Biochemical Pharmacology, Vol. 45, No. 3, pp. 585–592, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

INTERLEUKIN 2 INDUCTION OF CYTOCHROME P450-LINKED MONOOXYGENASE SYSTEMS OF RAT LIVER MICROSOMES

KAZUTAKA KUROKOHCHI,*† YOSHINORI MATSUO,* HIROHITO YONEYAMA,* MIKIO NISHIOKA† and YOSHIYUKI ICHIKAWA*‡

Departments of *Biochemistry and †Internal Medicine (3rd Division), Kagawa Medical School, 1750-1, Ikenobe, Miki-cho, Kita-gun, Kagawa, 761-07, Japan

(*Received* 14 *May* 1992; *accepted* 5 *November* 1992)

Abstract—The effects of interleukin 2 (IL-2), a pivotal cytokine for generating an effective immune response, on rat liver microsomal cytochrome P450-linked monooxygenase systems were investigated by measuring the contents of cytochromes b_5 and P450, and the activities of various xenobiotic-metabolizing enzymes [debrisoquine and bufuralol monooxygenases (CYP2D), 7-ethoxycoumarin *O*-deethylase, benzphetamine *N*-demethylase, aniline hydroxylase and *p*-nitroanisole *N*-demethylase]. The enzymatic activities except for *p*-nitroanisole *N*-demethylase and aniline hydroxylase were increased approximately to 1.3-fold of those of untreated liver microsomes following intraperitoneal infection of IL-2 (15 U/rat). However, the amount of immunoreactive b_5 protein, and the activities of aniline hydroxylase and *p*-nitroanisole *N*-demethylase were not changed by injection of IL-2. To elucidate further the mechanism of the induction of CYP2D by IL-2, quantitative analyses of immunoreactive CYP2D protein and its mRNA were conducted by western blot and slot blot hybridization analyses. The results indicated that IL-2 induced an increase in the amounts of immunoreactive CYP2D protein and its mRNA. These enzymatic activities were thus up-regulated at the mRNA level.

Interleukin-2 (IL-2§) produced by activated T lymphocytes is one of the best characterized cytokines. This cytokine was first recognized as a T cell growth factor in the supernatant of phytohemagglutinin stimulated lymphocytes [1], and is pivotal for the generation of an effective immune response. Much of its biological activity, gene structure and clinical potential on hepatitis and cancer immunotherapy has been documented [2–4]. Many immunomodulators such as interferon (IFN), IFN inducers, interleukin-1 (IL-1) and interleukin-6 (IL-6) depress the activities of liver cytochrome P450 (P450)-linked monooxygenase systems [5–8]. Among them, much attention has been focused on the depressive effects on P450-linked monooxygenase systems caused by inflammatory cytokines such as IL-1 and IL-6. However, there have been no reports regarding the effects of IL-2 on P450-linked monooxygenase systems. Therefore, we investigated the effects of IL-2 on P450-linked monooxygenase systems by measuring the content and the enzymatic activities of xenobiotic-metabolizing enzymes of rat liver microsomes. Furthermore, the effects of IL-2 on the immunoreactive CYP2D protein and gene expression of CYP2D subfamily members were investigated by performing quantitative analyses of the immunoreactive CYP2D protein of liver microsomes and its mRNA.

MATERIALS AND METHODS

Chemicals. IL-2 purified from the conditioned medium of a rat splenocyte culture stimulated by concanavalin A, benzphetamine and 7-ethoxycoumarin was purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Debrisoquine, 4-hydroxydebrisoquine, bufuralol and 1′-hydroxybufuralol were generous gifts from Dr H. Fukui at the Department of Pharmacology, Osaka University Medical School. The Histofine SAB-PO(R) kit for western blot analyses was purchased from the Nichirei Co. (Tokyo, Japan). Rabbit anti-human immunoglobulins (IgG, IgA and IgM) were purchased from the Funakoshi Chemical Co. (Tokyo, Japan). All other chemicals of the highest commercially available grade were purchased from Wako Pure Chemicals (Osaka, Japan) and Nacalai Chemical Co. (Kyoto, Japan).

Animals and IL-2 treatments. Male Wistar white rats weighing about 100 g (5 weeks old) were used throughout this study. They were housed in plastic cages and allowed free access to food and water. They were maintained under a controlled lighting cycle (12 hr on, 12 hr off) and deprived of food and water overnight before decapitation. IL-2 solutions (15 or 30 U/rat) dissolved in 1.5 mL saline and saline only for the controls were injected once intraperitoneally into the rats.

Preparation of rat liver microsomes. Twenty-four or 48 hr after the injection of IL-2, rats were killed by cervical dislocation and prior to removal their livers perfused via the portal vein with ice-cold 50 mM potassium phosphate buffer (pH 7.4) containing 1.15% (w/v) potassium chloride to remove as much blood as possible. The microsomal fractions were immediately prepared at below 4° by the method of Mitoma *et al.* [9].

Determination of the contents of P450, b_5 and

‡ Corresponding author. Tel. (81) 878 98-5111; FAX (81) 878 98-7109.

§ Abbreviations: IL-1, interleukin 1; IL-2, interleukin 2; IL-6, interleukin 6; P450, cytochrome P450; b_5, cytochrome b_5; IFN, interferon; SDS, sodium dodecyl sulfate.

protein. Protein concentration was determined by the method of Lowry *et al.* [10] using bovine serum albumin as a standard. A molar extinction coefficient of bovine serum albumin of $6.68 \times 10^3\ M^{-1}\ cm^{-1}$ at 280 nm was used [11].

The content of P450 was determined spectrophotometrically by a carbon monooxide difference spectrum of a dithionite-reduced sample with a molar extinction coefficient of $91 \times 10^3\ M^{-1}\ cm^{-1}$ at 450 minus 490 nm [12]. The content of b_5 was determined by a reduced and oxidized difference spectrum with a molar extinction coefficient of $185 \times 10^3\ M^{-1}\ cm^{-1}$ at 424 minus 409 nm [12].

Assays of the enzymatic activities. The activities of benzphetamine *N*-demethylase and *p*-nitroanisole *N*-demethylase of liver microsomes were determined by measuring the formation of formaldehyde according to the method of Nash [13]. The activity of 7-ethoxycoumarin *O*-deethylase was determined by the method of Greenlee and Poland [14]. The activity of aniline *p* hydroxylase was determined by measuring the formation of *p*-aminophenol from aniline by the method of Imai *et al.* [15]. These enzymatic reactions were performed at 37° for 5 min. The activities of debrisoquine and bufuralol monooxygenases were determined by the methods of Kronbach *et al.* [16] and Matsuo *et al.* [17] at 37° for 40 min. All measurements of enzymatic activities were carried out in a final volume of 1 mL of 50 mM potassium phosphate buffer (pH 7.4) containing substrate: 1 mM benzphetamine, 1 mM 7-ethoxycoumarin, 0.25 mM debrisoquine or 0.25 mM bufuralol, NADPH regenerating system (5 mM glucose 6-phosphate, 0.5 IU glucose 6-phosphate dehydrogenase, 4 mM $MgCl_2$ and 0.2 mM NADPH) and 1 mg microsomal protein.

Preparation of anticytochrome b_5 (b_5) *IgG*. b_5 was purified from bovine liver microsomes by the method of Spatz and Strittmatter [18]. This purified enzyme gave a single protein band on SDS–polyacrylamide gel at a molecular mass of 16 KDa. The immunization of rabbits with purified b_5 was performed by the method of Hamamoto *et al.* [19]; that is, the solution of highly purified b_5 in 0.5 mL of 50 mM potassium phosphate buffer, pH 7.4 containing 0.1 mM EDTA with 1.0 mL of Freund's complete adjuvant was injected into the footpads or a thigh muscle of rabbits once a week for 3 weeks. Two weeks after the last injection, a booster injection of 1.0 mg protein was given. The globulins were purified from the rabbit sera by ammonium sulfate precipitation.

Western blot analyses. Liver microsomes (5 μg of protein/lane) were subjected to SDS–PAGE and the proteins were transferred electrophoretically to nitrocellulose sheets and then immunoblotted with the serum of an autoimmune hepatitis patient containing the LKM-1 autoantibody (CYP2D6 antibody) and anti-b_5 IgG. The serum of an autoimmune hepatitis patient was received as a generous gift from Dr Michael P. Manns of the Department of Internal Medicine (1st Division) at the University of Mainz, Germany. The reactivity of anti-CYP2D IgG in this serum has already been confirmed by examining the expressed fusion protein of cDNA in *Escherichia coli* [20]. Low range molecular mass marker proteins were obtained from Bio Rad laboratory Co., Ltd (Richmond, CA, U.S.A.).

RNA slot blot hybridization. Liver specimens of approximately 500 mg were incubated in a solution consisting of 4 M guanidine thiocyanate, 25 mM sodium citrate, 0.1 M 2-mercaptoethanol and 0.5% (w/v) sarcosyl, for 10 hr at 37°. Total RNA extractions from the livers were conducted by the method of Chomczynski and Sacchi [21]. Five micrograms of RNA were used for the slot blot hybridization. Bovine CYP2D cDNA, cloned and sequenced in this laboratory and found to be approximately 70% homologous to the rat CYP2D subfamily, was used as the probe [22]. Under the conditions used, 70% homologous hybridization was possible with the cDNA probe, which means that the same subfamily, i.e. CYP2D subfamily members, should be detected. The labeling of CYP2D cDNA with [^{32}P]-dATP was carried out by the method of nick translation [23]. Hybridization was conducted in 4 × SETS buffer [0.6 M NaCl, 120 mM Tris–HCl (pH 7.5), 8 mM EDTA, 10 × Denhardt and 0.1% (w/v) SDS] containing 50 mg/mL heat-denatured salmon sperm DNA at 60° for 24 hr. A single wash was conducted in 4 × SETS buffer [0.15 M NaCl, 30 mM Tris–HCl (pH 7.5), 2 mM sodium EDTA and 0.1% (w/v) SDS] followed by a double wash in 1 × SETS buffer at 60° for 15 min.

Densitometrical analyses of western blot and RNA slot blot hybridization. Densitometrical analyses of immunoblots were performed optically by absorbance at 550 nm and those of RNA slot blots were performed at 610 nm using a Shimadzu Dual Wavelength TLC Scanner Model CS 910.

Statistical analyses. Statistical analyses were performed on Macintosh classic Stat-Work soft ware. The statistical significance was determined using the Student's *t*-test.

RESULTS

Initially, the effect of 15 and 30 U of IL-2 on the spectrophotometrical P450 content of rat liver microsomes was investigated. After 24 hr, the P450 content of the rat liver microsomes was comparable to that before injection but it was significantly increased to 118.6% and 118.8% of the original value 48 hr after the injection of 15 and 30 U of IL-2, respectively. (The mean content of P450 of untreated liver microsomes was 0.84 ± 0.11 nmol/mg of microsomal protein.) Consequently, it was decided to use rat liver microsomes 48 hr after injection of 15 U of IL-2 for the following experiments.

In addition, we examined the effect of IL-2 (15 U/rat) on the spectrophotometrical content of b_5 of rat liver microsomes 48 hr after the injection of IL-2. In contrast with P450, a significant change in the content of b_5 due to injection of IL-2 was not observed. The mean contents of b_5 of the liver microsomes of untreated rats and IL-2-treated rats were 0.40 ± 0.015 and 0.42 ± 0.027 nmol/mg of microsomal protein, respectively.

Furthermore, the immunoreactive b_5 proteins of liver microsomes were immunoquantitated using

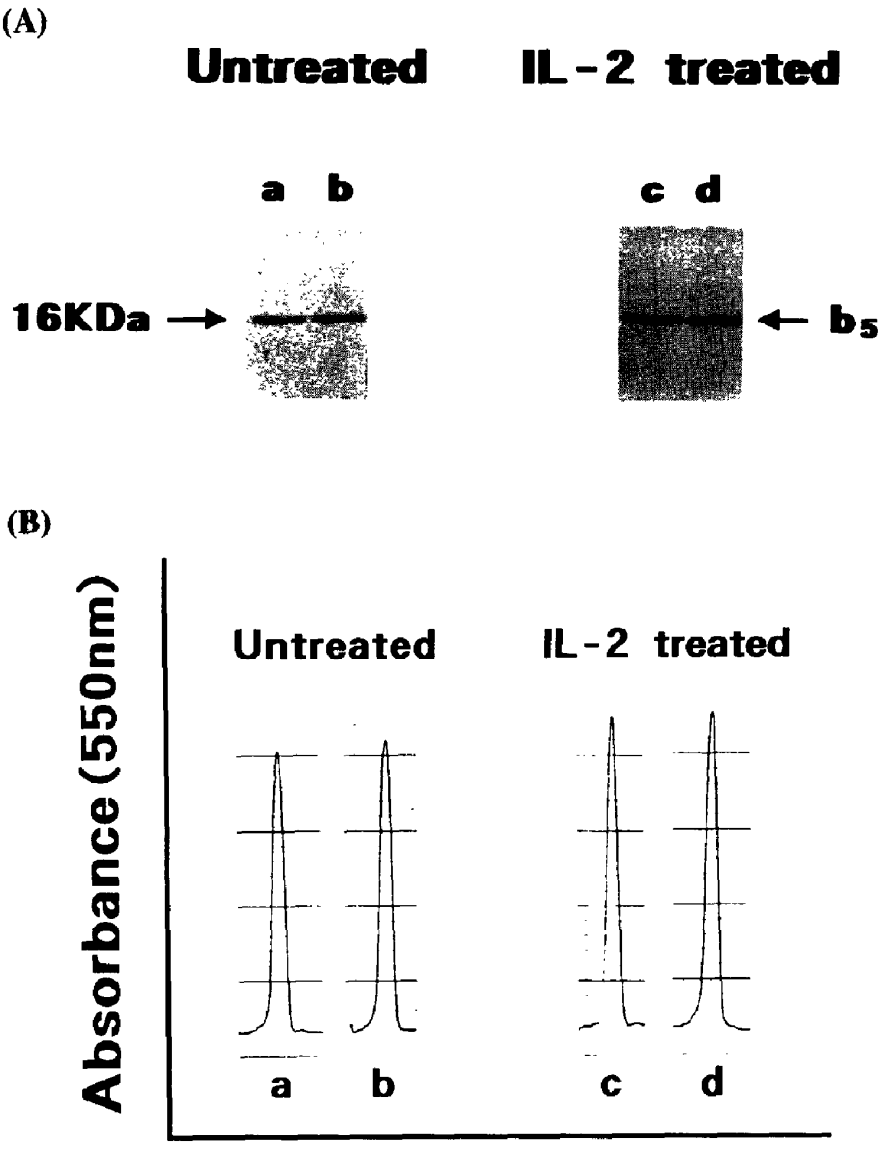

Fig. 1. (A) The effects of IL-2 on the immunoreactive b_5 protein of rat liver microsomes. Duplicate experiments are shown with IL-2 (15 U/rat) dissolved in 1.5 mL saline, or only saline for the controls, intraperitoneally injected into rats. After 48 hr the rats were killed and liver microsomes prepared. The proteins (5 μg) of the liver microsomes were subjected to SDS–PAGE, then transferred to nitrocellulose sheets. The blots were visualized using anti-b_5 IgG obtained from b_5-immunized rabbits. The relative molecular mass of the stained band is indicated as 16 kDa. Lanes a and b, untreated rats; lanes c and d, IL-2 treated rats. (B) Densitometric analyses of (A), performed as described in the text.

anti-b_5 IgG purified from b_5 immunized rabbits. The electrophoretical bands and their densitometrical analyses shown in Fig. 1 did not express differences between untreated and IL-2-treated rats.

Subsequently, the effects of IL-2 (15 U/rat) on the activities of several xenobiotic-metabolizing enzymes: debrisoquine 4-monooxygenase, bufuralol 1′-monooxygenase, 7-ethoxycoumarin *O*-deethylase, benzphetamine *N*-demethylase, aniline *p*-hydroxylase and *p*-nitroanisole *N*-demethylase, were examined. The activities of all the xenobiotic-metabolizing enzymes with the exception of aniline hydroxylase and *p*-nitroanisole *N*-demethylase were significantly enhanced by 15 U of IL-2. The inducible rates of these activities were approximately 1.3-fold of those in untreated liver microsomes. The activities of aniline *p*-hydroxylase and *p*-nitroanisole *N*-demethylase were not changed by the injection of IL-2. These results are shown in Fig. 2.

To elucidate further the induction mechanism of xenobiotic-metabolizing enzymes by IL-2, the amount of CYP2D protein was immunoquantitated by western blots and the amount of CYP2D mRNA was quantitated by RNA slot blot hybridization. The

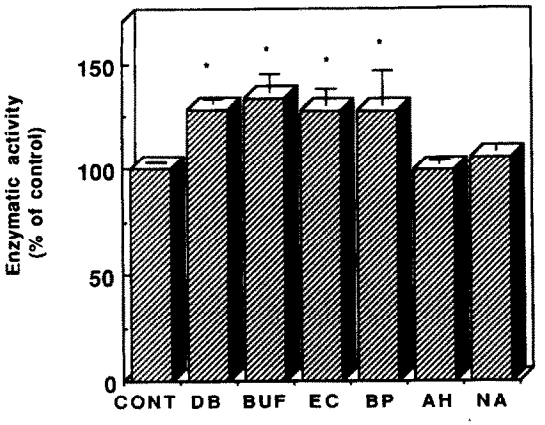

Fig. 2. The effects of IL-2 on the activities of debrisoquine 4-monooxygenase (DB), bufuralol 1′-monooxygenase (BUF), 7-ethoxycoumarin *O*-deethylase (EC), benzphetamine *N*-demethylase (BP), aniline *p*-hydroxylase (AH) and *p*-nitroanisole *N*-demethylase (NA) of rat liver microsomes. IL-2 (15 U) dissolved in 1.5 mL saline, or saline only for the controls, was injected intraperitoneally. The enzymatic activities are expressed as percentages of control values. Control values are DB, 0.28 ± 0.12; BUF, 0.62 ± 0.23; EC, 2.79 ± 0.88; BP, 4.25 ± 1.30; AH, 0.77 ± 0.17 and NA, 1.91 ± 0.08 (nmol/min/mg microsomal protein). Each value represents the mean ± SD of four rats and the values which are significantly different from controls are shown by *: *$P < 0.05$. Columns indicate the means and error bars express the standard deviations.

results of western blot analyses of liver microsomes using the serum containing the CYP2D antibody are shown in Fig. 3 and those of RNA slot blot hybridization are shown in Fig. 4. From the densitometrical analyses of stained protein bands of western blot and hybridized bands of RNA slot blot, significant changes ($P < 0.05$) were observed between the absorption peak areas of IL-2-treated rats and those of untreated rats. The inducible contents of CYP2D protein and its mRNA by IL-2 corresponded, indicating that IL-2-inducible CYP2D was up-regulated at the mRNA level.

DISCUSSION

In this study, the effects of IL-2 on the content of P450 and the activities and gene expression of several xenobiotic-metabolizing enzymes were investigated. Since IL-1 and IL-6 have depressive effects on the enzymatic activities of liver microsomal enzymes [6–8], the results in this study provided new evidence of induction effects on the content and enzymatic activities of P450 by IL-2. The relationship between some cytokines and the activities of xenobiotic-metabolizing enzymes of liver microsomes has been reported by many investigators [5–8]. Some cytokines such as IFN, IL-1 and IL-6 have been reported to depress the level of P450 as well as the activities of P450-dependent xenobiotic-metabolizing enzymes [3–6]. We confirmed that IL-1 suppressed the gene expression of CYP2D mRNA [24]. Attention has been focused on the depressive effects of IL-1 and IL-6 which are considered to be important mediators of the inflammatory response. Unexpectedly, the present study shows that P450 is induced by injection of the cytokine IL-2 *in vivo* in contrast with the reports of depressive effects of the other cytokines. Tominaga *et al.* [25] have reported that IL-2 directly stimulates glucocorticoid synthesis using rat adrenocortical cells in an *in vitro* study. However, there have been no reports on the induction of xenobiotic-metabolizing enzymes by IL-2 in an *in vivo* study.

It is well known that a variety of xenobiotics including 3-methylcholanthrene and phenobarbital induce the activities of xenobiotic-metabolizing enzymes [26, 27]. Our finding of induction by IL-2 of P450 is of interest, because IL-2 is not a xenobiotic but a cytokine physiologically produced with important immunological roles. We further analysed the effects of IL-2 on b_5 which is a major component of the P450-dependent mixed-function oxidase system, but no induction of b_5 protein was observed. The physiological relevance of the induction of xenobiotic-metabolizing enzymes by IL-2 is unclear. However, our results suggest that IL-2 may bear a close relation to the defense mechanism against xenobiotics via P450, which may enhance detoxification.

From the results of our experiments, induction of P450 was not observed after 24 but was observed after 48 hr with post intraperitoneal IL-2 injection. With other cytokines including IL-1, IL-6 and IFN depression of xenobiotic-metabolizing enzymes was observed 24 hr after injection. The fact that induction by IL-2 is delayed compared to that by other cytokines suggests that the effects of IL-2 on xenobiotic-metabolizing enzymes are indirect, possibly provoked by changes in endogenous substrates. The receptors of IL-2 exist mainly on the surface of T lymphocytes and are not found on hepatocytes. This is consistent with the suggestion that IL-2 indirectly affects the xenobiotic-metabolizing enzymes.

Though there is overlapping in the substrate specificity of the enzymatic activities of P450 isozymes [28–32], the major activities of individual P450 isozymes of rat liver microsomes are as follows: CYP2D1 is principally responsible for debrisoquine and bufuralol monooxygenation, CYP2B1 and CYP2C11 for benzphetamine N-demethylation, and CYP1A1 and CYP2B1 for 7-ethoxycoumarin O-deethylation. Aniline *p*-hydroxylation is mainly catalysed by CYP2E1 and CYP1A1, and *p*-nitroanisole N-demethylation is mainly catalysed by CYP2C11. However, the activity of CYP1A1 in 7-ethoxycoumarin *O*-deethylase is much higher than that in aniline *p*-hydroxylase [33].

Therefore, all activities of xenobiotic-metabolizing enzymes except for the activities of aniline *p*-hydroxylase and *p*-nitroanisole *N*-demethylase were induced significantly by IL-2, suggesting that IL-2 influences not just one specific isozyme but also some subfamilies of P450 isozymes such as CYP2D1, CYP2B1 and CYP1A1. IL-2 does not have much effect on CYP2E1 and CYP2C11 subfamily members.

Moreover, we investigated the effects of IL-2 on the content of CYP2D protein and its gene

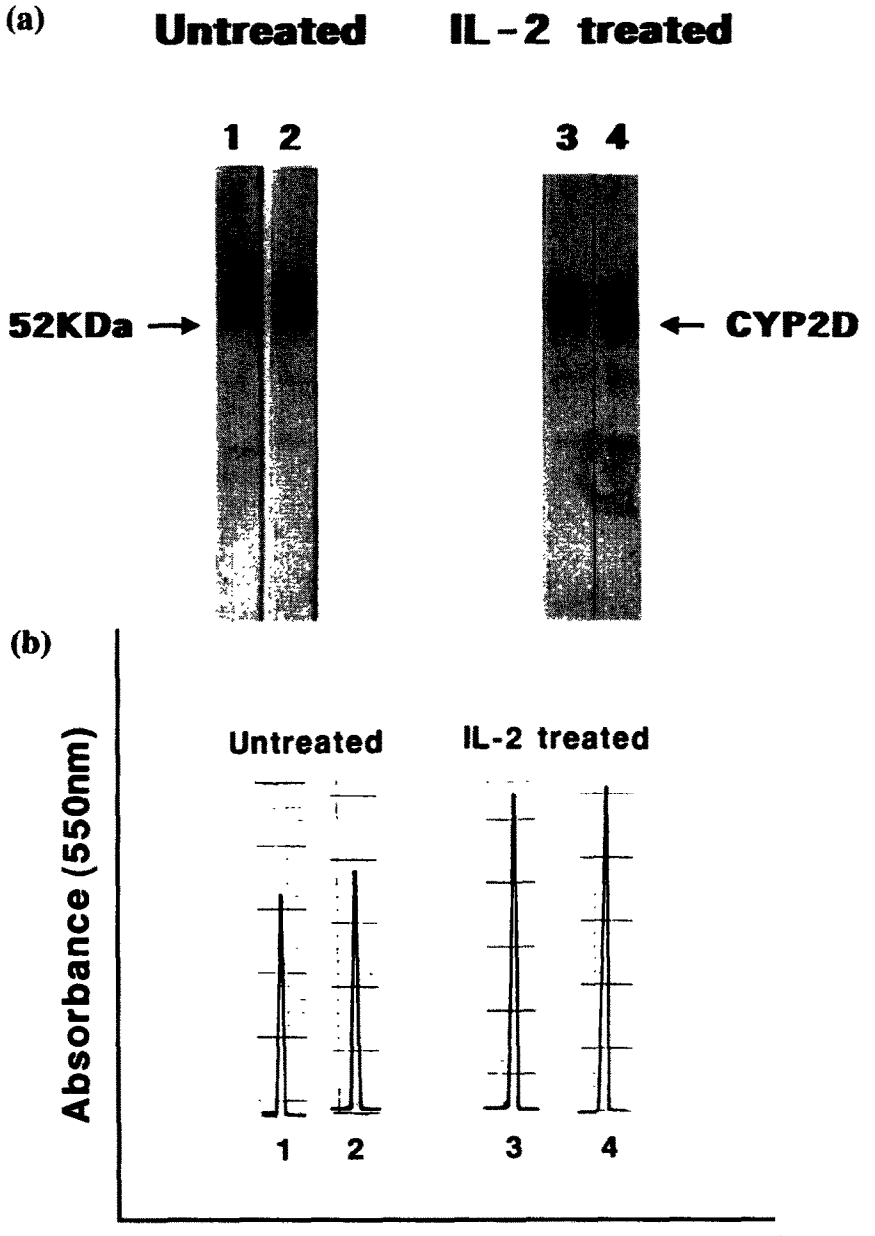

Fig. 3. (a) Western immunoblot analyses of CYP2D of rat liver microsomes. IL-2 (15 U/rat) dissolved in 1.5 mL saline or saline only for the controls, was injected intraperitoneally. The liver microsomal proteins (5 μg) were subjected to SDS–PAGE and transferred to nitrocellulose sheets, and the blots were visualized by the method described in Materials and Methods. The relative molecular mass of the stained protein band is indicated as 52 kDa. Lanes 1 and 2, untreated rats; lanes 3 and 4, IL-2 treated rats. (b) Densitometric analyses of (a), performed as described in the text.

expression. Serum of an autoimmune hepatitis patient containing a high titre of LKM-1 antibody (CYP2D6 antibody) was used for performing quantitative analyses of immunoreactive CYP2D of rat liver microsomes. It has already been demonstrated that CYP2D6 antibody in the serum of an autoimmune hepatitis patient recognizes the CYP2D of rat liver microsomes [33–35]. Therefore, the antibody against CYP2D6 used in this experiment should also react with the CYP2D protein of rat liver microsomes. A previous report demonstrated that CYP2D6 antibody was able to recognize both forms of P450, CYP2D1 and CYP2D2 of rat liver microsomes [34]. From the results of western blot analyses of our experiments, a difference in the induction rates of CYP2D1 and CYP2D2 protein could not be detected because the molecular masses of these proteins were similar and their electrophoretical bands overlapped. From the standpoint of enzymatic activities, CYP2D1 has

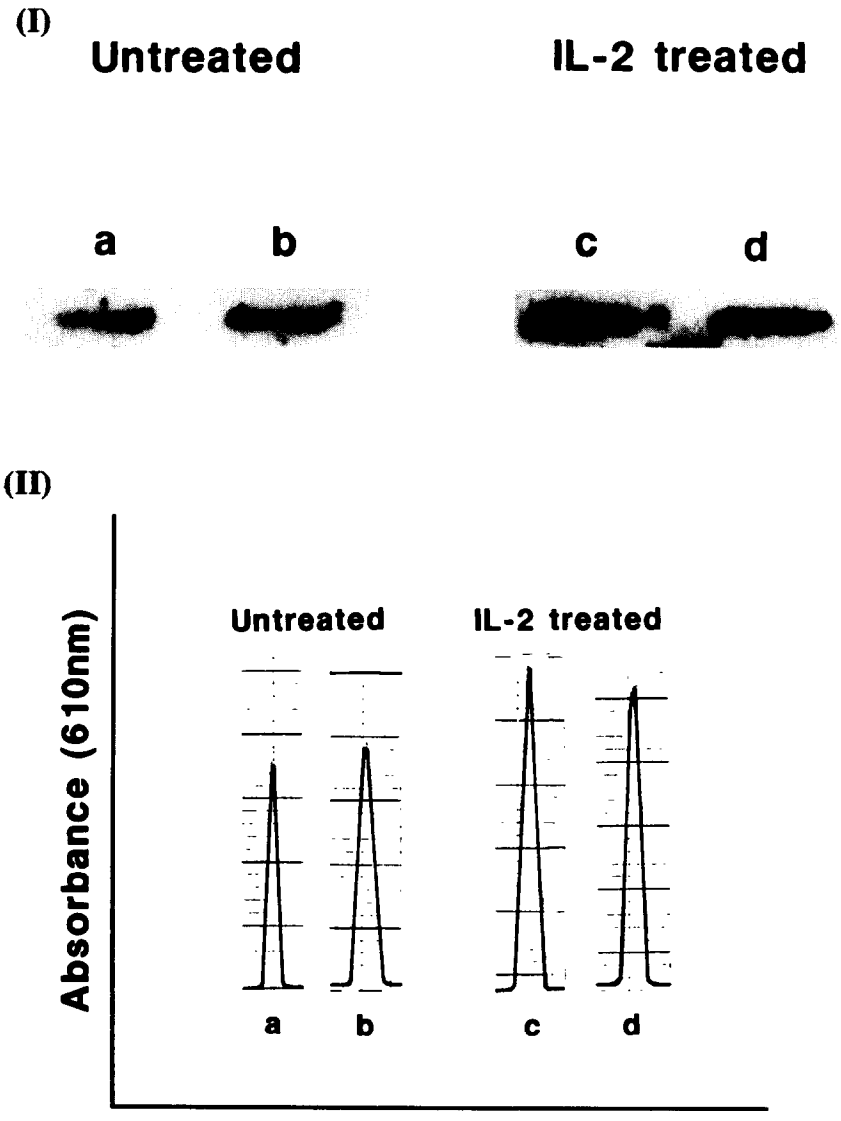

Fig. 4. (I) Slot blot analysis of CYP2D mRNA using total RNA extracted from livers. Duplicate RNA samples from individual livers are shown. Total liver RNA (5 μg) was bound to a nylon filter and hybridized with the CYP2D cDNA as described in Materials and Methods. Five micrograms of RNA extracted from livers were applied for slot blot hybridization. Lanes a and b, RNA from untreated rats; lanes c and d, RNA from IL-2 treated rats. (II) Densitometric analyses of (I), performed as described in the text.

activities towards both debrisoquine and bufuralol monooxygenases, while CYP2D2 has negligible catalytic activities toward debrisoquine compared with those of CYP2D1. [28]. Our data show that the induction rates of the activities of debrisoquine and bufuralol monooxygenases by IL-2 were the same. Taken together it seems that it was not CYP2D2 but CYP2D1 that was mainly induced by IL-2.

From the results of our western blot and RNA slot blot analyses, the inducible contents of immunoreactive CYP2D protein and its mRNA were comparable, suggesting that IL-2 does not alter significantly the translation rate from CYP2D mRNA to CYP2D protein but affects the mRNA level which was determined by the balance between the rate of mRNA synthesis and the stability of mRNA.

In conclusion, activities of xenobiotic-metabolizing enzymes and the CYP2D protein content of liver microsomes are enhanced by IL-2, and the induction of CYP2D is regulated at the mRNA level. The physiological relevance of inducible effects on xenobiotic-metabolizing enzymes awaits further elucidation.

Acknowledgements—We are grateful to Dr M. Manns (Department of Internal Medicine, 1st Division, University of Mainz, Germany) for providing us with the serum of autoimmune hepatitis patient containing the LKM-1

autoantibody and Dr I. Hamamoto (Department of Surgery, 1st Division, Kagawa Medical School, Japan) for giving us helpful advice regarding the purification of b_5.

REFERENCES

1. Morgan DA, Ruscetti FW and Gallo RC, Selective *in vitro* growth of T-lymphocytes from normal human bone marrows. *Science* **193**: 1007–1008, 1976.
2. Taniguchi T, Matsui M, Fujita T, Takaoka C and Kashima N, Structure and expression of a cloned cDNA for human interleukin 2. *Nature* **302**: 305–310, 1983.
3. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettighausen SE, Matory YL, Skibber JM, Shiloni E, Vetto JT, Siepp CA, Simpson C and Reichert CM, Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *N Engl J Med* **313**: 1485–1985.
4. Nishioka M, Kagawa H, Shirai M, Terada S and Watanabe S, Effects of human recombinant interleukin 2 in patients with chronic hepatitis B: a preliminary report. *Am J Gastroenterol* **82**: 438–442, 1987.
5. Mannering GJ, Renton KW, Azhary R and Deloria LB, Effects of interferon-inducing agents on hepatic cytochrome P450 drug metabolizing systems. *NY Acad Sci* **350**: 314–331, 1980.
6. Ghezzi P, Saccardo V, Villa P, Rossi V, Bianchi M and Dinarello CA, Role of interleukin-1 in the depression of liver drug metabolism by endotoxin. *Infect Immunol* **54**: 837–840, 1986.
7. Sheldofsky SI, Swim AT, Robinson JM, Gallicchio VS, Cohen DA and McClain CJ, Interleukin-1 (IL-1) depresses cytochrome P450 levels and activities in mice. *Life Sci* **40**: 2331–2336, 1987.
8. Wright K and Morgan ET, Regulation of cytochrome P450IIC12 expression by interleukin 1, interleukin 6, and dexamethasone. *Mol Pharmacol* **39**: 468–474, 1990.
9. Mitoma C, Posner HS, Reitz HC and Udenfriend S, Enzymatic hydroxylation of aromatic compounds. *Arch Biochem Biophys* **61**: 431–441, 1956.
10. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
11. Ryan MT and Chopra RK, The paradoxical effect of fatty acid on steroid–albumin interaction. *Biochim Biophys Acta* **427**: 337–349, 1976.
12. Omura T and Sato R, The carbon monooxide binding pigment of liver microsomes. *J Biol Chem* **239**: 2370–2385, 1964.
13. Nash T, Colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem J* **55**: 416–421, 1953.
14. Greenlee W and Poland A, An improved assay of 7-ethoxycoumarin *O*-deethylase activity: induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, 3-methylcholanthrene and 2378-tetrachloride-benzo-*p*-dioxin. *J Pharmacol Exp Ther* **205**: 596–605, 1978.
15. Imai Y, Ito A and Sato R, Evidence for biologically different types of vesicles in the hepatic microsomal fraction. *J Biochem* **60**: 417–428, 1966.
16. Kronbach T, Mathys D, Gut J, Catin T and Meyer UA, High-performance liquid chromatography assays for bufuralol 1′-hydroxylase, debrisoquine 4-hydroxylase, and dextrometrophan *O*-deethylase in microsomes and purified cytochrome P450 isozymes of human liver. *Anal Biochem* **162**: 24–32, 1986.
17. Matsuo Y, Iwahashi K and Ichikawa Y, Debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase activities in bovine and rabbit tissues. *Biochem Pharmacol* **43**: 1911–1919, 1992.
18. Spatz L and Strittmatter P, A form of reduced nicotinamide adenine dinucleotide-cytochrome b_5 reductase containing both the catalytic site and an additional hydrophobic membrane-binding segment. *J Biol Chem* **248**: 793–799, 1973.
19. Hammamoto I, Hiwatashi A and Ichikawa Y, Zonal distribution of cytochrome P-450 and related enzymes of bovine adrenal cortex-quantitative assay of concentrations and total contents. *J Biochem* **99**: 1743–1748, 1986.
20. Manns MP, Johnson EF, Griffin KJ, Tan EM and Sullivan KF, Major antigen of liver kidney microsomal autoantibodies in idiopathic autoimmune hepatitis is cytochrome P450db1. *J Clin Invest* **83**: 1066–1072, 1989.
21. Chomczynski P and Sacchi N, Single-step method of RNA isolation by acid guanidinum thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162**: 156–159, 1987.
22. Tsuneoka Y, Matsuo Y, Higuchi R and Ichikawa Y, Characterization of the cytochrome P-450IID subfamily in bovine liver: nucleotide sequences and micro-heterogeneity of the members of the cytochrome P-450IID subfamily. *Eur J Biochem* **208**: 739–746, 1992.
23. Rigby PWJ, Dieckmann M, Rhodes C and Berg P, Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase. *J Mol Biol* **113**: 237–251, 1977.
24. Kurokohchi K, Yoneyama H, Matsuo Y, Nishioka M and Ichikawa Y, Effects of interleukin 1α on the activities and gene expression of cytochrome P450IID subfamily. *Biochem Pharmacol* **44**: 1669–1674, 1992.
25. Tominaga T, Fukata J, Naito Y, Usui T, Murakami N, Fukushima M, Nakai Y, Hirai Y and Imura H, Prostaglandin-dependent *in vitro* stimulation of adrenocortical steroidogenesis by interleukins. *Endocrinology* **128**: 526–531, 1991.
26. Conney AH, Miller EC and Miller JA, The metabolism of methylated aminoazo dyes. V. Evidence for induction of enzyme synthesis in the rat by 3-methylcholanthrene. *Cancer Res* **16**: 450–460, 1956.
27. Orrenius S, Ericsson JLE and Ernster L, Phenobarbital-induced synthesis of the microsomal drug-metabolizing enzyme system and its relationship to the proliferation of endoplasmic reticulum. A morphometric and biochemical study. *J Cell Biol* **25**: 627–639, 1965.
28. Gonzalez FJ, Matsunaga T, Nagato K, Meyer UA, Nervert DW, Pastewka J, Kozak CA, Gillette JR, Gelboin HV and Hardwick JP, Debrisoquine 4-hydroxylase: characterization of a new P450 gene subfamily, regulation, chromosomal mapping, and molecular analysis of the DA rat polymorphism. *DNA* **6**: 149–161, 1987.
29. Guengerich FP, Dannan GA, Wright ST, Martin MV and Kaminsky LS, Purification and characterization of liver cytochromes P450: electrophoretic, spectral, catalytic, and immunochemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or b-naphtoflavone. *Biochemistry* **21**: 6019–6030, 1982.
30. Muller-Enoch D, Churchill P, Fleischer S and Guengerich FP, Interaction of liver microsomal cytochrome P-450 and NADPH-cytochrome P450 reductase in the presence and absence of lipid. *J Biol Chem* **259**: 8174–8182, 1984.
31. Yoshioka H, Morohashi KI, Sogawa K, Miyata T, Kawajiri K, Hirose T, Inayama S, Fujii-Kuriyama Y and Omura T, Structural analysis and specific expression of microsomal cytochrome P450(M-1) mRNA in male rat livers. *J Biol Chem* **262**: 1706–1711, 1987.
32. Morishima N, Yoshioka H, Higashi Y, Sogawa K and Fujii-Kuriyama Y, Gene structure of cytochrome

P450(M-1) specifically expressed in male rat liver. *Biochemistry* **26**: 8279–8285, 1987.
33. Ichikawa Y, The function and structure of cytochrome P-450 *Tanpakushitsukakusankouso* (Kyoritsu Publishing Co.) **32**: 290–303, 1987.
34. Gueguen M, Meunier-Rotival M, Bernard O and Alvarez F, Anti-liver kidney microsomal antibody recognizes a cytochrome P450 from the IID subfamily. *J Exp Med* **168**: 801–806, 1988.
35. Jacqz-Aigrain E, Gueguen M, Zanger UM, Robieux I and Alvarez F, Cytochrome CYP2D subfamily in non-human primates, catalytical and immunological characterization. *Biochem Pharmacol* **41**: 1657–1663, 1991.