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# Immunochemical Analysis of a Cytochrome P-450IA1 Homologue in Human Lung Microsomes

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## SUMMARY

The monoclonal antibody MAb 1-7-1, which specifically binds to cytochromes P-450IA1 and P-450IA2 in 3-methylcholanthrene-induced rat liver microsomes, was used to identify a cytochrome P-450IA1 homologue in human lung microsomes. Although MAb 1-7-1 had similar affinity constants for human and rat microsomes, the amount bound to human lung microsomes was severalfold lower than that bound to microsomes from untreated rat or rabbit lung and much lower than the amount bound to 3-methylcholanthrene-induced rat lung or liver microsomes. The amount bound to untreated baboon lung microsomes was similar to that bound to human lung microsomes. Three cytochrome P-450IA1-catalyzed activities, 7-ethoxyresorufin *O*-deethylase, 7-ethoxycoumarin, *O*-deethylase, and aryl hydrocarbon hydroxylase, were measurable in human lung microsomes, but the cytochrome P-450IA2-dependent activity acetanilide 4-hydroxylase was not. MAb 1-7-1 inhibited, and its binding correlated strongly with, 7-ethoxyresorufin *O*-deethylase activity ($r = 0.92$, $p < 0.01$) in human lung microsomes. 7-Ethoxyresorufin *O*-deethylase activities in human lung were similar to those measured in untreated baboon lung but considerably lower than those present in untreated rabbit lung, untreated or 3-methylcholanthrene-induced rat lung and liver, or human liver. We conclude that MAb 1-7-1 recognizes a cytochrome P-450IA1 homologue in human lung and that no cytochrome P-450IA2 homologue is detected. Cytochrome P-450IA1 is expressed in human lung at relatively low levels, similar to those observed in untreated primate (baboon) lung. The majority of the 19 human lung samples examined do not exhibit a permanent polycyclic aromatic hydrocarbon-induced state with respect to this isozyme.

The cytochrome P-450 superfamily, which comprises a large number of related hemoprotein isozymes that have varying immunochemical properties, substrate specificities, and tissue localizations (1, 2), can be further subclassified into at least 14 subfamilies of structurally related isozymes (3). Isozymes of the cytochrome P-450I subfamily are of particular toxicological importance because they bioactivate numerous xenobiotics, including PAHs, to reactive electrophiles, which may cause cytotoxicity or initiate carcinogenesis (4–7).

It has long been presumed that levels of cytochrome P-450 in the human lung, and specifically levels of isozymes of the P-450I family, may be a determining factor in the susceptibility of individuals to airborne environmental carcinogens (8). Multiple cytochrome P-450 isozymes, including members of the P-450I family, have been identified and well characterized in rat and rabbit lung (9). In animal lungs, these isozymes are inducible by exposure to PAHs. 3MC, a model PAH, induces cytochrome P-450I-related activities, specifically AHH, in rat and rabbit lung (10, 11). Furthermore, the PAH-containing component of cigarette smoke (12) induces pulmonary AHH and EROD activities in mice (13) and rats (14, 15).

Individual cytochrome P-450 isozymes in human lung have not as yet been well characterized. Although direct spectral demonstration of cytochrome P-450 in the human lung has not been reported, cytochrome P-450-catalyzed metabolic activity in human lung homogenates and cell fractions has been demonstrated (16–22). AHH and ECOD activities, both reputed to be catalyzed primarily by cytochrome P-450I isozymes, have been measured in the human lung (16–22). The response of the human lung to xenobiotic inducers of cytochrome P-450 has also been studied (23). A study examining lung tissue samples from lung cancer patients found a positive correlation between ECOD and AHH levels and the degree of recent exposure to cigarette smoke (24). The A*h* receptor, which is known to mediate the induction of cytochrome P-450I isozymes in animals, has been demonstrated in the human lung (25). Thus, it

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**ABBREVIATIONS:** PAH, polycyclic aromatic hydrocarbon; 3MC, 3-methylcholanthrene; MAb 1-7-1, monoclonal antibody 1-7-1; MAb 2-66-3, monoclonal antibody 2-66-3; EROD, 7-ethoxyresorufin *O*-deethylase; ECOD, 7-ethoxycoumarin *O*-deethylase; AHH, aryl hydrocarbon hydroxylase; PBS, phosphate-buffered saline.

has been inferred that isozymes of the P-450I subfamily are present in the human lung. As yet, no published studies have definitively demonstrated that homologues of either cytochrome P-450IA1 or cytochrome P-450IA2, or both, are present in human lung.

Although levels of both cytochrome P-450IA1 and cytochrome P-450IA2 are regulated at the *Ah* genetic locus, the two isozymes are not expressed in equal ratios in all tissues. Cytochrome P-450IA1 is present, as demonstrated immunochemically and catalytically, in both hepatic and extrahepatic tissues, including the lung, of 3MC-treated rats and rabbits (26, 27). However, cytochrome P-450IA2 is detectable only in the livers of these animals and is not seen in the lungs of either untreated or 3MC-treated animals (26, 27). In humans, expression of these two isozymes occurs in a pattern similar to that observed in rabbits and rats. Cytochrome P-450IA1 and P-450IA2 homologues have both been identified in the human liver (28); cytochrome P-450IA2 is the predominant P-450I subfamily member present (29). Because the levels of cytochrome P-450IA1 are very low in human liver and its levels in extrahepatic tissues are much higher than its levels in liver, cytochrome P-450IA1 is considered to be primarily an extrahepatic enzyme in humans (30). Cytochrome P-450IA1 and its associated activities have been demonstrated in human placenta (31, 32) and lymphocytes (33).

The present study utilizes immunochemical assay methods, namely Western blotting and solid-phase radioimmunoassay, to investigate whether isozymes of the P-450I family are present in human lung, and at what levels. The presence in human lung of a homologue to cytochrome P-450IA1 is demonstrated. MAb 1-7-1, which specifically recognizes the two known cytochrome P-450I isozymes in rat liver microsomes, cytochromes P-450IA1 and P-450IA2 (34), was used to identify and quantitate this cytochrome P-450I isozyme in human lung microsomes. By correlating metabolic selectivity with immunochemical analysis, we demonstrate that a cytochrome P-450IA1 but not a cytochrome P-450IA2 homologue is present at detectable levels in human lung microsomes. We compare the levels of these individual isozymes in the human lung with those present in other species and estimate the degree of variability of lung cytochrome P-450IA1 levels in the patient population available to us.

## Experimental Procedures

**Materials.** Bovine serum albumin, 7-ethoxyresorufin, $NADP^+$, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, [$^{14}$C]acetanilide (specific activity, 24.1 mCi/mmol), 4-acetamidophenol, trichloroacetic acid, potassium phosphate, sodium chloride, potassium chloride, and magnesium chloride were obtained from Sigma Chemical Co. (St. Louis, MO). Resorufin, umbelliferone, 7-ethoxycoumarin, high pressure liquid chromatography-grade methanol, and acetanilide, which was recrystallized twice in water/methanol before use, were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Newborn calf serum was obtained from Biologos Inc. (Naperville, IL). 4-[$^3$H]Hydroxyacetanilide, at a specific activity of 5.5 Ci/mmol, was obtained from New England Nuclear Research Products (Boston, MA). 3-MC and benzo[*a*]pyrene were purchased from Eastman Kodak Company (Rochester, NY). The BCIP/NBT phosphatase substrate system, consisting of 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium, was purchased from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD). Alkaline phosphatase-conjugated goat anti-mouse IgG (H + L), used for Western blotting, and the AffiniPure F(ab′)$_2$ fragment of goat anti-mouse IgG (H + L), used for radioimmunoassay, were purchased from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA). The F(ab′)$_2$ fragment was radioiodinated (35) in our laboratory to a specific activity of approximately 8 $\mu$Ci/$\mu$g, using IodoBeads purchased from Pierce (Rockford, IL). $^{125}$I (100 mCi/ml) was obtained from ICN Radiochemicals (Irvine, CA). All chemicals for electrophoresis were purchased from Bio-Rad Laboratories (Richmond, CA).

**Human lung tissue.** Human lung samples, weighing from 0.5 to 10 g, were obtained as surgical waste from patients undergoing partial lung resection, for diagnosis and therapy of pulmonary carcinoma, at the University of Illinois Hospital, Cook County Hospital, or West Side Veterans Administration Hospital. Lung samples were taken directly from the operating rooms, snap frozen within 30 min in liquid nitrogen, and maintained thereafter at −70°. Only samples that were remote from any tumor site and free of macroscopically or microscopically detectable cancer were used.

**Animal tissue.** Baboon lung samples were obtained from animals euthanized 6 to 8 weeks after recovery from experimental lumbar disc surgery. These animals were maintained on a normal diet of commercial monkey chow and were not exposed to any additional drugs or environmental chemicals. Liver and lung tissues were taken from adult male Sprague-Dawley rats treated with 3MC (30 mg/kg intraperitoneally, dissolved in corn oil), or with the equivalent amount of corn oil alone, for 3 days. Rabbit lung tissue was taken from untreated adult male New Zealand White rabbits.

**Preparation of microsomes.** Human lung microsomes were prepared by previously published methods (16, 36). The final pellet was resuspended in a volume of storage buffer (containing 0.2 M potassium phosphate, 1 mM EDTA, and 20% glycerol, at pH 7.4) equal to one half the original tissue weight and was either refrozen at −70° or used immediately. Antibody binding levels remained constant for at least 1 year when microsomes were stored at −70°, as determined by removal and assay of selected samples at bimonthly intervals. One gram of human lung tissue yielded 2–4 mg of microsomal protein. Protein concentrations were determined by the method of Lowry *et al.* (37). Microsomes from other tissues were prepared by the same method used for human lung, to control for the effects of the additional washing steps on microsome recovery and antibody binding affinity.

**Immunochemical preparations.** Monoclonal antibodies were prepared and selected as described (38). MAb 1-7-1 is selective for isozymes of the cytochrome P-450I subfamily; in 3MC-induced rat liver microsomes it binds only to cytochromes P-450IA1 and P-450IA2 (38). MAb 2-66-3 is selective for those cytochrome P-450 isozymes induced in rat liver microsomes by phenobarbital (39). A nonspecific mouse IgG preparation, NBS 1-48-5, which was produced by hybridoma cells (38) but does not bind selectively to any known cytochrome P-450 isozyme, was used as a control. MAb 1-7-1, MAb 2-66-3, and NBS 1-48-5 were obtained from peritoneal ascites fluid produced in tumor-bearing mice. The ascites fluid was dialyzed with PBS, diluted, and frozen at −70° until it was used.

**Solid-phase radioimmunoassay.** Radioimmunoassays were carried out by a modification of previously described methods (34, 40), using 96-well polyvinyl chloride microtiter plates (Dynatech Laboratories, Inc., Chantilly, VA). The wells were coated overnight at 4° with 20 $\mu$l of microsome suspension in PBS containing 1 mM magnesium chloride and 0.02% sodium azide. The microsomal suspensions were removed by aspiration and the wells were incubated with 150 $\mu$l of 10% newborn calf serum in PBS, with 0.02% sodium azide, for 1 hr at room temperature to block nonspecific binding sites. The wells were then exposed to 20 $\mu$l of monoclonal antibody suspension (250 $\mu$g of ascites protein/ml of PBS), containing 10% newborn calf serum and 0.02% sodium azide, for 1 hour at room temperature. The wells were then washed three times with PBS containing 0.1% bovine serum albumin and 0.02% sodium azide. Subsequently, 20 $\mu$l ($8 \times 10^4$ cpm) of $^{125}$I-labeled F(ab′)$_2$ fragment of goat anti-mouse IgG were added to the wells and incubated at room temperature for 1 hr. After four washes with PBS containing 0.1% bovine serum albumin and 0.02% sodium azide, the microtiter plates were dried under a heat lamp and individual

wells were cut with a hot wire and put in counting vials. The vials were counted in a TM Analytic γ-counter. Specific binding was determined by subtracting the radioactivity of the sample from that of a blank obtained by substituting 10% newborn calf serum in PBS for microsomal protein.

**Western blotting.** Lung microsomal proteins (25 μg of microsomal protein/lane) were separated by the method of Laemmli (41). Some lanes contained commercial molecular weight standards. Proteins were transferred to a nitrocellulose membrane (Schleicher & Schuell, Keene, NH) at 5°, using a Hoeffer Transfor unit operating at constant voltage (100 V, 1.5 A) (42). The transfer was complete at 45 min, as judged by Amido black staining of the nitrocellulose membrane. One centimeter-wide strips were cut from the membrane and placed in a Hoeffer multiwell incubation tray, where the nonspecific binding sites were blocked with 3% ovalbumin in PBS for 1 hr at room temperature (43). The blocking solution was aspirated and monoclonal antibody, at a concentration of 250 μg of ascites protein/ml of PBS containing 1% ovalbumin, was added and incubated overnight at 4°. This concentration of monoclonal antibody is 5 times higher than that normally used for animal or liver microsomes (44); a concentration of 50 μg of ascites protein/ml was used with the strip obtained from 3MC-treated rat liver microsomes. After several washes with PBS, the strips were immersed in PBS containing alkaline phosphatase-conjugated goat anti-mouse IgG, at 1:10,000 dilution, for 1 hr at room temperature. The membrane strips were washed twice with PBS containing 0.05% Tween 20 and twice with PBS alone. Bands corresponding to immunoreactive proteins were detected by addition of a phosphatase substrate system (5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium) and development for up to 45 min.

**Metabolic activities.** ECOD activity was quantitated by fluorometric determination of the conversion of 7-ethoxycoumarin to umbelliferone (45). EROD activity was assayed by detecting the conversion of 7-ethoxyresorufin to resorufin (46). Fluorometric measurement (47) of phenolic metabolites of benzo[*a*]pyrene was used to assay AHH activity. Acetanilide 4-hydroxylase activity was determined by a dual-label radiometric method, which uses high pressure liquid chromatography to separate the hydroxylated metabolites of acetanilide (48). The following constituents were included in incubations for all four assays described above: 0.25 ml of 0.2 M potassium phosphate, pH 7.4, 50 μl of 100 mM magnesium chloride, 25–100 μl of microsomes (2–5 mg of protein/ml), 100 μl of cofactor mix (consisting of 100 mM glucose-6-phosphate and 10 mM $NADP^+$), 2 units of glucose-6-phosphate dehydrogenase, 10 μl of substrate dissolved in appropriate solvent, and water to bring the total reaction volume to 1 ml. Tissues with low enzyme activity were incubated for 30 min, whereas tissues with high activity were incubated for 10 min, after which the reaction was stopped by the addition of either 0.3 M trichloroacetic acid or methanol. Assays were run in duplicate or triplicate when tissue amount permitted.

For antibody inhibition studies, saturating levels of monoclonal antibody suspension (100 μg of ascites protein/incubation) were incubated with the microsomal preparations for 15 min on ice before the addition of the other reaction components, and assays were performed as described above. Negative controls, with substitution of an equivalent amount of NBS 1-48-5 ascites protein or buffer for the monoclonal antibody, were included in each experiment.

## Results

Fig. 1 shows that MAb 1-7-1 binds to human lung microsomes (Fig. 1A) as well as to rat liver and lung microsomes (Fig. 1B). This binding is dependent upon microsomal protein in amounts below 10 μg/well. Linearity appears to be limited by the amount of microsomal protein rather than by the amount of antigen present; cpm can be a linear function of antigen level up to at least 7500 cpm (Fig. 1B). In routine binding assays, wells were coated with 20 μl of microsomal suspension at a concentration of 250 μg of protein/ml, or 5 μg of microsomal protein/well.

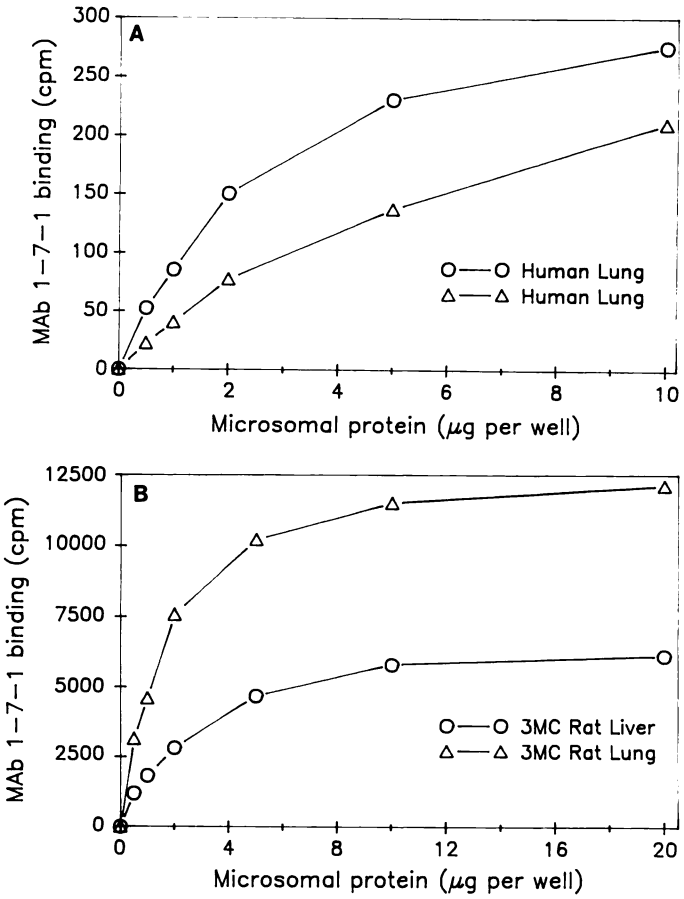


**Fig. 1.** Solid-phase radioimmunoassay of human and rat microsomes using MAb 1-7-1. Microtiter plates were coated with 20 μl of microsomal suspensions, at varying concentrations (0–1000 μg of protein/ml), overnight at 4°. MAb 1-7-1 was diluted in PBS containing 10% newborn calf serum, and 20 μl (5.0 μg of ascites protein) were added/well. Binding was detected by addition of 20 μl ($8 \times 10^4$ cpm/well) of $^{125}$I-conjugated $F(ab')_2$ fragment of goat anti-mouse IgG (H + L). Specific binding was obtained by subtraction of the radioactivity of blanks obtained by substitution of 10% newborn calf serum for microsomal protein. Values are reported as mean cpm/well of duplicate determinations. A, Microsomes from two different human lung samples. B, Microsomes from liver and lung of 3MC-treated rat.

Fig. 2 shows the dependence of detected counts on the amount of MAb 1-7-1 employed. Wells were coated with 5 μg of the indicated microsomal protein. When more than 5 μg of ascites protein/well are used, binding sites are saturated and the number of counts depends only on the amount of antigen present. This amount of MAb 1-7-1 was used for routine assay. Under these conditions, the number of counts in each well is a linear function of the amount of microsomal antigen, whereas the amount of MAb 1-7-1 added is in excess and does not limit the number of detectable counts.

Cross-species comparisons of cytochrome P-450I levels using MAb 1-7-1 binding requires that the affinity of the antibody be approximately equivalent for the species compared and that a saturating amount of antibody be used in all cases. Antibody binding curves were prepared for several different tissues and analyzed by the computer program EQUIL.[1] In Fig. 2, A and B, the relative affinities of MAb 1-7-1 for 3MC-treated rat liver

[1] R. F. Goldstein and E. Leung. EQUIL: simulation and data analysis of binding reactions with arbitrary chemical models. Submitted for publication.

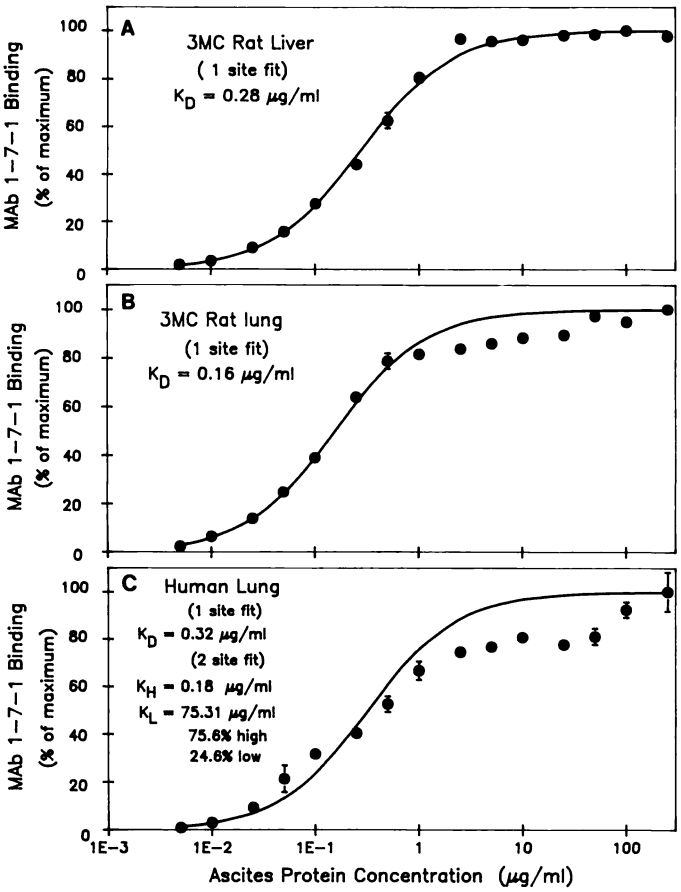


**Fig. 2.** Solid-phase radioimmunoassay of 3MC-induced rat liver (A), 3MC-induced rat lung (B), and human lung (C) microsomes with MAb 1-7-1. Assays were performed as described in the legend to Fig. 1, except that wells were coated with a fixed amount of microsomal protein (5 μg in 20 μl) and the amount of MAb 1-7-1 added/well was varied from 0 to 500 μg of ascites protein, in a volume of 20 μl.

and lung microsomes are analyzed. Using a single binding site model, a $K_D$ of 0.28 μg of ascites protein/ml was calculated for 3MC-induced rat liver microsomes. MAb 1-7-1 has a slightly higher affinity, with a $K_D$ of 0.16 μg of ascites protein/ml, for immunoreactive protein in 3MC-induced rat lung microsomes. In human lung, a higher $K_D$, 0.32 μg of ascites protein/ml, is seen (Fig. 2C). Affinity constants have also been calculated for human lung microsomes using a two-site binding model. They are 0.18 μg of ascites protein/ml for the high affinity site and 75 μg of ascites protein/ml for the low affinity site. The similarity of the affinity constants among the three tissues suggests that epitopes recognized by MAb 1-7-1 in rat and human microsomes have similar affinities for the antibody.

Western blots provide additional evidence that a cytochrome P-450I species is present in human lung microsomes. Fig. 3 shows that a single band, corresponding to a protein with a molecular weight of 55,000, is recognized by MAb 1-7-1 in human lung microsomes. This band migrates in similar fashion to the $M_r$ 56,000 and 57,000 bands recognized by MAb 1-7-1 in 3MC-treated rat liver microsomes (34). A single band of similar molecular weight is also recognized by MAb 1-7-1 in baboon lung microsomes. No bands were identified by the nonspecific hybridoma-produced IgG ascites preparation NBS 1-48-5 in rat, baboon, or human lung microsomes (data not shown).

Nineteen human lung samples were subjected to solid-phase

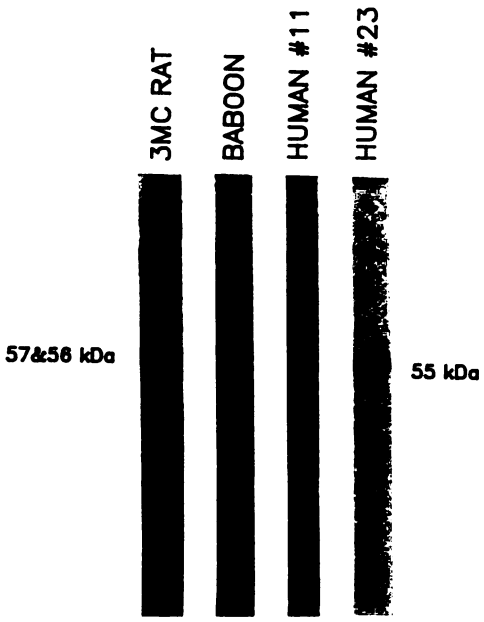


**Fig. 3.** Western blot analysis of human and untreated baboon lung microsomes using MAb 1-7-1. Western blotting was performed as described in Experimental Procedures. Twenty-five micrograms of microsomal protein were electrophoresed in each lane. Proteins with a molecular weight of 55,000 were stained in lanes containing microsomal protein from baboon lung (*lane 2*) and two human lung samples identified in Table 1 (*lanes 3* and *4*). *Lane 1* contains protein from 3MC-treated rat liver microsomes.

TABLE 1

**MAb 1-7-1 binding and metabolic activities in individual human lung microsome samples**

MAb 1-7-1 binding was determined in triplicate wells for each microsomal sample, by solid-phase radioimmunoassay, and are reported as mean cpm. EROD, ECOD, and AHH activities (pmol of product formed/min/mg of microsomal protein) are reported as mean values of duplicate determinations. Patient age, sex, and smoking status are indicated (+, active smoker; −, nonsmoker; NA, smoking history not available).

| Patient number | Patient history | | | MAb 1-7-1 binding | Metabolic activities | | |
|---|---|---|---|---|---|---|---|
| | Age | Sex | Smoker | | EROD | ECOD | AHH |
| | | | | cpm | pmol/min/mg | | |
| 2 | 50 | F | + | 230 | 0.62 | 9.80 | NT[a] |
| 3 | 65 | M | + | 220 | 0.35 | 3.43 | NT |
| 4 | 36 | M | − | NT | 0.89 | 4.82 | NT |
| 5 | 67 | M | NA | 170 | 1.21 | 6.40 | 0.81 |
| 6 | 50 | F | + | NT | 0.92 | 7.67 | NT |
| 7 | 68 | M | + | 170 | 1.53 | 5.22 | 1.22 |
| 8 | 53 | M | + | 170 | 1.55 | 4.18 | NT |
| 9 | 61 | F | − | NT | 1.63 | 3.59 | NT |
| 10 | 40 | M | + | 290 | 5.21 | 5.74 | 1.67 |
| 11 | 61 | F | + | 260 | 3.73 | 6.43 | 4.12 |
| 12 | 43 | F | + | 77 | 0.78 | 5.28 | 1.11 |
| 13 | 59 | M | NA | 86 | 1.07 | 8.33 | 1.87 |
| 14 | 51 | F | NA | 210 | 2.19 | 4.03 | 1.75 |
| 15 | 58 | M | + | 130 | 1.65 | 6.19 | NT |
| 16 | 60 | F | + | 140 | 0.85 | 3.91 | NT |
| 17 | 53 | F | − | 120 | 2.34 | 3.67 | 2.73 |
| 18 | 33 | M | − | 180 | 1.06 | 1.95 | 2.51 |
| 19 | 54 | F | − | 140 | 0.74 | 3.14 | 0.63 |
| 20 | 26 | M | + | 200 | 0.60 | 2.52 | 1.65 |
| 21 | 44 | M | + | 190 | 0.68 | 2.80 | 1.29 |
| 22 | 67 | M | NA | 150 | 1.31 | 3.66 | 0.73 |
| 23 | 39 | M | + | 690 | 8.34 | 6.25 | 4.26 |

[a] NT, not tested.

radioimmunoassay (Table 1). MAb 1-7-1 binding was observed with all 19 samples. This binding, measured as cpm of labeled second antibody bound/well, ranged 9-fold, from 77 to 690 cpm/well, with a mean value ± SD of 200 ± 130 cpm/well. Table 2 shows that the mean level of antibody binding to human lung microsomes was 4-fold lower than to untreated rat lung microsomes and 9-fold lower than to untreated rat liver microsomes. MAb 1-7-1 binding to human lung microsomes was at least 25-fold and 50-fold lower, respectively, than its binding to 3MC-induced rat lung and liver microsomes. MAb 1-7-1 binding to human lung microsomes was also lower than to microsomes from untreated rabbit lung. Antibody binding levels for untreated baboon lung microsomes were similar to the mean value for human lung microsomes.

All 22 human lung microsome samples were capable of deethylating 7-ethoxyresorufin to resorufin (Table 1). There was a 24-fold variation in EROD activity (0.35 to 8.34 pmol/min/mg of protein) in the patient population. Fig. 4 shows that the frequency distribution of pulmonary EROD activity is tightly clustered for 20 of the 22 samples but 2 lung samples have EROD values greater than the mean by more than 1 SD. The mean value (2.05 ± 2.02 pmol/min/mg of protein) of EROD activity is greatly shifted by sample 23; the median value is 1.14 pmol/min/mg of protein. EROD activity levels (Fig. 5) correlate strongly with MAb 1-7-1 binding ($r = 0.92$, $p < 0.01$). Baboon lung microsomes had a mean EROD activity of 1.27 ± 0.37 pmol/min/mg of protein (Table 2), which is not statistically different from the mean value obtained for the human lung samples. Untreated rabbit lung, 3MC-treated rat lung, and 3MC-treated rat liver had 3, 80, and 6500 times higher EROD activities, respectively, than did human lung (Table 2).

ECOD activity was also detected in every human lung sample tested (Table 1). ECOD activity in human lung samples ranged 5-fold, from 1.95 to 9.80 pmol/min/mg of protein, with a mean value ± SD of 4.69 ± 1.72 pmol/min/mg of protein. ECOD activity levels in individual human lung samples were distributed normally about the mean (Fig. 6). MAb 1-7-1 binding correlated poorly ($r = 0.26$) with ECOD activity (Fig. 7), indicating that the cytochrome P-450 isozyme recognized by MAb 1-7-1 does not account for all of the ECOD activity measured in the individual human lung samples. The four baboon lung samples had ECOD activity levels similar to those seen in human lung, with a mean ± SD of 5.73 ± 2.56 pmol/min/mg of protein (Table 2). Far higher ECOD activities of 160, 225, and 270 pmol/min/mg of protein were observed in lung microsomes from untreated rat, 3MC-treated rat, and untreated rabbit, respectively (Table 2). 3MC-induced rat liver microsomes contained the highest ECOD activity (6400 pmol/min/mg of protein) of any tissue tested. Human liver microsomal ECOD activity was 68 pmol/min/mg of protein.

AHH activity was measurable in all 14 human lung microsomal fractions assayed (Table 1). Individual activities were normally distributed (Fig. 8). The relatively poor correlation between AHH activity and MAb 1-7-1 binding ($r = 0.60$) also indicates that the immunoreactive protein recognized by MAb 1-7-1 is not the sole form of cytochrome P-450 responsible for benzo[*a*]pyrene hydroxylation in the human lung (Fig. 9). Human and baboon lung microsomes had mean activities of 1.89 ± 1.15 and 1.32 ± 0.43 pmol/min/mg of protein, respectively, which were not significantly different. Untreated rabbit and rat lung microsomes had AHH activities of 12.0 and 4.2 pmol/min/mg of protein. As expected, the 3MC-treated rat liver and lung microsomes possessed considerably higher AHH activity, 1800 and 40 pmol/min/mg of protein, respectively (Table 2).

Human lung microsomes contained no detectable acetanilide 4-hydroxylase activity (Table 3). The lowest level of detection of activity by this assay is 0.05 pmol/min/mg of protein. Both untreated baboon and rat lung microsomes were without acetanilide 4-hydroxylase activity as well. Acetanilide 4-hydroxylase activity was measurable in untreated rabbit lung and in 3MC-treated rat liver and lung at levels of 0.2, 148, and 0.3 pmol/min/mg of protein, respectively (Table 3).

The fact that EROD activity in these human lung samples correlates well with MAb 1-7-1 binding suggests that EROD activity is catalyzed principally by those species of cytochrome P-450 that bind to MAb 1-7-1. Therefore, the ability of MAb 1-7-1 to inhibit EROD activity in human lung microsomes was also determined. Fig. 10 shows that EROD activity in both human and 3MC-treated rat lung is inhibited by MAb 1-7-1. The inhibition curves parallel one another and activities from both species are maximally inhibited in the presence of 50 μg of ascites protein/reaction tube. Human lung microsomes were preincubated with saturating amounts of MAb 1-7-1 (100 μg of ascites protein/reaction tube) and inhibition of EROD activity was determined for each of seven samples. EROD activity after inhibition of MAb 1-7-1 ranged from 81 to 22% of control levels, with a mean value of 47 ± 24% of control (Table 4).

TABLE 2

**MAb 1-7-1 binding and metabolic activities in microsomes from different species**

MAb 1-7-1 binding was determined in duplicate (animal tissue) or triplicate (human tissue) wells for each of the number of samples given in parentheses, by solid phase radioimmunoassay, and are reported as mean cpm ± standard deviation. EROD, ECOD, and AHH activities were measured in duplicate determinations from the number of microsomal samples given in parentheses and are reported as mean (pmol of product/min/mg of microsomal protein) ± standard deviation.

| Tissue | MAb 1-7-1 binding | Metabolic activities | | |
|---|---|---|---|---|
| | | EROD | ECOD | AHH |
| | *cpm* | *pmol/min/mg* | | |
| Human lung | 200 ± 130 (19) | 2.05 ± 2.0 (22) | 4.69 ± 1.7 (22) | 1.89 ± 1.1 (14) |
| Baboon lung | 240 ± 38 (4) | 1.27 ± 0.37 (4) | 5.73 ± 2.6 (4) | 1.32 ± 0.43 (3) |
| Rabbit lung | 1500 ± 59 (3) | 9.20 ± 3.9 (3) | 295 ± 65 (3) | 12.3 ± 2.8 (3) |
| Rat lung | 840 (2) | 8.27 ± 1.9 (3) | 160 ± 18 (3) | 4.2 ± 1.8 (3) |
| 3MC-treated rat lung | 4700 ± 150 (3) | 147 ± 15 (3) | 245 ± 14 (3) | 55.5 ± 18 (3) |
| Rat liver | 1900 (2) | NT[a] | NT | NT |
| 3MC-treated rat liver | 10000 ± 410 (3) | 12800 ± 900 (3) | 6400 ± 450 (3) | 1800 ± 150 (3) |
| Human liver | 320 ± 25 (4) | NT | NT | NT |

[a] NT, not tested.

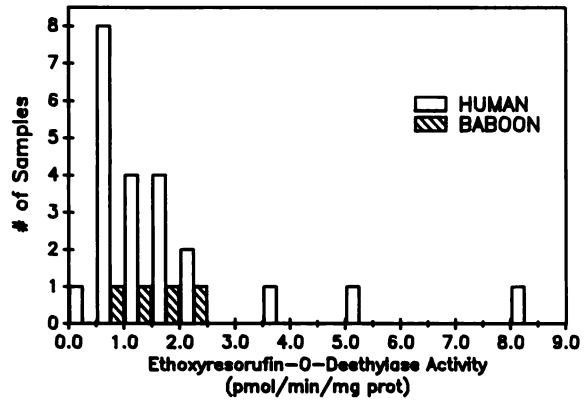


**Fig. 4.** Distribution of EROD activities from individual human and baboon lung samples. *Ordinate*, the number of individual microsomal samples with specific metabolic activities falling between values indicated by *marks* on the *abscissa*.

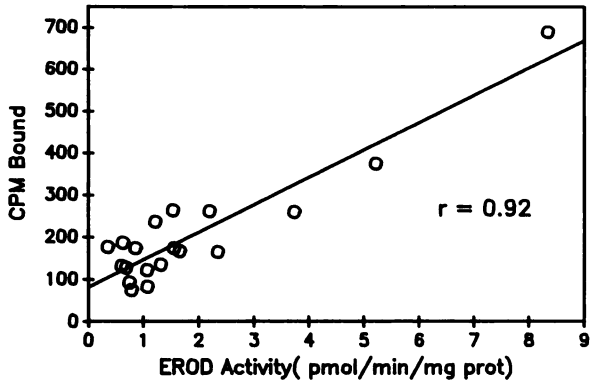


**Fig. 5.** Correlation of EROD activity with MAb 1-7-1 binding in individual human lung samples. EROD activities (pmol of resorufin formed/min/mg of protein) are indicated in Table 1. MAb 1-7-1 binding to human lung microsomes was determined by solid-phase radioimmunoassay (20). A correlation coefficient of 0.92 was calculated.

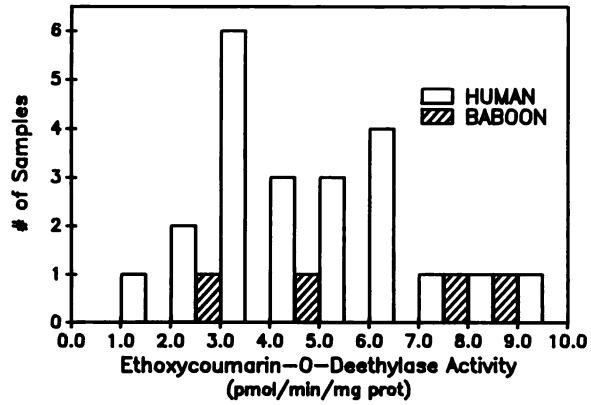


**Fig. 6.** Distribution of EROD activities from individual human and baboon lung samples. *Ordinate*, the number of individual microsomal samples with specific metabolic activities falling between values indicated by *marks* on the abscissa.

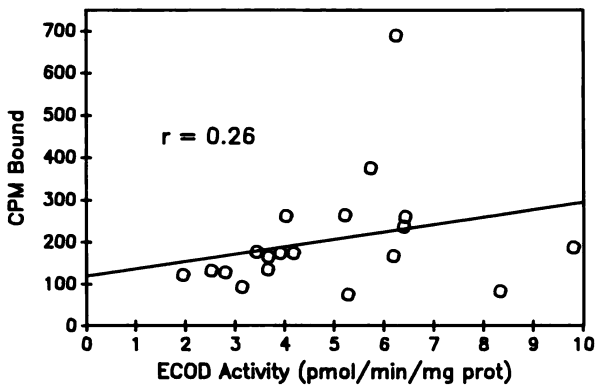


**Fig. 7.** Correlation of ECOD activity with MAb 1-7-1 binding in individual human lung samples. ECOD activities (pmol of umbelliferone formed/min/mg of protein) are indicated in Table 1. MAb 1-7-1 binding to human lung microsomes was determined by solid-phase radioimmunoassay (20). A correlation coefficient of 0.26 was calculated.

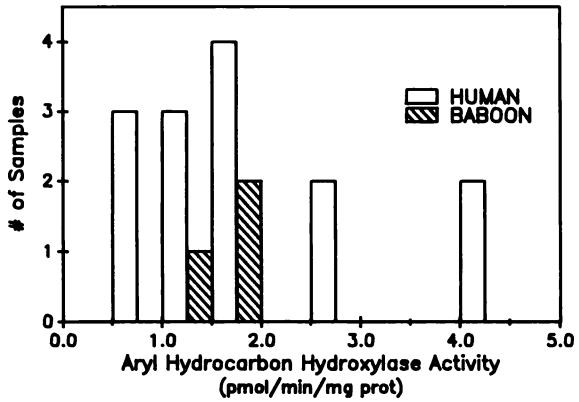


**Fig. 8.** Distribution of AHH activities from individual human and baboon lung samples. *Ordinate*, the number of individual microsomal samples with specific metabolic activities falling between values indicated by *marks* on the *abscissa*.

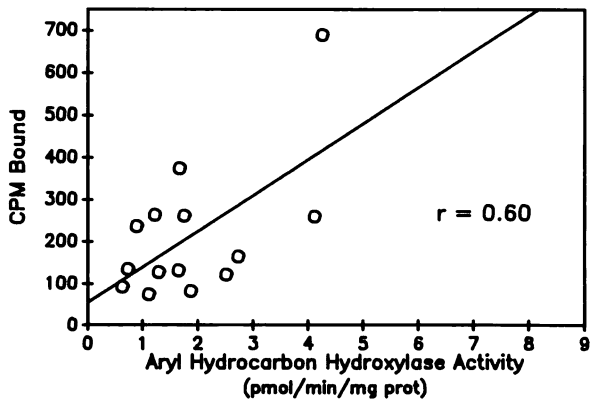


**Fig. 9.** Correlation of AHH activity with MAb 1-7-1 binding in individual human lung samples. AHH activities (pmol of 3-hydroxybenzo[*a*]pyrene formed/min/mg of protein) are indicated in Table 1. MAb 1-7-1 binding to human lung microsomes was determined by solid-phase radioimmunoassay (20). A correlation coefficient of 0.60 was calculated.

MAb 1-7-1 also inhibited EROD activity in baboon lung to similar levels, 51 ± 11% of control. EROD activity was inhibited in 3MC-treated rat lung and untreated rabbit lung microsomes to 20 and 46% of control levels, respectively. MAb 1-7-1 also inhibited EROD activity in all three human liver samples to a mean level of 73 ± 6.8% of control. MAb 2-66-3, a monoclonal antibody that recognizes one or more phenobarbital-inducible rat liver cytochrome P-450 isozymes (39, 49), was also used in an attempt to inhibit EROD activity. At concentrations of 100 μg of ascites protein/reaction tube, MAb 2-66-3 and NBS 1-48-5, a nonspecific hybridoma-produced IgG preparation, had no inhibitory effect on EROD activity in any sample tested (Table 4).

TABLE 3

**Acetanilide 4-hydroxylase activities in microsomes from different species**

Acetanilide 4-hydroxylase activity was measured in 10 individual human lung microsomal samples and in microsomes from different species. Activities are reported as the mean value (pmol of 4-acetamidophenol formed/min/mg of protein) of duplicate determinations from the number of different microsomal samples given in parentheses. The lowest activity detectable by the assay was estimated to be 0.05 pmol/min/mg of protein.

| Tissue | Acetanilide 4-hydroxylase activity |
|---|---|
| | *pmol/min/mg* |
| Human lung | |
| 11 | <0.05 |
| 12 | <0.05 |
| 13 | <0.05 |
| 17 | <0.05 |
| 18 | <0.05 |
| 19 | <0.05 |
| 20 | <0.05 |
| 21 | <0.05 |
| 22 | <0.05 |
| 23 | <0.05 |
| Untreated baboon lung | <0.05 (3) |
| Untreated rabbit lung | 0.2 (2) |
| Untreated rat lung | <0.05 (2) |
| 3MC-treated rat lung | 0.3 (2) |
| Untreated rat liver | 2.9 (2) |
| 3MC-treated rat liver | 148 (2) |
| Human liver | 1.3 ± 0.6 (4) |

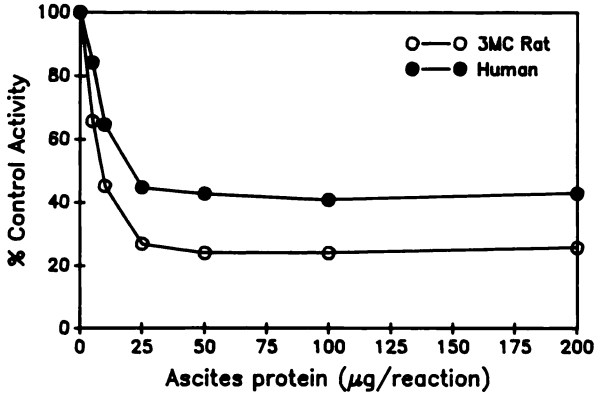


**Fig. 10.** Immunotitration of EROD activity in human lung and 3MC-induced rat lung microsomes by MAb 1-7-1. Lung microsomes were incubated with varied amounts (0–200 μg of ascites protein) of MAb 1-7-1 for 15 min before the addition of the other reaction components. Data shown are the means of duplicate incubations and are expressed as percentage of control activity, measured in the absence of monoclonal antibody.

MAb 1-7-1 did not inhibit ECOD activity in the human lung, nor was ECOD activity inhibited by MAb 1-7-1 in baboon lung or human liver microsomes (Table 5). MAb 1-7-1 did inhibit ECOD activity in 3MC-treated rat and rabbit lung microsomes, to levels of 65 and 88% of control, respectively. MAb 2-66-3 did not inhibit ECOD activity in human lung, baboon lung, or human liver microsomes. MAb 2-66-3 inhibited ECOD activity in untreated rat and rabbit lung to levels of 67 and 72% of control, respectively. NBS 1-48-5 had no effect on ECOD activity in any sample tested (Table 5). Because of the low amount of tissue available, the inhibition of AHH activity by MAb 1-7-1 was not determined.

## Discussion

We have used solid-phase radioimmunoassay to directly measure human lung cytochrome P-450 isozymes that have structural homology to those of the cytochrome P-450I family. The fact that the association constants of MAb 1-7-1 are similar for immunoreactive proteins in both human lung and 3MC-induced rat liver (Fig. 2) suggests that cytochrome P-450I isozymes with significant structural homology are present in microsomes from both species. The presence of an isozyme of the cytochrome P-450I family in the human lung is further demonstrated by Western blot analysis (Fig. 3). A single protein band in human lung microsomes, with an apparent molecular weight of 55,000, is recognized by MAb 1-7-1. This protein has a molecular weight characteristic of a cytochrome P-450 isozyme but differs slightly from the $M_r$ 56,000 and 57,000 proteins recognized by

TABLE 4

**Inhibition of EROD activity by monoclonal antibodies**

Inhibition by MAb 1-7-1, MAb 2-66-3, and nonspecific mouse hybridoma IgG NBS 1-48-5 of EROD activity in microsomes from human lung samples and other tissues was determined. Monoclonal antibodies (100 μg of ascites fluid protein) were incubated with microsomes for 15 min before the addition of other reaction components. The activities are reported as mean values of the percentage of control (uninhibited) EROD activity for duplicate determinations from the number of different microsomal samples per group given in parentheses.

| Tissue | EROD activity | | |
|---|---|---|---|
| | MAb 2-66-3 | MAb 1-7-1 | NBS 1-48-5 |
| | *% of control* | | |
| Human lung | | | |
| 10 | 99 | 22 | 100 |
| 11 | 102 | 39 | 97 |
| 13 | 103 | 24 | 100 |
| 19 | 100 | 81 | 96 |
| 21 | 108 | 71 | 100 |
| 22 | 100 | 60 | 100 |
| 23 | 98 | 30 | 100 |
| Baboon lung | 93 (3) | 51 (3) | 103 (3) |
| Rabbit lung | 95 (2) | 48 (2) | 99 (2) |
| Untreated rat lung | 100 (2) | 46 (2) | 100 (2) |
| 3MC-treated rat lung | 97 (2) | 20 (2) | 98 (2) |
| Human liver | | | |
| A | 100 | 77 | 100 |
| C | 103 | 77 | 100 |
| D | 98 | 65 | 98 |

TABLE 5

**Inhibition of ECOD activity by monoclonal antibodies**

The inhibitory effect of MAb 1-7-1, MAb 2-66-3, and nonspecific mouse hybridoma IgG NBS 1-48-5 on ECOD activity in microsomes from human lung samples and other tissues was determined. Monoclonal antibodies (100 μg of ascites fluid protein) were incubated with microsomes for 15 min before the addition of other reaction components. The activities are reported as mean values of the percentage of control (uninhibited) ECOD activity for duplicate determinations from the number of different microsomal samples per group given in parentheses.

| Tissue | ECOD activity | | |
|---|---|---|---|
| | MAb 2-66-3 | MAb 1-7-1 | NBS 1-48-5 |
| Human lung | | | |
| 10 | 102 | 100 | 100 |
| 11 | 100 | 95 | 95 |
| 13 | 100 | 98 | 98 |
| 19 | 105 | 100 | 100 |
| 21 | 100 | 105 | 100 |
| 22 | 104 | 108 | 102 |
| 23 | 98 | 106 | 95 |
| Baboon lung | 104 (3) | 107 (3) | 105 (3) |
| Rabbit lung | 72 (2) | 88 (2) | 100 (2) |
| Untreated rat lung | 67 (2) | 90 (2) | 99 (2) |
| 3MC-treated rat lung | 87 (2) | 65 (2) | 100 (2) |
| Human liver | | | |
| A | 102 | 100 | 104 |
| C | 104 | 100 | 103 |
| D | 106 | 100 | 103 |

MAb 1-7-1 in 3MC-treated rat liver microsomes. A $M_r$ 55,000 protein is also recognized in microsomes from untreated baboon lung microsomes. It should be noted that, although several different human lung microsome samples were examined by Western blot analysis, only those two samples that showed the highest amount of antibody binding by radioimmunoassay (samples 10 and 23) produced visible bands. The other samples tested (samples 16, 20 and 22) produced no visible bands. Also, in order to visualize these bands in human and baboon lung microsomes, a 5-fold increase in the amount of MAb 1-7-1 was required. Although 3MC-induced rat liver (Fig. 3; see also Ref. 44) and 3MC-induced rat lung (data not shown) produce visible bands when a 50 μg/ml dilution of MAb 1-7-1 is used, a 250 μg/ml antibody concentration is required for visualization of the cytochrome P-450 species in these two human lung microsome samples.

In rat and rabbit, cytochrome P-450IA1 is expressed and inducible in both the lung and liver, whereas cytochrome P-450IA2 is expressed and inducible only in the liver and not in the lung (26, 27). In over 90% of human liver samples, cytochrome P-450IA2 is the sole cytochrome P-450I isozyme expressed, and no cytochrome P-450IA1 homologue is detectable (28). MAb 1-7-1 binding is seen in every human liver sample we tested (Table 2); we, therefore, presume that in our assay MAb 1-7-1 identifies a cytochrome P-450IA2 homologue in human liver. But because MAb 1-7-1 recognizes both cytochromes P-450IA1 and P-450IA2 in rat liver, the number and identities of cytochrome P-450I subspecies present in the human lung cannot be ascertained by our radioimmunoassay alone. Ideally, monoclonal antibodies such as 1-36-1 and 1-31-2, which are monospecific for rat P-450IA1 but not for rat P-450IA2 (34, 50), could be utilized to provide a more definitive identification of the isozyme species present. However, when we used either of these monoclonal antibodies in our radioimmunoassay of human lung microsomes, no binding was observed (data not shown). We infer that MAb 1-7-1 recognizes an epitope that is present on rat and human P-450IA1 and P-450IA2 homologues, whereas antibodies 1-36-1 and 1-31-2 recognize an epitope specific for rat P-450IA1 that is not present on human P-450IA1. Therefore, because no monoclonal antibody exists that is monospecific for human P-450IA1, the cytochrome P-450-dependent metabolism of four substrates was measured in human lung microsomes, to determine the levels of specific isozymes of the cytochrome P-450I family present. Of the four activities measured, only EROD, ECOD, and AHH were measurable in the human lung. All human lung samples tested contained cytochrome P-450 isozymes that were able to metabolize all three substrates.

Only EROD activity correlated significantly ($r = 0.92$) with MAb 1-7-1 binding to individual human lung samples. MAb 1-7-1 also inhibited EROD activity in human lung (Fig. 10). Reconstitution studies using purified cytochrome P-450 preparations have shown that cytochrome P-450IA1 is the primary isozyme that catalyzes the deethylation of 7-ethoxyresorufin in rodent liver (51, 52). Although cytochrome P-450IA2 possesses some EROD activity, its specific EROD activity is only 5% that of cytochrome P-450IA1. Other cytochrome P-450 isozymes have negligible EROD activity (52, 53). The ability of MAb 1-7-1 to inhibit cytochrome P-450I-linked activities in PAH-induced animal tissues has previously been reported (38, 54). It is not unexpected, then, that MAb 1-7-1 binding correlates with, and inhibits, EROD activity in the human lung. EROD activity was not inhibited by MAb 2-66-3, a monoclonal antibody that binds cytochrome P-450 isozymes present in phenobarbital-induced rat liver (39). The degree of inhibition of EROD activity was variable; this observation contradicts somewhat our finding that EROD activity and MAb 1-7-1 binding, as detected by radioimmunoassay, are highly correlated (Fig. 5). Variability in inhibition of enzymatic activity may reflect a multiplicity of isozymes that catalyze the examined reaction. However, previous observations that greater than 90% of all EROD activity is cytochrome P-450IA1 dependent (51–53), coupled with our data showing strong correlation of EROD activity with MAb 1-7-1 binding (Fig. 5) and absence of measurable P-450IA2-dependent activity in these samples (Table 3), leads us to conclude that the experiments measuring inhibition of EROD activity by MAb 1-7-1 are less precise than the radioimmunoassay analysis. This high degree of variability in enzyme inhibition by MAb 1-7-1 has previously been observed with human liver (55) and lymphocytes (56). We conclude that the human lung contains a homologue of rat cytochrome P-450IA1 that binds MAb 1-7-1 and probably is the principal isozyme present that catalyzes the metabolism of 7-ethoxyresorufin. Therefore, both EROD activity and radioimmunoassay of MAb 1-7-1 binding provide good estimates of P-450IA1 enzyme levels, as well as P-450IA1-linked catalytic activity, in the human lung.

Both AHH and ECOD activities have previously been used to estimate the levels and catalytic activity of cytochrome P-450I isozymes in the human lung (19–19). Although cytochrome P-450IA1 has been shown to have the highest amount of ECOD activity relative to other isozymes in rodent liver (52), other cytochrome P-450 isozymes also contribute significantly to this activity (52, 53). Every human lung sample tested possessed measurable AHH and ECOD activities, but levels of these activities correlated poorly with MAb 1-7-1 binding. Coefficients of 0.60 and 0.26 were calculated for the correlation of AHH and ECOD activities, respectively, with MAb 1-7-1 binding. The finding that MAb 1-7-1 binding to human lung microsomes does not accurately reflect ECOD activity is further illustrated by the inability of MAb 1-7-1 to inhibit ECOD activity in the human lung. The fact that MAb 2-66-3 inhibits ECOD activity in rabbit and rat lung microsomes indicates that cytochrome P-450 isozymes other than P-450IA1 or P-450IA2 contribute to ECOD activity in these tissues. MAb 2-66-3 did not inhibit ECOD activity in the human lung, indicating that cytochrome P-450 isozymes identified by MAb 1-7-1 or MAb 2-66-3 do not contribute to ECOD activity in the human lung. Due to the limited quantity of individual lung samples, inhibition of AHH activity in the lung by MAb 1-7-1 was not measured. Previous studies have shown that AHH activity, which is measured as the rate of formation of phenolic metabolites of benzo[*a*]pyrene, is catalyzed by several cytochrome P-450 isozymes, including members of the P-450I and P-450II subfamilies, in both rodent and human tissues (57). We conclude that neither ECOD nor AHH activities provide the most accurate measure of the levels of cytochrome P-450I isozymes in the human lung and that EROD activity is a preferable indicator.

Acetanilide 4-hydroxylase activity was not above the level of detection of 0.05 pmol/min/mg of protein in any human lung microsomal sample tested. In contrast to EROD activity, acet-

anilide 4-hydroxylase activity is catalyzed selectively by cytochrome P-450IA2 and not by cytochrome P-450IA1 (58, 59). The absence of acetanilide 4-hydroxylase activity in human lung suggests either that cytochrome P-450IA2 is not expressed in this tissue or that it is expressed at levels much lower than those found in lungs of untreated rats or rabbits. We conclude that, although MAb 1-7-1 binding reflects levels of both cytochromes P-450IA1 and P-450IA2 in rat liver (34, 54), in human lung MAb 1-7-1 binds primarily to a homologue of cytochrome P-450IA1. MAb 1-7-1 binding in this tissue, therefore, quantitatively measures the levels of cytochrome P-450IA1 present. Both cytochromes P-450IA1 and P-450IA2 are present in human liver, as are their associated activities (28–30). The absence of cytochrome P-450IA2 from the human lung suggests that the two cytochrome P-450I isozymes are not co-expressed in this tissue in humans. As is the case for rats and rabbits (26, 27), in humans both isozymes are present, and presumably inducible, in the liver, but only cytochrome P-450IA1 is detectable in the lung. Thus, the human lung is similar to both animal lung (26, 27) and another human extrahepatic tissue, placenta (31, 32), in that cytochrome P-450IA1 is the principal cytochrome P-450I isozyme expressed and cytochrome P-450IA2 levels are not detectable.

Both solid-phase radioimmunoassay and EROD activity measurements demonstrate that cytochrome P-450IA1 levels are low in human lung, relative to other species. In accord with previous studies (20, 24), ECOD and AHH activities in the human lung microsomes are also much lower than levels measured in either untreated rabbit or untreated rat lung. Untreated baboon lung microsomes have metabolic capabilities very similar to those of human lung microsomes for all four metabolic activities measured. The presence of a cytochrome P-450I homologue in untreated baboon lung suggests that a constitutive form of cytochrome P-450I is present at low levels in primate lung, even under conditions that are "noninduced", i.e., when the subject is isolated as much as possible from exogenous airborne and dietary chemicals. The high degree of similarity in both EROD and MAb 1-7-1 binding levels between the baboon and human lungs implies that cytochrome P-450IA1 levels are similar in both species. The median EROD activity in the human lung samples, 1.14 pmol/min/mg of protein, is nearly identical to that median EROD activity measured in the untreated baboon lung, 1.28 pmol/min/mg of protein. The data further indicate that baboon or other primate lungs may provide a good model for estimating the ability of human lungs to metabolize xenobiotics but that other commonly employed animal models (i.e., rat and rabbit) greatly overestimate the rather low levels of pulmonary cytochrome P-450I isozymes present in humans and, therefore, do not provide the best toxicological models.

The fact that comparable levels of cytochrome P-450IA1, measured by both immunochemical and catalytic criteria, are seen in human lung and untreated baboon lung suggests that this isozyme is not present at highly induced levels in the majority of human lungs and that human lung cytochrome P-450IA1 levels in most patients tested, somewhat surprisingly, do not reflect a permanent "PAH-induced" state. Two individual human lung samples (samples 10 and 23) have EROD activities and MAb 1-7-1 binding levels that are significantly higher than the mean value. The reason for the high levels in these two samples is not known. Both patients were active cigarette smokers at the time of biopsy. Cigarette smoking generally induces monooxygenase activities in the human lung (24) and cigarette smoke induces, in particular, cytochrome P-450I-dependent activities in rat lung (14, 15). Cytochrome P-450I isozymes and related enzyme activities are inducible by cigarette smoking in human placenta, lymphocytes, and fetal liver (29, 32, 55, 60). A time-dependent induction by cigarette smoke of the cytochrome P-450I-related activities AHH and ECOD in lung has been demonstrated in human lung cancer patients (24). In our study, cytochrome P-450I levels did not appear to be induced in the majority of the human lung samples tested, even though 12 of the 19 tissue samples were from cigarette smokers. This may be due to cessation of smoking for a sufficiently long period of time before hospitalization, combined with a relatively rapid return of isozyme levels to normal values. A combination of factors, both environmental (but nonsmoking-related) and genetic, may also influence cytochrome P-450IA1 and EROD activity levels in these two atypical lung samples.

In conclusion, we demonstrate that the cytochrome P-450I isozyme that binds MAb 1-7-1 in human lung is a P-450IA1 homologue and that a homologue to cytochrome P-450IA2 is not expressed in human lung. This cytochrome P-450IA1 homologue is present at relatively low levels and does not appear to exist in a permanently induced state. Through its resistance to cytochrome P-450IA1 induction, the human lung is afforded some degree of protection from exposure to xenobiotics that readily induce this toxicologically important cytochrome P-450 isozyme in other species and tissues.

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**References**

1. Lu, A. Y. H., and S. B. West. Multiplicity of mammalian cytochrome P-450. *Pharmacol. Rev.* **31**:277–295 (1980).
2. Guengerich, F. P. Cytochromes P-450. *Comp. Biochem. Physiol. C Comp. Pharmacol.* **89c**:1–4 (1988).
3. Nebert, D. W., D. R. Nelson, M. Adesnik, M. J. Coon, R. W. Estabrook, F. J. Gonzalez, F. P. Guengerich, I. C. Gunsalus, E. F. Johnson, B. Kemper, W. Levin, I. R. Phillips, R. Sato, and M. R. Waterman. The P-450 gene superfamily: update on listing of all genes and recommended nomenclature of chromosomal loci. *DNA* **8**:1–14 (1989).
4. DePierre, J. W., and L. Ernster. The metabolism of polycyclic hydrocarbons and its relationship to cancer. *Biochim. Biophys. Acta* **473**:149–186 (1978).
5. Guengerich, F. P., and D. C. Liebler. Enzymatic activation of chemicals to toxic metabolites. *Crit. Rev. Toxicol.* **14**:259–307 (1985).
6. Ionannides, C., and D. V. Parke. The cytochromes P-448: a unique family of enzymes involved in chemical toxicity and carcinogenesis. *Biochem. Pharmacol.* **36**:4197–4207 (1987).
7. Guengerich, F. P. Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Res* **48**:2946–2954 (1988).
8. Oesch, F. Significance of various enzymes in the control of reactive metabolites. *Arch. Toxicol.* **60**:174–178 (1987).
9. Domin, B. A., C. J. Serabjit-Singh, R. R. Vanderslice, T. T. Devereux, J. R. Fouts, J. R. Bend, and R. M. Philpot. Tissue and cellular differences in the expression of cytochrome P-450 isozymes in *Proceedings, IUPHAR 9th International Congress of Pharmacology* (W. Paton, J. Mitchell, and P. Turner, eds.), Vol. 3. Macmillan Press, London, 219–224 (1984).
10. Ueng, T.-H., and A. P. Alvares. Pulmonary cytochromes P-450 from rabbits treated with 3-methylcholanthrene. *Mol. Pharmacol.* **22**:221–228 (1982).
11. Sagami, I., and M. Watanabe. Purification and characterization of pulmonary cytochrome P-450 from 3-methylcholanthrene-treated rats. *J. Biochem.* **93**:1499–1508 (1983).
12. Dawson, G. W., and R. E. Vestal. Smoking and drug metabolism. *Pharmacol. Ther.* **15**:207–221 (1982).
13. Kouri, R. E., T. H. Rude, R. D. Curren, K. R. Brandt, R. G. Sosnowski, L. M. Schechtman, W. F. Benedict, and C. J. Henry. Biological activity of tobacco smoke and tobacco smoke-related chemicals. *Environ. Health Perspect.* **29**:63–69 (1979).
14. Dansette, P. M., K. Alexandrov, R. Azerad, and C. Frayssinet. The effect of some mixed function oxidase inducers on aryl hydrocarbon hydroxylase and epoxide hydrase in nuclei and microsomes from rat liver and lung: the effect of cigarette smoke. *Eur. J. Cancer* **15**:915–922 (1979).
15. Godden, P. M. M., G. Kass, R. T. Mayer, and M. D. Burke. The effects of

cigarette smoke compared to 3-methylcholanthrene and phenobarbitone on alkoxyresorufin metabolism by lung and liver microsomes from rats. *Biochem. Pharmacol.* **36**:3393–3398 (1987).
16. McManus, M. E., A. R. Boobis, G. M. Pacifici, R. Y. Frempong, M. J. Brodie, G. C. Kahn, C. Whyte, and D. S. Davies. Xenobiotic metabolism in the human lung. *Life Sci.* **26**:481–487 (1980).
17. Lorenz, J., H. Schmassmann, E. Ohnhaus, and F. Oesch. Activities of polycyclic hydrocarbon activating and inactivating enzymes in the human lungs of smokers, non-smokers, lung cancer, and non-cancer patients. *Arch. Toxicol.* (Suppl 2) 483–489 (1979).
18. Prough, R. A., V. W. Patrizi, R. T. Okita, B. S. S. Masters, and S. W. Jakobsson. Characteristics of benzo[*a*]pyrene metabolism by kidney, liver, and lung microsomal fractions from rodents and humans. *Cancer Res.* **39**:1199–1206 (1979).
19. Jakobsson, S. W., R. T. Okita, N. I. Mock, B. S. S. Master, L. M. Buja, and R. A. Prough. Monooxygenase activities of human liver, lung, and kidney microsomes: a study of 42 *post mortem* cases. *Acta Pharmacol. Toxicol.* **50**:332–341 (1982).
20. Lorenz, J., H. R. Glatt, R. Fleischmann, R. Ferlinz, and F. Oesch. Drug metabolism in man and its relationship to that in three rodent species: monooxygenase, epoxide hydrolase, and glutathione *S*-transferase activities in subcellular fractions of lung and liver. *Biochem. Med.* **32**:43–56 (1984).
21. Devereux, T. R., T. E. Massey, M. R. Van Scott, J. Yankaskas, and J. R. Fouts. Xenobiotic metabolism in human alveolar type II cells isolated by centrifugal elutriation and density gradient centrifugation. *Cancer Res.* **46**:5438–5443 (1986).
22. Pacifici, G. M., M. Franchi, C. Bencini, F. Repetti, N. Di Lascio, and G. B. Muraro. Tissue distribution of drug-metabolizing enzymes in humans. *Xenobiotica* **7**:849–856 (1988).
23. Ohnhaus, E. E., and R. C. Bluhm. Induction of the monooxygenase enzyme system in human lung. *Eur. J. Clin. Invest.* **17**:488–492 (1987).
24. Petruzzelli, S., A.-M. Camus, L. Carrozzi, L. Ghelarducci, M. Rindi, G. Menconi, C. A. Angeletti, M. Ahotupa, E. Hietanen, A. Aitio, R. Saracci, H. Bartsch, and C. Giuntini. Long-lasting effects of tobacco smoking on pulmonary drug-metabolizing enzymes: a case control study on lung cancer patients. *Cancer Res* **48**:4695–4700 (1988).
25. Roberts, E. A., C. L. Golas, and A. B. Okey. *Ah* receptor mediating induction of aryl hydrocarbon hydroxylase detection in human lung by binding of 2,3,7,8-[$^3$H]tetrachlorodibenzo-*p*-dioxin. *Cancer Res.* **46**:3739–3743 (1986).
26. Liem, H. H., U. Muller-Eberhard, and E. F. Johnson. Differential induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of multiple forms of rabbit microsomal cytochrome P-450: evidence for tissue specificity. *Mol. Pharmacol.* **18**: 565–570 (1980).
27. Goldstein, J. A., and D. Linko. Differential induction of two 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible forms of cytochrome P-450 in extrahepatic verses hepatic tissues. *Mol. Pharmacol.* **25**:185–191 (1984).
28. Wrighton, S. A., C. Campanile, P. E. Thomas, S. L. Maines, P. B. Watkins, G. Parker, G. Mendez-Picon, M. Haniu, J. E. Shively, W. Levin, and P. S. Guzelian. Identification of a human liver cytochrome P-450 homologous to the major isosafrole-inducible cytochrome P-450 in the rat. *Mol. Pharmacol.* **29**:405–410 (1986).
29. Sesardic, D., A. R. Boobis, R. J. Edwards, and D. S. Davies. A form of cytochrome P-450 in man, orthologous to form *d* in the rat, catalyses the *O*-deethylation of phenacetin and is inducible by cigarette smoking. *Br. J. Clin. Pharmacol.* **26**:363–372 (1988).
30. Guengerich, F. P. Characterization of human microsomal cytochrome P-450 enzymes. *Annu. Rev. Pharmacol. Toxicol.* **29**:241–264 (1989).
31. Wong, T. K., B. A. Domin, P. E. Bent, T. E. Blanton, M. W. Anderson, and R. M. Philphot. Correlation of placental microsomal activities with protein detected by antibodies to rabbit cytochrome P-450 isozyme 6 in preparations from humans exposed to polychlorinated biphenyls, quaterphenyls, and dibenzofurans. *Cancer Res* **46**:999–1004 (1986).
32. Pasanen, M., F. Stenback, S. S. Park, H. V. Gelboin, and O. Pelkonen. Immunohistochemical detection of human placental cytochrome P-450-associated mono-oxygenase system inducible by maternal cigarette smoking. *Placenta* **9**:267–275 (1988).
33. Friedman, F. K., R. C. Robinson, B.-J. Song, S. S. Park, C. L. Crespi, M. A. Marletta, and H. V. Gelboin. Monoclonal antibody-directed determination of cytochrome P-450 types expressed in a human lymphoblastoid cell line. *Mol. Pharmacol.* **27**:652–655 (1985).
34. Cheng, K.-C., H. V. Gelboin, B.-J. Song, S. S. Park, and F. K. Freidman. Detection and purification of cytochrome P-450 in animal tissues with monoclonal antibodies. *J. Biol. Chem.* **259**:12279–12284 (1984).
35. Markwell, M. K. A new solid-state reagent to iodinate proteins. I. Conditions for the efficient labeling of antiserum. *Anal. Biochem.* **125**:427–432 (1982).
36. Guenthner, T. M., and T. A. Karnezis. Multiple epoxide hydrolases in human lung. *Drug Metab. Dispos.* **14**:208–213 (1986).
37. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275 (1951).
38. Park, S. S., T. Fujino, D. West, F. P. Guengerich, and H. V. Gelboin. Monoclonal antibodies that inhibit enzyme activity of 3-methylcholanthrene-induced cytochrome P-450. *Cancer Res.* **42**:1798–1808 (1982).
39. Park, S. S., T. Fujino, H. Miller, F. P. Guengerich, and H. V. Gelboin. Monoclonal antibodies to phenobarbital-induced rat liver cytochrome P-450. *Biochem. Pharmacol.* **33**:2071–2081 (1984).
40. Howard, F. D., J. A. Ledbetter, S. Q. Medhi, and L. A. Herzenberg. A rapid method for the detection of antibodies to cell surface antigens: a solid phase radioimmunoassay using cell membranes. *J. Immunol. Methods* **38**:75–84 (1980).
41. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage $T_4$. *Nature* (*Lond.*) **227**: 680–685 (1970).
42. Towbin, H., T. Staehelin, and J. Gordon. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**: 4350–4354 (1979).
43. Domin, B. A., C. J. Serabjit-Singh, and R. M. Philpot. Quantitation of rabbit cytochrome P-450, form 2, in microsomal preparations bound to nitrocellulose paper using a modified peroxidase-immunostaining procedure. *Anal. Biochem.* **136**:390–396 (1984).
44. Kloepper-Sams, P. J., S. S. Park, H. V. Gelboin, and J. J. Stegeman. Specificity and cross-reactivity of monoclonal and polyclonal antibodies against cytochrome P-450E of the marine fish scup. *Arch. Biochem. Biophys.* **253**:268–278 (1987).
45. Greenlee, W. F., and A. J. Poland. An improved assay of 7-ethoxycoumarin *O*-deethylase activity: induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, 3-methyl-cholanthrene, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Pharmacol. Exp. Ther.* **205**:596–605 (1978).
46. Burke, M. D., and R. T. Mayer. Inherent specificities of purified cytochromes P-450 and P-448 toward biphenyl hydroxylation and ethoxyresorufin deethylation. *Drug Metab. Dispos.* **3**:245–253 (1976).
47. Nebert, D. W., and H. V. Gelboin. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. *J. Biol. Chem.* **243**:6242–6249 (1968).
48. Guenthner, T. M., M. Negishi, and D. W. Nebert. Separation of acetanilide and its hydroxylated metabolites and quantitative determination of "acetanilide 4-hydroxylase activity" by high-pressure liquid chromatography. *Anal. Biochem.* **96**:201–207 (1979).
49. Friedman, F. K., R. C. Robinson, S. S. Park, and H. V. Gelboin. Monoclonan antibody-directed immunopurification and identification of cytochromes P-450. *Biochem. Biophys. Res. Commun.* **116**: 859–865 (1983).
50. Åstrom, A., and J. W. De Pierre. Metabolism of 2-acetylaminofluorene by eight different forms of cytochrome P-450 isolated from rat liver. *Carcinogenesis* (*Lond.*) **6**:113–120 (1985).
51. Cheng, K.-C., S. S. Park, H. C. Krutzsch, P. H. Grantham, H. V. Gelboin, and F. K. Friedman. Amino-terminal sequence and structure of monoclonal antibody immunopurified cytochromes P-450. *Biochemistry* **25**:2397–2402 (1986).
52. Guengerich, F. P., G. A. Dannan, S. T. Wright, and M. V. Martin. Purification and characterization of microsomal cytochrome P-450s. *Xenobiotica* **12**:701–716 (1982).
53. DeLuca, J. G., G. R. Dysart, D. Rasnick, and M. O. Bradley. A direct, highly sensitive assay for cytochrome P-450 catalyzed *O*-deethylation using a novel coumarin analog. *Biochem. Pharmacol.* **37**:1731–1739 (1988).
54. Fujino, T., D. West, S. S. Park, and H. V. Gelboin. Monoclonal antibody-directed phenotyping of cytochrome P-450-dependent aryl hydrocarbon hydroxylase and 7-ethoxycoumarin deethylase in mammalian tissues. *J. Biol. Chem.* **259**:9044–9050 (1984).
55. Pelkonen, O., M. Pasanen, H. Kuha, B. Gachalyi, M. Kairaluoma, E. A. Sotaniemi, S. S. Park, F. K. Friedman, and H. V. Gelboin. The effect of cigarette smoking on 7-ethoxyresorufin *O*-deethylase and other monooxygenase activities in human liver: analyses with monoclonal antibodies. *Br. J. Clin. Pharmacol.* **22**:125–134 (1986).
56. Fujino, T., S. S. Park, D. West, and H. V. Gelboin. Phenotyping of cytochromes P-450 in human tissues with monoclonal antibodies. *Proc. Natl. Acad. Sci. USA* **79**: 3682–3686 (1982).
57. Hall, M., L. M. Forrester, D. K. Parker, P. L. Grover, and C.R. Wolf. Relative contribution of various forms of cytochrome P-450 to the metabolism of benzo[*a*]pyrene by human liver microsomes. *Carcinogenesis* (*Lond.*) **10**:1815–1821 (1989).
58. Negishi, M., and D. W. Nebert. Structural gene products of the *Ah* locus: genetic and immunochemical evidence for two forms of mouse liver cytochrome P-450 induced by 3-methylcholanthrene. *J. Biol. Chem.* **254**:11015–11023 (1979).
59. Gonzalez, F. J., R. H. Tukey, and D. W. Nebert. Structural gene products of the *Ah* locus: transcriptional regulation of cytochrome $P_1$-450 and $P_3$-450 mRNA levels by 3-methylcholanthrene. *Mol. Pharmacol.* **26**:117–121 (1984).
60. Song, B. J., H. V. Gelboin, S. S. Park, G. C. Tsokos, and F. K. Friedman. Monoclonal antibody-directed radioimmunoassay detects cytochrome P-450 in human placenta and lymphocytes. *Science* (*Washington D.C.*) **228**:490–492 (1985).

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