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DIFFERENTIAL EFFECT OF CYTOKINES ON THE PHENOBARBITAL OR 3-METHYLCHOLANTHRENE INDUCTION OF P450 MEDIATED MONOOXYGENASE ACTIVITY IN CULTURED RAT HEPATOCYTES

MICHELLE A. CLARK,* BETTYE A. BING, PAUL E. GOTTSCHALL and JOSEPH F. WILLIAMS†

Department of Pharmacology and Therapeutics, University of South Florida College of Medicine, Tampa, FL 33612, U.S.A.

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Abstract—Cultured rat hepatocytes have been used to compare the relative activities of cytokines to inhibit the phenobarbital (PB) or 3-methylcholanthrene (MC) induction of cytochrome P4502B1 and 2B2 (P4502B1/2) or P4501A1 and 1A2 (P4501A1/2), respectively. Recombinant cytokines tested were human interleukin-6 (IL-6), interleukin-1α and -β (IL-1α and IL-1β, respectively), and rat γ-interferon (INFγ). Hepatocytes were cultured in the presence of 2 mM PB or 1 μg MC/mL culture medium for 24 hr with or without the cytokines. Benzyloxyresorufin and ethoxyresorufin *O*-dealkylase (BROD and EROD, respectively) activities were determined as indices of P4502B1/2 and P4501A1/2, respectively. All cytokines produced a concentration-dependent inhibition of the PB induction of BROD activity. IL-1β and IL-6 were approximately equipotent with IC_{50} values of 1–2 U/mL, causing greater than 90% inhibition of PB induction of BROD activity at a concentration of 50 U/mL culture medium. IL-1α tended to be less active. PB induction of BROD activity was also inhibited by INFγ, but higher concentrations (52.5 to 500 U/mL culture medium) were required. All cytokines were less effective in inhibiting the MC induction of EROD activity than the PB induction of BROD activity. IL-1β and IL-6, at 50 U/mL culture medium, inhibited EROD induction by only 35% compared with the greater than 90% inhibitory effect on the PB induction of BROD activity. INFγ was ineffective in inhibiting EROD activity at the concentrations studied. Western immunoblot analysis indicated that the cytokines prevented the ability of the inducers to increase the expression of P4502B1/2 and P4501A1/2 immunoreactive proteins, and this effect correlated with their inhibitory effect on induction of enzyme activity. The results suggest that inducible isoforms of cytochrome P450 differ in their susceptibility to regulation by the cytokines, and that cytokines possess differential activity to inhibit the induction of P450 isoforms, with IL-1β and IL-6 being the most effective.

Key words: cytochrome P4502B1/2; cytochrome P4501A1/2; phenobarbital; 3-methylcholanthrene; hepatocytes; cytokines

It is now recognized that the level and activity of P450‡, a family of hemoproteins responsible for the monooxygenation of a wide variety of drugs as well as various endogenous substrates, are affected markedly by inflammation, infection, and other conditions that invoke the acute phase response [1–3]. In animals, decreased drug-metabolizing activity is seen following the injection of bacteria, LPS, viruses, or other inflammatory agents such as turpentine or carrageenan. Clinical studies have also shown that monooxygenase activity is decreased in patients during acute viral infection or other inflammatory conditions [4–6].

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 [7]. Mediators implicated in the acute phase response include INFγ, IL-1, TNF, and IL-6. IL-6 is considered to be the major mediator of the acute phase response, affecting the production of most of the acute phase proteins. Although IL-1, INFγ, and TNF affect the production of a limited number of the acute phase proteins, they can influence the production, release and effects of IL-6. Injection of these cytokine mediators into experimental animals or their administration to humans has been shown to decrease P450-dependent drug-metabolizing activity [8–15], suggesting that the decreased P450 activity seen during inflammation is due to these endogenously released cytokines. *In vivo* studies have suggested that the various cytokines

* Corresponding author: Ms. Michelle Clark, Department of Pharmacology and Therapeutics, MDC Box 9, USF College of Medicine, 12901 Bruce B. Downs Blvd., Tampa, FL 33612. Tel. (813) 974-2565; FAX (813) 974-3081.

† Deceased, 7 April 1994.

‡ Abbreviations: P450, cytochrome P450; BROD, 7-benzyloxyresorufin *O*-dealkylase; EROD, 7-ethoxyresorufin *O*-dealkylase; P4501A1/2, P4501A1 and P4501A2; P4502B1/2, P4502B1 and P4502B2; IL-1, interleukin-1; IL-6, interleukin-6; INF, interferon; TNF, tumor necrosis factor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PB, phenobarbital sodium; MC, 3-methylcholanthrene; LPS, bacterial lipopolysaccharide; and HBSS, Hanks' balanced salt solution.

differ with respect to their ability to decrease P450-mediated monooxygenase activity, and that the expression of various P450 isoforms may be differentially regulated by the cytokines [12, 16].

Although the effects of inflammation and the cytokine mediators to decrease the levels and activity of constitutive P450 isoforms are well-established, studies suggest that inflammation and cytokines may also regulate the expression and activity of the inducible isoforms of P450. LPS administration to rats differentially affects the monooxygenase activity [17] and the levels of P4502B1/2 and P4501A1/2 induced by PB or MC, respectively [18]. Differential effects of INFα preparations on the induction of P450 monooxygenase activity in hamsters [19] and mice [16] have also been reported. Knickle *et al.* [20] recently showed that when the interferon-inducer poly rI-rC is administered to mice it suppresses the clofibrate induction of P4504A.

Because of the complex interplay among the cytokines [7], it is difficult to determine from *in vivo* experiments which of the agents is primarily involved in the regulation of the various P450 isoforms. During the acute phase response and after cytokine administration, changes in the secretion of various hormones occur that may also influence the expression of P450. Primary cultures of hepatocytes offer an excellent system to investigate the direct effect of cytokines to regulate P450 isoform expression. It has been shown that IL-6 markedly suppresses the PB induction of P4502B1/2-mediated BROD activity and the level of immunoreactive P4502B1/2 protein in cultured rat hepatocytes [21]. Furthermore, IL-6 was also shown to prevent the PB-induced increase in P4502B1 mRNA, suggesting that the cytokine affects the PB induction at the transcriptional level. Barker *et al.* [22] showed that IL-1β, but not IL-6, suppresses TCDD induction of P4501A1/2 mRNA in rat hepatocyte cultures. In primary mouse hepatocytes, mouse INFγ inhibited the TCDD induction of EROD activity, an index of P4501A1/2 [23].

The present study has used primary cultured rat hepatocytes to compare the relative activities of various cytokines on the induction of P450 isoforms by PB and MC. Cytokines included in the study are human IL-1α and β, IL-6, and rat INFγ. BROD and EROD activities were determined as indices of P4502B1/2 and P4501A1/2, respectively. Western blot analysis was performed to evaluate the effect of the cytokines on the level of P450 isoform immunoreactive protein.

MATERIALS AND METHODS

Materials. Human recombinant IL-1α (2×10^4 U/mL) and IL-6 (5.1×10^5 U/mg, LPS content <0.1 pg/U) were provided by the Genetics Institute (Cambridge, MA). A second human recombinant IL-1α (1×10^6 U/mg, LPS content <0.1 pg/U) preparation, recombinant rat INFγ (4×10^6 U/mg, LPS content <12 ng/U), Williams E medium, and calcium- magnesium-free HBSS were purchased from GIBCO BRL (Grand Island, NY). Human recombinant IL-1β (2×10^7 U/mg, LPS content <100 pg/mg) was obtained from the Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). Matrigel was provided by Dr. P. Sinclair (VA Hospital, White River Junction, VT) or was purchased from Collaborative Research (Bedford, MA). Dr. Sinclair also provided a rabbit polyclonal anti-mouse P4501A1/2, and a mouse monoclonal H-8 antibody against P4502B1/2 was a gift from Dr. M. Adesnick (New York University Medical Center, New York, NY). Dexamethasone sodium phosphate was obtained from Elkin–Sinn, Inc. (Cherry Hill, NJ). 7-Benzyloxyresorufin, 7-ethoxyresorufin, and resorufin were purchased from Molecular Probes Inc. (Eugene, OR). Collagenase was obtained from Boehringer–Mannheim Biochemicals (Indianapolis, IN) or the Worthington Biochemical Corp. (Freehold, NJ). SDS was purchased from Pierce (Rockford, IL), acrylamide from Fisher Scientific (Fair Lawn, NJ), and *N'*,*N'*-methylenebisacrylamide from the Eastman Kodak Co. (Rochester, NY). Other chemicals were obtained from either the Sigma Chemical Co. (St. Louis, MO) or Bio-Rad Life Sciences (Richmond, CA).

Preparation of hepatocytes. Adult male Fischer 344 rats (200–250 g, Harlan Industries, Indianapolis, IN) were used throughout the study. Animals were maintained on a 12-hr light/dark cycle with food and water available *ad lib*. All animal use procedures were in accordance with the "NIH Guide for the Care and Use of Laboratory Animals" and were approved by the University of South Florida Animal Care and Use Committee. Hepatocytes were prepared and cultured essentially as previously described [24]. Animals were lightly anesthetized with ether, and the livers were perfused *in situ* through a cannula inserted through the right atria into the inferior vena cava; a calcium-, magnesium-free HBSS containing 0.5 mM EGTA and 26 mM Tricine buffer, pH 7.6, was perfused for 8 min (30 mL/min), and then HBSS containing 26 mM Tricine buffer, pH 7.4, 1 mM $CaCl_2$, 0.05% collagenase, and 0.005% soy bean trypsin inhibitor was perfused for 10 min (20 mL/min). Throughout the perfusions, both solutions were bubbled with 100% oxygen. The liver was removed, and 18 gauge sterile needles were used to tease apart the digested liver. Hepatocytes were suspended in 40 mL of Williams E culture medium containing ascorbic acid (50 μg/mL), insulin (10 μg/mL), 6 nM selenium, 0.1 μM dexamethasone, and antibiotics (100 U penicillin G, 100 μg streptomycin, and 0.25 μg amphotericin B/mL culture medium), and centrifuged at 50 *g* for 1 min. The cells were resuspended in medium and washed twice by centrifugation at 50 *g*. Cell yield was typically in excess of 2×10^8 cells/liver, and viability was above 85% as determined by trypan blue exclusion. Tissue culture plates were precoated with Matrigel (120 μL/6-cm plate), and hepatocytes were plated at approximately 3 million cells/6-cm plate in 3 mL of Williams E medium. Cultures were maintained at 37° in an atmosphere of 5% CO_2 in air, and the culture medium was changed every 24 hr. Chemicals were added to cultures 48 hr after inoculation, and each treatment was performed in duplicate. Cytokines were diluted with Williams E medium, PB was dissolved in water, and MC was dissolved in DMSO. The final concentration of

DMSO in the culture was 0.1% (v/v). After a 24-hr exposure to the chemicals, cells were harvested in 20 mM Tris–HCl, pH 7.8, and sonicated twice for 5 sec with a Heat Systems W375 sonicator (Plainview, Long Island, NY) equipped with a microtip. The protein in the sonicate was determined using the method of Bradford [25] with bovine serum albumin as standard.

Enzymatic assays. BROD and EROD activities were determined on hepatocyte sonicates as described [26] with slight modification. The assay (total volume 150 μL) contained: 0.2 mg sonicated protein in 20 mM Tris–HCl, pH 7.8; 10 μM dicumarol to inhibit diaphorase activity; 3.2 mg bovine serum albumin/mL; 20 mM nicotinamide; 20 μM substrate, and an NADPH-regenerating system (10 mM glucose-6-phosphate, 0.2 mM NADPH, 10 mM $MgCl_2$, and 0.4 U glucose-6-phosphate dehydrogenase/mL). After a 15-min incubation at 37°, the reaction was terminated by the addition of 1.0 mL cold methanol. The sample was centrifuged at 1000 *g* for 5 min, and the product, resorufin, was measured fluorometrically using a Farrand Spectrofluorometer (Farrand Optical Corp., Inc., Valhalla, NY) with excitation and emission wavelengths of 528 and 590 nm, respectively. Authentic resorufin was used as standard. Determinations were performed in duplicate on sonicates from each of the individual culture plates, and the results were averaged.

Immunoblots. Samples were immunoblotted as described [24]. Sonicated protein was separated by electrophoresis in SDS–10% polyacrylamide (SDS–PAGE) under reducing conditions, and transferred to nitrocellulose sheets (100 V for 1 hr). The sheets were blocked for 1 hr with a 0.3% Tween 20–PBS solution, rinsed, and incubated with H-8 mouse monoclonal anti-P4502B1/2 or rabbit polyclonal anti-P4501A1/2 in 0.05% Tween 20 in PBS solution. The sheets were treated with conjugated alkaline phosphatase anti-mouse or anti-rabbit IgG, and color was developed using 5-bromo-4-chloro-3-indoletoluidine phosphate and *p*-nitroblue tetrazolium chloride according to the manufacturer's instructions (Bio-Rad). The intensity of the immunoreactive bands was determined by scanning with a Bio-Rad Video densitometer.

Data analysis. Representative concentration–response curves for the inhibition of BROD and EROD (expressed as a percentage of PB-induced activity or MC-induced activity, respectively) activities are shown in Figs. 1 and 2. The IC_{50} values for the concentration–response curves from individual experiments were derived using FLEXFIT for the Macintosh (Peter J. Munson, NIH). For each fit, parameters A and D (maximum and minimum response) were constrained at 100 and 0, respectively. Differences among mean IC_{50} values were determined using analysis of variance and the Tukey–Kramer multiple comparison test (SUPERANOVA; Abacus Concepts, Berkeley, CA). Differences between immunoreactive protein levels were determined using a one-sample *t*-test (StatView, Abacus), i.e. testing whether the mean of a treatment group is different from the PB-induced control by setting the control at 100%. A level of $P < 0.05$ was accepted as evidence of a statistically significant difference between two treatment groups.

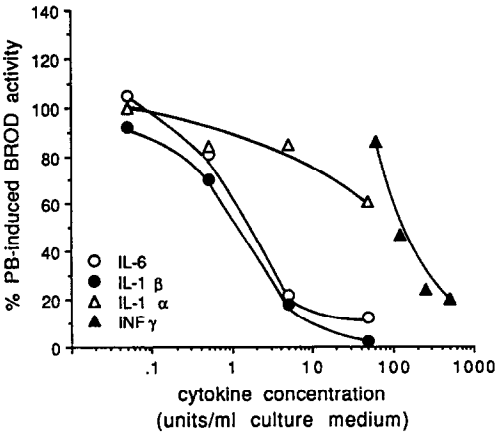

Fig. 1. Effects of increasing concentrations of IL-6, IL-1β, IL-1α and INFγ on the PB induction of BROD activity in cultured rat hepatocytes. Results are expressed as a percentage of the PB-induced control, and the data shown are representative curves for each cytokine. PB (2 mM) and the cytokines at the indicated concentrations were added (duplicate plates/treatment) to hepatocytes 48 hr after inoculation, and cells were harvested 24 hr later. BROD activity was determined in duplicate on the cell sonicate from each individual culture plate. The PB-induced BROD value was 164.78 ± 22.86 pmol resorufin formed/min/mg sonicated protein (N = 12).

RESULTS

In agreement with previous studies [21, 24], treatment of hepatocytes for 24 hr with PB (2 mM) caused a significant increase in BROD activity above that observed in untreated hepatocytes. BROD activity of untreated hepatocytes (N = 12) was 8.91 ± 0.65 pmol resorufin formed/min/mg sonicated protein. After incubation with PB for 24 hr, hepatocyte BROD activity was 164.78 ± 22.86 pmol resorufin formed/min/mg sonicated protein (N = 12). The effect of PB to induce BROD activity was somewhat variable among the experiments, ranging from 10- to 50-fold. Therefore, the effect of the cytokines was calculated as the percentage of the respective PB-induced BROD activity in each experiment. Figure 1 shows representative concentration–response curves for the effects of IL-6, IL-1α, IL-1β, and rat INFγ on the PB induction of BROD activity. IL-6, IL-1β and IL-1α (0.05 to 50 U/mL culture medium) caused a concentration-dependent inhibition of PB-induced BROD activity. PB induction of BROD activity was inhibited, on average, more than 90% by 50 U/mL culture medium of IL-1β or IL-6; IL-1α was less active than the other two interleukins, causing only 36% inhibition at the highest dose, and the results were more variable. Recombinant rat INFγ (62.5 to 500 U/mL culture medium) caused a concentration-dependent decrease in the PB induction of BROD activity, but higher concentrations were required. At a concentration of

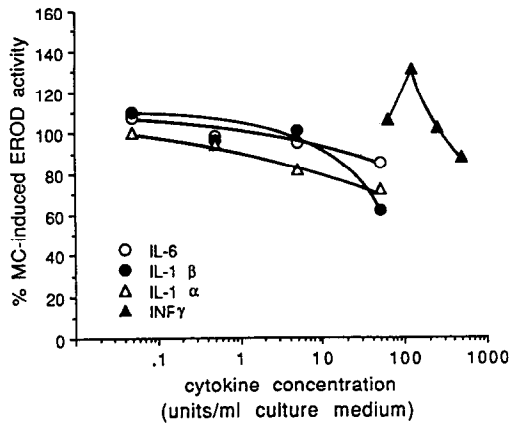

Fig. 2. Effects of increasing concentrations of IL-6, IL-1β, IL-1α and INFγ on the MC induction of EROD activity in cultured rat hepatocytes. Results are expressed as a percentage of the MC-induced control, and the data shown are representative curves for each cytokine. Each experiment was repeated at least three times with similar results. MC (1 μg/mL culture medium) and the cytokines at the indicated concentrations were added (duplicate plates/treatment) to hepatocytes 48 hr after inoculation, and cells were harvested 24 hr later. EROD activity was determined in duplicate on the cell sonicate from each individual culture plate. The MC-induced EROD value was 120.53 ± 20.15 pmol resorufin formed/min/mg sonicated protein (N = 12).

500 U/mL culture medium, rat INFγ caused an approximately 75% inhibitory effect. The mean IC_{50} values for each cytokine were derived from concentration–response curves for each cytokine from at least three independent experiments. The IC_{50} values for PB in the presence of IL-6, IL-1β, IL-1α or INFγ were 2.0 ± 0.6, 1.6 ± 0.0, 5.3 ± 2.2 and 97.3 ± 26.5 pmol resorufin formed/min/mg sonicated protein, respectively. IL-6, IL-1β and IL-1α were all significantly more potent than rat INFγ in inhibiting PB-induced BROD activity.

Exposure of rat hepatocytes to MC for 24 hr significantly increased EROD activity, an index of P4501A1/2. In hepatocytes treated with vehicle (DMSO), EROD activity was 5.45 ± 2.67 pmol resorufin formed/min/mg sonicated protein (N = 12), and after MC treatment EROD activity increased approximately 25-fold (120.53 ± 20.15 pmol resorufin formed/min/mg sonicated protein; N = 12). As with PB induction of BROD activity, the effect of MC to induce EROD activity varied among the experiments, and the effects of the cytokines are expressed as a percentage of the MC-induced EROD activity. Figure 2 depicts representative effects of increasing concentrations of IL-6, IL-1β, IL-1α, and INFγ on the MC induction of EROD activity in cultured rat hepatocytes. IL-6, IL-1β and IL-1α were about equally effective in inhibiting MC induction of EROD activity. At a concentration of 50 U/mL culture medium, IL-6, IL-1β, and IL-1α caused only a 35% inhibition of EROD induction. Thus, IL-6 and IL-1β were less active in inhibiting the MC induction of EROD activity (Fig. 2) than in inhibiting the PB induction of BROD activity (Fig. 1). Rat INFγ, in contrast to its inhibitory effect on BROD induction, was ineffective in inhibiting the MC induction of EROD activity, and the results suggest a slight stimulatory effect at 62.5 and 125 U/mL culture medium (Fig. 2).

Hepatocyte sonicates from the previous experiments were subjected to SDS–PAGE and western blot analysis to determine the effect of the cytokines on the PB or MC induction of P4502B1/2 or P4501A1/2 immunoreactive protein, respectively. The intensity of the stained bands was determined by densitometric analysis, and results are expressed as the intensity of the band from sonicate with both inducer and cytokines as a percentage of the band intensity obtained for sonicate from cultures exposed to PB or MC alone (Fig. 3). Figure 3A shows that IL-6, IL-1β and IL-1α at concentrations of 50 U/mL culture medium significantly inhibited the PB induction of immunoreactive P4502B1/2. Similar to the effect of these cytokines on BROD activity, IL-6 and IL-1β tended to be more potent in inhibiting BROD immunoreactive protein levels compared with IL-1α, since 5 U/mL of IL-6 and IL-1β also were effective in reducing BROD protein levels. Figure 3B shows that INFγ at 250 and 500 U/mL culture medium suppressed the PB induction of P4502B1/2 immunoreactive protein, but to a lesser extent than the other cytokines. The inhibitory effects of these cytokines on PB-induced P4502B-1/2 immunoreactive protein is in agreement with their activity to suppress PB induction of BROD activity (Fig. 1). The MC-stimulated increase in the level of P4501A1/2 immunoreactive protein was affected only slightly by IL-6 or IL-1β (Fig. 3C), and closely matched their effects to decrease MC induction of EROD activity (Fig. 2). Figure 4 is an example of a western blot showing the effects of the cytokines to inhibit P4502B1/2 and P4501A1/2 immunoreactive protein levels.

DISCUSSION

The results of the present study using cultured rat hepatocytes indicate a role for each of the cytokines in directly regulating the PB induction of P4502B-1/2. The ability of the cytokines to prevent MC induction of P4501A1/2 was markedly less than their effect on the PB-inducible isoforms. The data suggest that cytokines differ in their activity to affect P450 regulation, and that P450 isoforms may be differentially sensitive to regulation by specific cytokines. Within the concentration range examined, IL-6, IL-1β, and IL-1α were more active than INFγ in inhibiting the PB induction of BROD activity. There was a tendency for IL-1β to have a greater potency in inhibiting BROD activity compared with IL-1α, which agrees with studies in other systems [27, 28]. Previously, Williams [29] reported that human INFγ was ineffective in inhibiting the PB induction of BROD activity. The ineffectiveness of human INFγ is consistent with the known species specificity of this agent, and with the reports of Franklin and Finkle [30] and Craig *et al.* [31] that

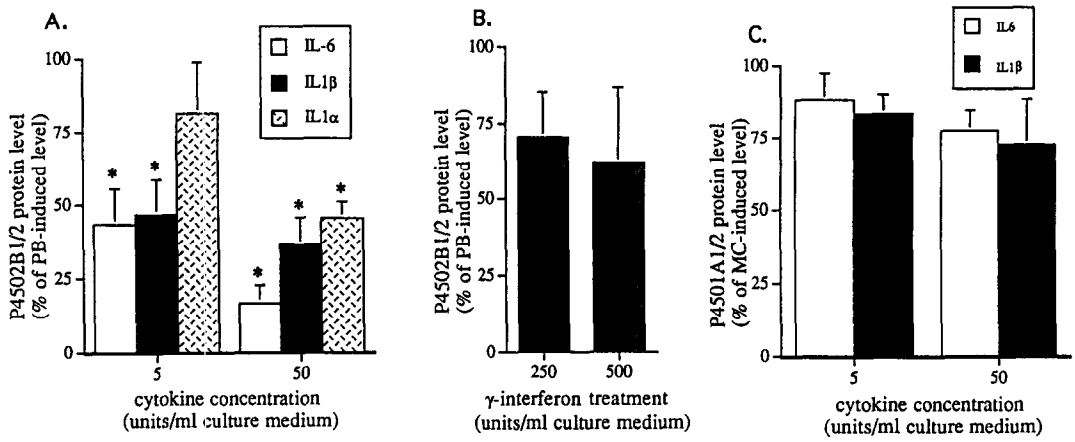

Fig. 3. Western immunoblot analysis of the effects of cytokines to inhibit the PB or MC induction of P4502B1/2 or P4501A1/2 immunoreactive protein. (A) Effects of IL-6, IL-1β and IL-1α, at 5 and 50 U/mL culture medium to inhibit the PB induction of P4502B1/2. (B) Effect of INFγ at 250 and 500 U/mL culture medium to inhibit the PB induction of P4502B1/2. (C) Effects of IL-6 and IL-1β at 5 and 50 U/mL culture medium to inhibit the MC induction of P4501A1/2. Sonicated protein (20 μg/lane) was separated by SDS–PAGE, and immunoblotted as described in Materials and Methods. The relative density of the bands was determined, and expressed as a percentage of the intensity of the band obtained for the PB- or MC-induced hepatocytes in the absence of cytokine. Values are the means ± SEM from three experiments. Key: (*)$P < 0.05$ compared with 100% activity.

the hepatic effects of INFγ to decrease P450 do not cross species. Since previous results [21] showed that IL-6 inhibits the PB induction of P4502B1 mRNA, it is reasonable to assume that the effects of IL-1α, IL-1β, and rat INFγ may also be due to effects on transcription. Experiments are in progress to compare the relative abilities of the cytokines to affect the transcriptional regulation of P4502B1/2.

IL-6 and IL-1β were less active in inhibiting the MC induction of EROD activity than in preventing the PB induction of BROD activity, and INFγ was ineffective at the concentrations studied. In other studies [17], MC induction of P450 has been shown to be less sensitive to the inhibitory effects of LPS compared with PB-induced isoforms. On the other hand, IL-1α was about equally effective in inhibiting both PB and MC induction of the respective monooxygenase activities. The slight effect of INFγ to augment the MC induction of EROD activity is similar to that reported for LPS administered to mice to potentiate MC induction of P4501A1/2 [18]. Using a human recombinant INFγ preparation, Williams [29] demonstrated a slight inhibitory effect on the MC induction of EROD activity. The reason(s) for the differences between the effects of rat and human INFγ in the present and previous studies is unknown, but may reflect species selectivity of INFγ to inhibit selective P450 isoforms.

The relative activities of IL-6, IL-1β, and INFγ to influence the induction of P4501A1/2 immunoreactive protein and EROD activity in cultured rat hepatocytes obtained in the present study differ from the results of Barker *et al.* [22], who examined the effects of IL-6 and IL-1β on the TCDD induction of P4501A1 and 1A2 mRNA, and of Jeong *et al.* [23], who studied the effects of mouse INFγ on the TCDD induction of EROD activity in mouse hepatocytes. Using cultured rat hepatocytes, Barker *et al.* [22] reported that low concentrations of IL-1β caused an 85% inhibition of the TCDD induction of P4501A1 and 1A2 mRNAs, respectively, and that IL-6 was ineffective even at high concentrations (3000 U/mL culture medium). Half-maximal effect of IL-1β to decrease P4501A1 and 1A2 induction was achieved at concentrations of 2.0 and 0.5 U/mL culture medium, respectively. Barker *et al.* [22] did not measure TCDD induction of EROD activity or P4501A1/2 immunoreactive protein. In the present study, IL-1β and IL-6 were about equally effective in preventing the MC induction of EROD activity and P4501A1/2 immunoreactive protein, but the effect was seen at higher concentrations of IL-1β than reported by Barker *et al.* [22] to decrease P4501A1/2 mRNA accumulation. It is possible that the cytokines may affect various steps of the induction process to different degrees. Morgan [32] has shown previously that LPS may affect the expression of P4502C11 mRNA independently of an effect on P4502C11 protein. Also, the apparent differences between the effect of the two cytokines in the present study and that of Barker *et al.* [22] may be related to differences in the culture conditions. In the study by Barker *et al.* [22], the culture medium contained 100 μM dexamethasone and 10% fetal bovine serum as compared with the present study that was conducted in the absence of serum and with 0.1 μM dexamethasone. Ferrari *et al.* [33] using fetal rat hepatocytes reported that 1.0 μM dexamethasone itself decreased EROD activity, but protected against the effect of IL-1. Previous studies [34] have shown that dexamethasone can cause a concentration-related synergistic augmentation of the polycyclic aromatic hydrocarbon induction of P4501A1/2. We have not observed an

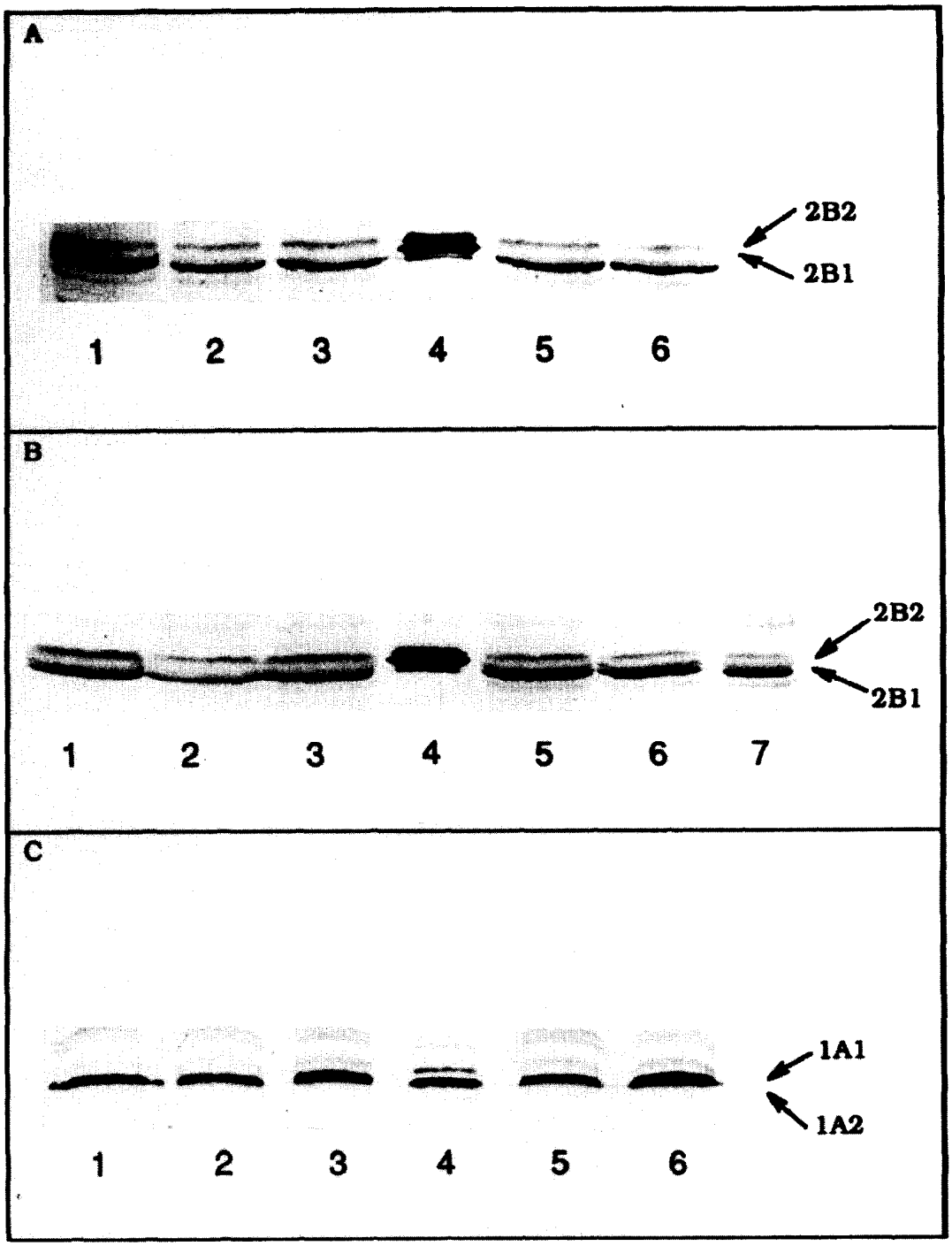

Fig. 4. Western blot analysis showing the effects of IL-6, IL-1β, IL-1α and INFγ to inhibit the PB or MC induction of P4502B1/2 and P4501A1/2 immunoreactive protein, respectively. (A) The inhibitory effects of IL-6 and IL-1β at 50 and 5 U/mL culture medium on the levels of P4502B1/2 immunoreactive protein. Lane 1, PB (2 mM); lanes 2 and 3, PB and 50 and 5 U IL-6/mL culture medium, respectively; lanes 5 and 6, PB and 50 and 5 U IL-1β/mL culture medium, respectively; lane 4 contains 1.5 μg hepatic microsomal protein from a PB-induced rat. (B) The effect of INFγ at 500 and 250 U/mL culture medium and IL-1α at 50 and 5 U/mL culture medium to inhibit P4502B1/2 immunoreactive protein. Lanes 1 and 5 contain sonicates from hepatocytes cultured with PB (2 mM) alone; lanes 2 and 3, PB and 500 and 250 U INFγ/mL culture medium, respectively; lanes 6 and 7, PB and 50 and 5 U IL-1α/mL culture medium, respectively; lane 4 contains 1.5 μg hepatic microsomal protein from a PB-induced rat. (C) The effects of IL-6 and IL-1β on the MC induction of P4501A1/2. Lane 1 contains MC (1 μg/mL); lanes 2 and 3, MC and 50 and 5 U IL-6/mL culture medium, respectively; lanes 5 and 6, MC and 50 and 5 U IL-1β/mL culture medium, respectively; lane 4 contains 1.0 μg hepatic microsomal protein from an MC-induced rat.

effect of 0.1 μM dexamethasone to augment MC induction of EROD activity, but have confirmed the report of Mathis *et al.* [34] using 10 μM dexamethasone. Furthermore, IL-1 can inhibit the binding of steroids to the glucocorticoid receptor, decreasing the dexamethasone induction of phosphoenolpyruvate carboxykinase (PEPCK) [35]. Thus, it may be that the results seen by Barker *et al.* [22] are due to a selective effect of IL-1β, but not IL-6, to modulate the effects of 100 μM dexamethasone to augment TCDD induction of P4501A1/2 as opposed to the effects of TCDD *per se*. It is also possible that differences between our work and that of Barker *et al.* [22] may be due to strain-related differences. The ineffectiveness of rat INFγ in the present study to inhibit the MC induction of EROD activity also differs from the results of Jeong *et al.* [23] who showed a concentration-dependent (5–500 U/mL) effect of mouse INFγ to suppress the TCDD induction of EROD activity in cultured mouse hepatocytes. The difference between the present results and those of Jeong *et al.* [23] might possibly indicate a species difference in the action of INFγ to regulate P450.

The present study indicates that rat hepatocyte cultures should be useful in unraveling the mechanism and specific effects of various cytokines either alone or in combination to regulate P450 expression and activity. One concern with the use of cultured hepatocyte preparations is the contamination with Kupffer cells, the resident liver macrophage. Since IL-1 and INF are known to induce the production and secretion of IL-6 by macrophages, it is possible that effects of these cytokines in the present and previous studies may be partially or totally mediated by IL-6 elicited from Kupffer cells. The use of cytokine antibodies and/or receptor antagonist may be of value in delineating the effects of these cytokines. However, a direct effect of IL-6 to regulate P450 is supported by the study of Fukuda *et al.* [36] in which IL-6 reduced mRNA levels for P4501A1 and 1A2, and for P4503A3 in human hepatoma cell cultures, which do not contain Kupffer cells.

In addition to the production of cytokines during inflammation and infection, cytokines, particularly IL-1 and INFs, are being used as immunotherapeutic agents for the treatment of chronic hepatitis B and C, cancer, and other diseases. Since clinical studies also indicate that patients administered these cytokines have reduced hepatic monooxygenase activity [15], altered therapeutic and/or toxic effects of co-administered agents might be expected. Further studies are necessary to identify which P450 isoforms are affected by the various cytokines, and their mechanism of action.

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