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Detection of human lung cytochromes P450 that are immunochemically related to cytochrome P450IIE1 and cytochrome P450IIIA

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Abstract—We have used monoclonal antibodies that were prepared against and specifically recognize human hepatic cytochromes P450 as probes for solid phase radioimmunoassay and Western immunoblotting to directly demonstrate the presence in human lung microsomes of cytochromes P450 immunochemically related to human liver cytochromes P450IIE1 (CYP2E1) and P450IIIA (CYP3A). The detected levels of these cytochromes are much lower than levels in human liver microsomes, but similar to the levels seen in microsomes from untreated baboon lung. Proteins immunochemically related to two other constitutive hepatic cytochromes P450, cytochrome P450IIC8 (CYP2C8) and cytochrome P450IIC9 (CYP2C9), were not detectable in lung microsomes.

The characterization of cytochrome P450 in the human lung, and particularly of individual cytochromes P450, is of interest to many pharmacologists and toxicologists because of the role these enzymes play in the bioactivation of numerous xenobiotics to cytotoxic or mutagenic electrophiles [1–5]. However, in contrast to the relatively detailed knowledge concerning individual cytochromes P450 in animal lung, individual forms in the human lung have not been extensively characterized until recently. Immunochemical and metabolic studies have identified a CYP1A1* species in human lung microsomes [7]. Antibodies that recognize rat liver CYP2B1, CYP2B2 and CYP1A2, and human liver CYP3A3 were used to seek immunochemically related forms in human lung microsomes; in all cases, no related cytochrome was

* Individual cytochromes P450 are abbreviated using the "CYP" nomenclature suggested by Nebert *et al.* [6]. In all cases, these abbreviations refer to enzyme protein, and not to genetic loci.

Table 1. Radioimmunoassay of individual cytochromes in lung and liver microsomes

Sample No.*	Smoking status†	CPM in RIA‡ K03 (CYP2E1)	2F7 (CYP3A)	6H5 (CYP2C9)	4D4 (CYP2C8)
7	+	470 ± 40§	960 ± 50§	170 ± 9	130 ± 20‖
10	+	490 ± 20§	1020 ± 110§	210 ± 10§	150 ± 30§
11	+	390 ± 10§	790 ± 20§	200 ± 10‖	120 ± 8
18	−	450 ± 40§	1070 ± 20§	160 ± 20	80 ± 10
20	+	470 ± 20§	820 ± 40§	150 ± 4	70 ± 20
21	+	500 ± 60§	980 ± 40§	220 ± 20§	140 ± 30‖
22	n.r.	440 ± 50§	1140 ± 30§	180 ± 20‖	90 ± 10
23	+	360 ± 20§	760 ± 30§	240 ± 30§	180 ± 10§
Baboon lung	−	500 ± 40§	1280 ± 50§	190 ± 20‖	110 ± 10
Blank		180 ± 10	210 ± 10	140 ± 20	70 ± 10
Human lung (mean − blank; N = 8)		260	730	60	50
Human liver (− blank)		2480	9600	350	1880

* Patient identifier.

† Reported by patient at time of surgery. Key: (+) smoker, (−) nonsmoker, and n.r. not reported.

‡ Counts per minute (mean ± SD) were calculated from triplicate determinations for each sample (5 μg protein). Blank cpm, obtained by substituting 10% newborn calf serum in phosphate-buffered saline for microsomal protein, have not been subtracted.

§ Sample cpm significantly different from blank, $P < 0.01$.

‖ Sample cpm significantly different from blank, $P < 0.05$.

detected [7–9]. CYP1A1 mRNA has been detected in lung tissue from cigarette smokers, but not in tissue from nonsmokers [10]. Oligonucleotide probes have identified cDNA sequences in human lung that code for CYP4B1 and CYP2F1 [11, 12]. In this paper, we report the detection of two additional cytochromes P450 in human lung microsomes. We have employed monoclonal antibodies that were raised to and specifically recognize four human liver cytochromes P450 to perform solid phase radioimmunoassay and Western immunoblotting. We have also compared the levels of the forms detected in human lung microsomes to levels of the same cytochromes detected in human liver and baboon lung.

Methods

Human lung samples 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. The human liver sample was surgical waste from a biopsy for the diagnosis of metastatic malignant melanoma. Only samples remote from any tumor site and free of macroscopically or microscopically detectable cancer were used. Baboon lung was obtained from an animal euthanized 6–8 weeks after recovery from experimental lumbar disc surgery. This animal was maintained on a normal diet of commercial monkey chow and was not exposed to any additional drugs or environmental chemicals. Microsomes were prepared as previously described [7, 13].

Monoclonal antibodies 4D4, 6H5, KO3, and 2F7, which were prepared against and specifically recognize, respectively, human liver CYP2C8 (HLx), CYP2C9 (P450MP), CYP2E1 (HLj), and CYP3A* (HLp), were characterized as previously described [14]. Solid phase radioimmunoassays and immunoblotting were carried out by previously described methods [7].

Results and Discussion

Table 1 shows the results of radioimmunoassay of microsomes from human and baboon lung and human liver. Proteins reactive with antibodies KO3 and 2F7 were detected in all eight human lung samples tested. According to previous characterization of these antibodies [14], the proteins that were recognized are thus immunochemically related to human liver CYP2E1 and CYP3A, respectively. Related cytochromes were also detected in baboon lung microsomes. In the case of antibody 4D4, which specifically detects CYP2C8, four samples produced values that were significantly different, at the $P = 0.05$ level, from cpm obtained from blank incubations, and four samples produced values that were not different from the blank cpm. Only two samples were different from the blank at the $P = 0.01$ level. In the case of antibody 6H5, which specifically detects CYP2C9, five lung samples produced cpm that were significantly different from blank values at the $P = 0.05$ level, while three did not. Only three samples were different from the blank at the $P = 0.01$ level. For baboon lung, the cpm obtained with antibody 6H5 were different from blank cpm ($P < 0.05$), while cpm obtained with antibody 4D4 were not. All four antibodies detected high levels of cytochromes in human liver microsomes. CYP2C8 and CYP2C9 are known to be primarily hepatic, and not extrahepatic, cytochromes [6, 14]. However, the fact that they were not definitively detected in whole lung homogenates by this method does not prove their absence from human lung. Given the cellular heterogeneity of the

* Human liver CYP3A3, CYP3A4, and CYP3A5 are highly homologous, and all three are recognized by this antibody. Therefore, cytochromes detected by this antibody are referred to as CYP3A without implication of specificity for any one of the three forms.

lung, it is not possible at present to conclude whether or not these two cytochromes may be expressed in any subpopulation of human lung cells.

Figure 1 shows immunoblot analysis of human lung and liver, and baboon lung, microsomes. Positive staining bands were obtained for human liver microsomes with all four antibodies. Staining of lung microsomal proteins was more difficult to analyze. In the case of antibody KO3, lightly stained bands, indicated by arrows, were detected with both human and baboon lung proteins. Antibody 2F7 produced a band with one human lung microsome sample that was faintly visible on the transfer membrane strip, but was not photographically reproduced. Two other human lung microsomes samples, as well as the baboon lung microsome sample, produced no visible band in reaction with antibody 2F7. In the case of antibodies 6H5 and 4D4, no bands were visible in any blots of either human or baboon lung microsomes. Rabbit lung microsomes were also tested with all four antibodies; none produced any visible bands (data not shown). Because the antibodies that we have employed may have different specific binding affinities for their respective antigens, the data presented here should not be used to directly compare the relative levels of one or more different P450 species in the same tissue sample. The data do not necessarily indicate that CYP3A is present at a higher specific content than CYP2E1. Nor can the densities of immunoreactive bands be used to directly compare levels of two specific cytochromes; the corresponding antibodies may bind much less well to sodium dodecyl sulfate (SDS)-denatured proteins than they do to microsomal proteins *in situ*. This may explain why antibody 2F7 yielded positive radioimmunoassay results, but negative immunoblots with human and baboon lung microsomes.

The two cytochromes whose expression has been detected may be toxicologically important to the lung in that they participate in the biotransformation and activation to toxic or carcinogenic species of a number of inhaled environmental toxicants. CYP2E1 metabolizes a number of volatile solvents, and is also inducible by acetone, benzene, and ethanol [15–20]. CYP3A4 has been implicated as the primary form responsible for diol-epoxide formation in human liver [21]. Human lung microsomes can activate benzo[*a*]pyrene 7,8-dihydrodiol to a mutagenic species by a mechanism that is not inhibitable by 7,8-benzoflavone (thus presumably not CYP1A1-dependent) [21]. It is possible that this activity reflects the presence in human lung of the CYP3A species identified by our monoclonal antibody. The demonstration of the presence of a CYP3A-related form in human lung is significant in that it shows that both forms known to be responsible for polycyclic aromatic hydrocarbon activation to diol-epoxides, that is, CYP1A1 and CYP3A4, may reside in the human lung.

The levels in baboon lung microsomes of the individual cytochromes studied here were similar to the levels in human lung microsomes. This is further evidence that baboon, and possibly other primate species, are probably far better models for human pulmonary metabolic and toxicity studies than are rabbit or rat; the mean amount of total cytochrome P450 per mg of microsomal protein was 1.23 ± 0.92 pmol/mg for human lung, and 1.9 pmol/mg for baboon lung. Average total P450 content of the human lung samples is, therefore, only 0.55 and 3.7% of the contents of rabbit and rat lung, respectively [22, 23]. Furthermore, significant differences for either total P450 levels, or levels of individual cytochromes, were not detected when the baboon lung sample (taken from an animal that was not exposed to known dietary or inhaled

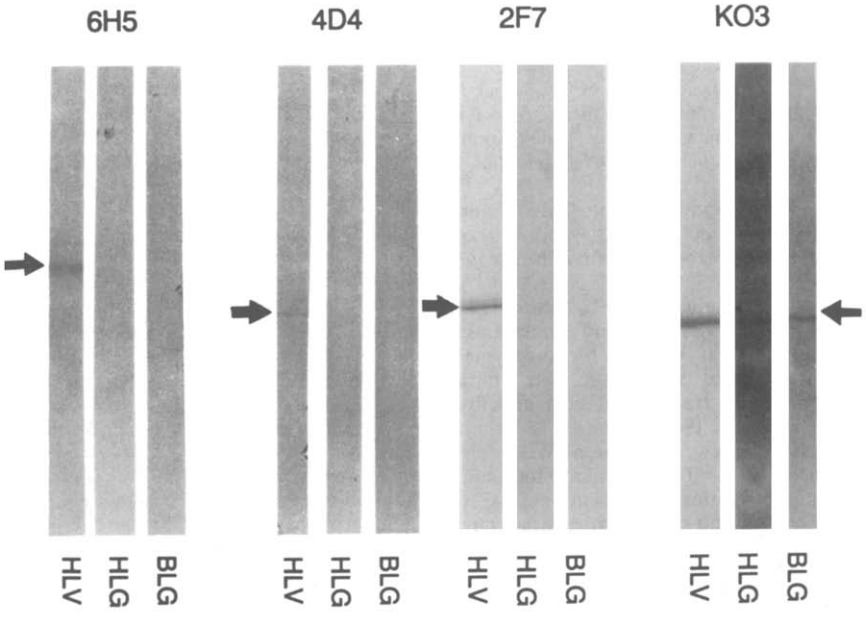

Fig. 1. Immunoblot analysis of human lung and liver, and baboon lung, microsomes with monoclonal antibodies to specific human liver cytochromes P450. A 75–150 μg sample of microsomal protein was analyzed after electrophoretic separation, using 2 mL of a 100/1 dilution of the indicated ascites fluid in isotonic buffered saline. Abbreviations: HLV, human liver microsomes; HLG, human lung microsomes; and BLG, baboon lung microsomes. Arrows indicate the positions of stained bands on the transfer membranes.

inducing agents) was compared to the human sample population, which contained lungs from both smokers and nonsmokers.

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