ECOD activity [from 2.20 ± 0.04 in controls (N = 4) to 1.18 ± 0.07 in the three treated groups (N = 12)] and EROD activity (from 36.32 ± 2.74 in controls to 20.47 ± 1.04 in the treated groups) to a similar extent whatever the route of administration.

DISCUSSION

Host defense mechanisms against tissue damage and infection are associated with impaired drug metabolism. The wide variety of agents capable of inducing the same alterations in P450-dependent monoxygenase activities has led various authors to search for a common mediator produced under these conditions. TNF and IL1 were both subsequently shown to reproduce the effect of inflammatory processes on drug-metabolizing enzymes [22–27]. In this study we found that parenteral administration of rhIL6 to male rats down-regulated hepatic microsomal monoxygenase activities, suggesting that the effects of Il1 and TNF on P450-dependent enzymes may result partly from the induction of IL6 by both cytokines *in vivo*.

Some enzyme activities were reduced to the same extent by 200 μg IL6/kg (corresponding to 80×10^4 U/kg, tested in a B9 clone), 10 μg IL1β/kg (corresponding to 25×10^4 U/kg, tested in the

Table 4. Effects of rhIL6 on hepatic microsomal P450 levels and related enzyme activities as a function of route of administration

	Control i.v.	IL6 (100 μg/kg) i.v.	i.p.	s.c.
P450 (nmol/mg protein)	1.21 ± 0.05	0.94 ± 0.05† (77%)	0.86 ± 0.03† (71%)	1.08 ± 0.07§ (89%)
ECOD (nmol/min/mg protein)	2.26 ± 0.09	1.57 ± 0.09† (70%)	1.47 ± 0.08† (65%)	1.83 ± 0.04† (81%)
Ethylmorphine demethylase (nmol/min/mg protein)	31.83 ± 1.27	24.69 ± 1.63* (77%)	22.25 ± 1.39† (70%)	25.81 ± 1.13* (81%)
EROD (pmol/min/mg protein)	31.44 ± 1.16	27.35 ± 0.59* (87%)	20.84 ± 1.01‡ (66%)	28.64 ± 2.01§ (91%)
Aniline hydroxylase (nmol/min/mg protein)	3.36 ± 0.22	2.56 ± 0.23* (76%)	2.29 ± 0.23† (68%)	2.62 ± 0.24* (78%)

Each group contained four animals.
Data are the means ± SEM.
Statistical analysis was done by Student's *t*-test (* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$). The results in brackets are expressed as % of control. § Not statistically different from control group (*t*-test).

lymphocyte-activating factor assay) and 2.5 mg/kg LPS. Therefore, rhIL6 appears to be nearly as potent as IL1β. It is also known that LPS-stimulated macrophages generally produce greater quantities of IL6 than IL1. Moreover, high concentrations of IL6 have been found *in vivo* under various conditions [47, 48].

The sensitivity of various enzyme activities to IL6 administration was variable. Deethylation of ethoxycoumarin appeared to be the least specific marker since all the cytokines, including interferons, so far tested in mice and rats *in vivo* are capable of depressing its activity. This is true for IL1α [25, 26], IL1β [25], TNF and lymphotoxin [22, 25], interferons α and γ [25, 49], and IL6 (our results). Moreover, inhibition of ECOD activity does not correlate with increased serum concentrations of fibrinogen [25] or AGP (our results), both of which are acute-phase proteins synthesized by hepatocytes under the control of inflammatory cytokines.

The results obtained for PROD, an enzyme activity reflecting P450 IIB 1/2 in rats and inducible by phenobarbital, are particularly interesting since PROD activity was very sensitive to endotoxin (33.7% of control values 24 hr after injection) and rhIL1β (29.9% of controls) but refractory to rhIL6 (96.8% of controls after injection of 200 μg/kg). IL1 and LPS inhibited P450 IIB 1/2 immunoreactive protein (Fig. 4).

Similar, although less marked, results were obtained for EROD activity which was reduced to about 50% of controls by LPS, TNF and IL1β but was hardly modified by IL6 (Tables 2 and 3). Western blotting also indicated that P450 IA1 protein was sensitive to LPS, IL1β and TNF (Fig. 4). Results obtained in mice showed that EROD activity was inhibited by LPS to only 70% of control values. In this species, recombinant IL1 [24] and interferon α [49] decrease its activity to only 80 and 70% of control values, respectively.

Other enzymes, including ethylmorphine-*N*-demethylase, were inhibited to the same extent (about 50%) after LPS, IL1β and IL6 administration. In the work by Renton and Mannering [18], ethylmorphine demethylation was depressed to 27% of controls by LPS and to about 40–50% of controls by various interferon inducers in male Holtzman rats. Other P450 IIIA2-dependent activities, microsomal levels of the apoprotein and P450 IIIA2 mRNA were also decreased by recombinant rat interferon γ administered to Wistar rats [50].

Overall, our results suggest that at least two mechanisms are involved in the impairment of drug metabolism during the acute-phase response and that inflammatory cytokines may regulate P450 activities by a mechanism similar to that involved in the regulation of acute-phase proteins. Indeed, strong similarities between the induction of acute-phase proteins and the inhibition of P450-dependent activities by the three cytokines, IL1, TNF and IL6, have been observed: (i) *In vivo*, some acute-phase proteins are predominantly induced in the rat by IL1 and TNF but are little affected by IL6 (AGP, haptoglobin, complement C3) [12, 27]. Similarly, some P450-dependent activities are inhibited by IL1 and TNF but little, if at all, by IL6 administration to rats (EROD and PROD activities) (our results). Interestingly, taking into account all our results, the increase in serum AGP concentrations induced by LPS and the three cytokines was correlated with the decrease in EROD and PROD activities ($r = -0.86$, $N = 36$, $P < 0.01$, between AGP and EROD activity; $r = -0.88$, $N = 24$, $P < 0.01$, between AGP and PROD activity). (ii) Other acute-phase proteins (e.g. fibrinogen) [12, 13, 15, 51, 52] and some enzyme activities (e.g. ECOD, ethylmorphine-*N*-demethylase) are modified after the administration of IL6, IL1 or TNF. (iii) Some acute-phase proteins (AGP, α2-macroglobulin) [12] and some P450-dependent activities (P450 IIC12) [53] are modified more strongly by the co-administration of a low dose of dexamethasone with IL6.

The biological properties of cytokines observed *in vivo* partially differ from those described *in vitro* in cultured rat hepatocytes. *In vitro*, contrary to IL1, IL6 is the major inducer of acute-phase proteins

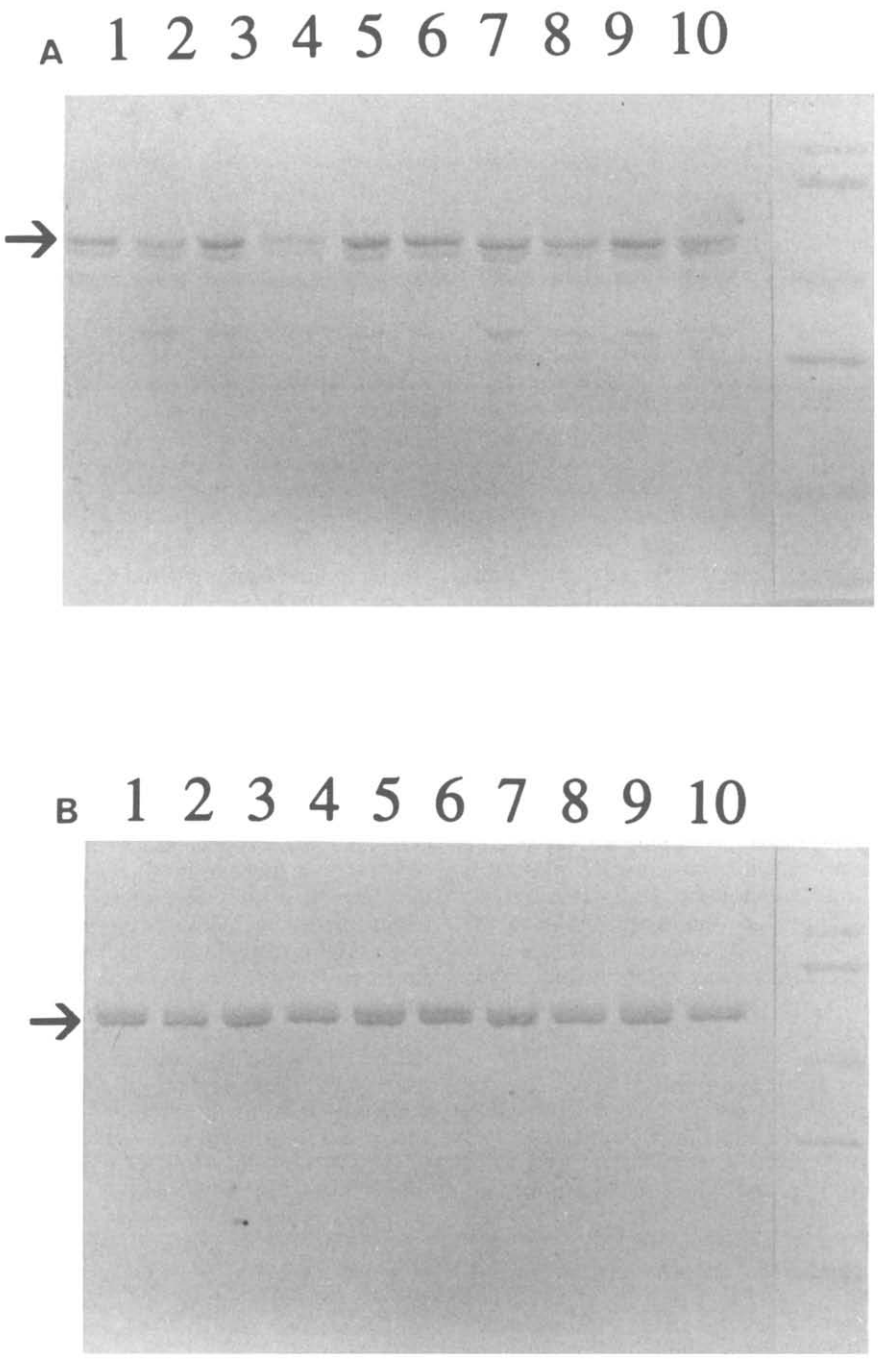

Fig. 4. Western blots of P450 apoproteins. Western blot was performed as described in Materials and methods with 20 μg of protein. Lanes 1, 5 and 9: saline with 1% of heat decomplemented fetal calf serum, lane 2: IL1 10 μg/kg, lane 3: IL6 100 μg/kg, lane 4: IL1 10 μg/kg and IL6 100 μg/kg, lane 6: IL6 200 μg/kg, lane 7: saline, lane 8: LPS 2.5 mg/kg, lane 10: TNF 150 μg/kg. The arrow indicates the corresponding apoprotein. (A) P450 IA1; (B) P450 IIB1/2; (C) P450 IIIA1/2.

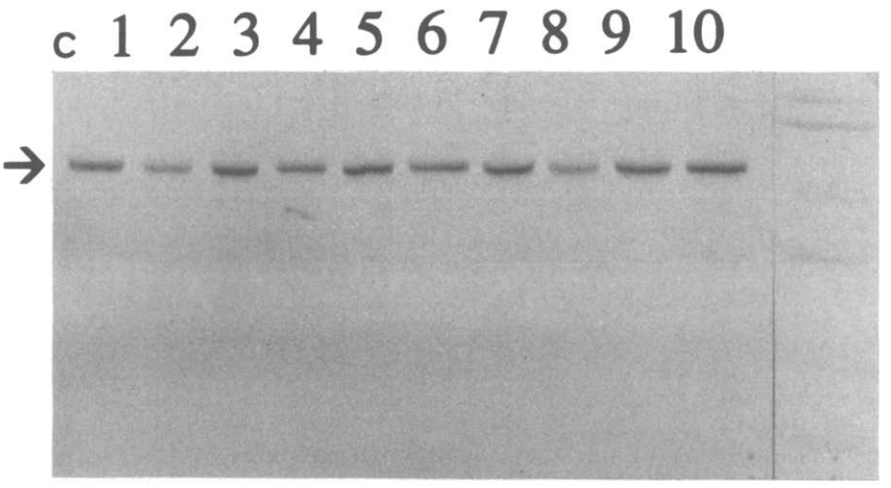

Fig. 4C.

such as α2-macroglobulin [14, 54]. With regard to P450-dependent activities, until very recently only IL1 was found to be active in hepatocyte cultures, in terms of ECOD activity and hepatocyte P450 content [13, 55]. However, IL6 has been found recently to be active toward aminopyrine-*N*-demethylase activity [56] and Williams *et al.* [37] have just described the potent inhibitory effect of rhIL6 on phenobarbital-induced P450 IIB-dependent activity and on P450 IIB 1/2 apoprotein in rat hepatocytes. This indicates that IL6 shares with IL1 the property to depress mixed-function oxidases *in vitro*.

It is not clear why IL6 is inactive *in vivo* toward P450 IIB 1/2-dependent activity and the corresponding isozyme revealed by immunoblot (Fig. 4) but active *in vitro* toward the same isozyme [57]. In this last study, rat hepatocytes were cultured in the presence of 0.1 μM dexamethasone [57, 58] and it is possible that glucocorticoids are an essential requirement for the effect of IL6 on P450 IIB, as they are necessary for the *in vitro* and *in vivo* effects of IL6 on some acute-phase proteins [12–14]. This hypothesis is reinforced by data indicating that co-administration of dexamethasone (at a dose 100-fold lower than that used to induce some P450 isoforms) and rhIL6 potentiates the suppression of P450 IIC12 in female rats [53]. It is necessary to determine *in vivo* the effect of rhIL6 associated with a low dose of dexamethasone to test this hypothesis.

The poor AGP-inducing capacity of IL6 observed by us and some of the results for microsomal enzymes

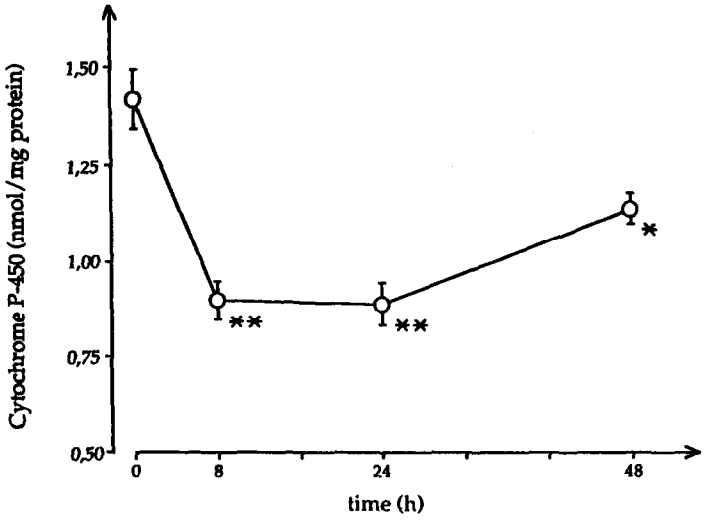

Fig. 5. Time course of hepatic microsomal P450 concentrations after i.v. administration of 200 μg/kg rhIL6. *P < 0.05; **P < 0.01.

cannot be attributed to a lack of activity of the rh molecule. The endogenous molecule is submitted to post-translational modifications including glycosylation and phosphorylation [29, 59], but rhIL6 expressed in *E. coli* retains all its major biological activities and Baumann *et al.* [29] have shown that the ligand-binding chain of the rat liver IL6 receptor presents more than 50% homology with the human leukocyte IL6 receptor. In addition, Marinkovic *et al.* [12] using rhIL6 of the same origin found that it stimulated strongly the synthesis of thiostatin and fibrinogen, but only minimally induced AGP (2.5-fold increase) after the injection of 2×33 μg/kg IL6 to rats. Finally, on a more general level, interleukin activities are not species-specific, contrary to interferons for which species identity has to be respected.

The optimal conditions for cytokine effectiveness toward the whole organism are unknown. The most active route of administration seems to depend on the cytokine used and possibly also on the effect considered. For instance, it has been found that s.c. injection or rhIL1β to rats is more lethal than the i.v. or i.p. routes of administration [60] while we found that IL1β depressed P450-dependent monoxygenases to the same extent using each of the three parenteral routes. No conclusion can be drawn concerning mice because Ghezzi *et al.* [22] used i.v. administration of IL1 and Shedlofsky *et al.* [24] the i.p. route [24] to determine different enzyme activities. Concerning rhIL6, the i.p. route of administration is preferred for studying the induction of acute-phase proteins in the rat [12]. Our finding that the order of potency for drug metabolism impairment by IL6 was s.c. < i.v. < i.p. (Table 4) indicates that the i.p. route is best for studying these effects. It is tempting to speculate that the lower activity of IL6 injected s.c. may result from its catabolism by skin fibroblasts [61, 62].

One important question is the pathophysiological and pharmacological relevance of the doses of cytokines administered. Endotoxin administration to experimental animals is a model of endotoxin shock in man characterized by a large increase in serum concentrations of TNF and IL6 (fewer data are available for IL1) during the hours following LPS injection [9–11, 47]. We have observed that 90 min after 2 mg/kg LPS injection to Fischer rats, serum TNF concentrations reach 40,000 U/mL (Chauvelot-Moachon *et al.*, to be submitted). Assuming a distribution volume of 10 mL for a rat weighing 200–250 g, i.v. injection of 150 μg/kg TNF (240,000 U/rat) gives a maximal plasma concentration of 24,000 U/mL, i.e. approximately half the TNF concentration obtained after LPS injection. By contrast, injection of 2 mg/kg LPS to Fischer rats leads to a maximal plasma concentration of 400 U/ml IL6 while 200 μg rhIL6/kg gives a concentration extrapolated at time zero of 16,000 U/mL. However, IL6 plasma levels do not necessarily reflect what happens in particular tissues or biological liquids. For instance, IL6 concentrations in the synovial fluid of patients with various kinds of arthritis can reach 50,000 U/mL [63, 64]. On the other hand, serum levels of IL6 can exceed 50,000 U/mL in mice in which cytokine release is induced by monoclonal antiCD3 antibody, a molecule used clinically during organ transplantation [65].

Depression of P450-dependent mixed-function oxidases secondary to increased levels of inflammatory cytokines could account for the decreased metabolism of low- and high-clearance drugs in various circumstances such as influenza vaccination [66], acute viral respiratory tract illness [67], turpentine oil-induced inflammation [68], indwelling catheters [69] etc. The potential clinical importance concerns drugs with a low therapeutic ratio. The effects of high interleukin and TNF concentrations on drug pharmacokinetics is a wide field for investigation, given the direct administration of cytokines, and the use of agents inducing cytokine release in the body and under pathological states associated with acute-phase responses.

Acknowledgements—This work was supported by funds from INSERM, Grant 892010 entitled "Relationships between hepatocyte functions and inflammation, consequences for drug pharmacokinetics" and from Association pour la Recherche sur le Cancer, Grant 6095. We thank D. Young for reading the manuscript. We thank Dr P. Kremers for the gift of the monoclonal KO3.

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