0026-895X/92/010076-07$03.00/0

MOLECULAR PHARMACOLOGY, 41:76-82

Two Monoclonal Antibodies Recognizing Different Epitopes on Rat Cytochrome IIB1 React with Human IIE1

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Received July 10, 1991; Accepted October 14, 1991

SUMMARY

To identify human cytochromes P450 (P450) in the CYP2B subfamily, 14 human liver microsomal samples were screened by immunoblots developed with monoclonal antibodies that recognized seven distinct epitopes on rat IIB1. Two of these antibodies recognized a protein in all of the samples. This protein was termed P450BE. Using video-imaging densitometry, the levels of P450BE were determined and compared with levels of other P450s. An excellent correlation was seen ($r = 0.87$) between P450BE and human IIE1. However, rat IIE1 did not react in immunoblot and enzyme-linked immunosorbant assays with the two anti-rat IIB1 monoclonal antibodies. As previously observed, the levels of IIE1 in the samples correlated well ($r = 0.88$) with the ability of these human liver microsomes to *N*-demethylate *N*-nitrosodimethylamine. The levels of P450BE also correlated well ($r = 0.91$) with the ability of the microsomes to *N*-demethylate *N*-nitrosodimethylamine. In addition, excellent correlations were obtained when the levels of P450BE and IIE1 were compared with the ability of the microsomes to *O*-deethylate ethoxycoumarin ($r = 0.87$ and $r = 0.85$, respectively). To identify the protein recognized by the anti-rat IIB1 antibodies, P450BE was purified from microsomes prepared from human liver D. Amino-terminal amino acid sequence analyses of P450BE revealed that the 18-amino acid sequence obtained matched the corresponding sequence of human IIE1. In addition, purified human IIE1 and P450BE migrated with the same apparent molecular weight in polyacrylamide gels. Furthermore, proteolytic maps of P450BE and IIE1, generated with two proteases, were found to be identical. Sequence alignments and antigenicity calculations identified three regions of rat IIB1 as likely candidates for the epitopes shared in common with human IIE1. In conclusion, this study indicates that caution must be taken when interpreting the results of immunochemical assays when species lines are crossed.

The P450s are a multigene family of enzymes that are the terminal oxidases of the mixed function oxidase system. The hepatic P450s comprising the first three families of the P450s are primarily responsible for the conversion of lipophilic endobiotics (e.g., prostagladins, steroids, and fatty acids) and xenobiotics (e.g., drugs and pesticides) to more hydrophilic metabolites that are readily eliminated from the body. The evolution of this superfamily of oxidative enzymes appears to have involved gene duplication, divergence, and convergence events (1). Interindividual differences in the forms and levels of the P450s expressed influence the toxicologic and pharmacologic response of humans to drugs and toxicants. In addition, interspecies differences in the regulation of expression and the metabolic capabilities of the P450s are responsible for species-specific metabolism of drugs and other xenobiotics.

Among the most widely studied P450s are the major forms of P450 induced by phenobarbital. These P450s comprise the *CYP2B*[1] gene subfamily. In the rat, phenobarbital treatment induces the expression in the liver of two forms of P450, only one of which is expressed in the untreated animal (2–4). In rabbits and mice, however, only one form is highly induced in the liver by phenobarbital treatment (2–4). Phenobarbital treatment has been shown to induce the accumulation of CYP2B subfamily members primarily by increasing gene transcription (2–4). Several human proteins have been shown to cross-react with antibodies prepared against CYP2B proteins isolated from the experimental species (5, 6). In addition, molecular biologic techniques have been used to identify and characterize a human gene, referred to as *CYP2B6*, that codes for a protein structurally related to rat IIB1 (5–7). However, whether the human *CYP2B6* gene is expressed at the protein level has not been positively demonstrated (5–7). In fact, unlike what has been found in the rat, where the expression of the *CYP2B* genes has been shown to be transcriptionally controlled (1, 2), no correlation was observed by Yamano *et al.* (5) between

This research was supported in part by National Institutes of Health Grant GM-38261 to S.D.B.

[1] Nomenclature used for the P450s is that of Nebert *et al.* (30).

ABBREVIATIONS: P450, cytochrome P450; SDS, sodium dodecyl sulfate.

CYP2B6 mRNA levels and the level of a protein recognized by a rabbit anti-rat IIB1 antibody in 13 human liver specimens.

In an effort to identify a P450(s) in the human CYP2B subfamily, immunoblots of human liver microsomes were developed with monoclonal antibodies that have been shown to recognize seven distinct epitopes on rat IIB1 (8). Two of these monoclonal antibodies recognized a P450 in each of the 14 human liver specimens examined. This P450 was isolated and characterized and found not to be a human IIB subfamily member. Surprisingly, the two monoclonal antibodies to different epitopes on rat IIB1 recognized human IIE1. The results of this study illustrate the potential problems associated with using an immunochemical probe generated against a P450 of one species to phenotype the P450s of another species.

Experimental Procedures

Materials. Cholic acid, Tergitol NP-10, ethoxycoumarin, pentoxyresorufin, *N*-nitrosodimethylamine, papain, and *Staphylococcus aureus* V8 were purchased from Sigma Chemical Co. (St. Louis, MO). Electrophoresis and immunoblot reagents and hydroxylapatite (Bio-Gel, HTP) were purchased from Bio-Rad (Richmond, CA). Sepharose 4B-CL and CNBr-activated Sepharose 4B were obtained from Pharmacia (Piscataway, NJ). Whatman DE51, DE52, DE53, and CM52 celluloses were purchased from Bodman (Doraville, GA).

Liver specimens. The human liver specimens were obtained from the Liver Transplant Unit at the Medical College of Wisconsin, under a protocol approved by the Committee for the Conduct of Human Research. Livers were also obtained from male Fischer 344 rats that were treated with phenobarbital as previously described (9). Microsomes were prepared from the various liver specimens by differential centrifugation (10).

Antibody preparation and immunoblot analysis. Monoclonal antibodies BE26, BEA33, BE28, BEF29, BE46, B50, and B51 were produced and characterized as previously described by Reik *et al.* (8), from mice immunized with rat IIB1. In addition to recognizing rat IIB1, BEA33 and BEF29 have been shown to react with rat P450IIA1 and P450IIC7, respectively.

Antibodies that specifically recognize the human P450s IIE1, IA2, IIC8, IIC9, or IIA were prepared and characterized as previously described (11, 12). Antiserum recognizing P450IID6 was graciously supplied by Dr. Christine Hunt (Duke University, Durham, NC). Immunoblots were performed as described (9).

Purification of human P450BE. All procedures were carried out at 4°, unless otherwise noted. Liver microsomes prepared from specimen D (4732 mg of protein) were solubilized and applied to aminooctyl-Sepharose 4B-CL columns as previously described (13). The columns were washed (1 liter each) with equilibration buffer (13) containing 0.42% cholate, and P450BE was eluted with equilibration buffer containing 0.33% cholate and 0.06% Tergitol NP-10. The 417-nm-absorbing material eluted by this buffer was monitored for relative purity by silver-stained SDS-polyacrylamide gels and for P450BE by immunoblot analyses developed with monoclonal antibody BE26. The majority of P450BE was found to elute in the latter half of the peak of 417-nm-absorbing material, and these fractions were combined. The combined fraction was concentrated to one tenth its original volume, in a Amicon ultrafiltration unit equipped with a PM30 membrane.

The concentrated fraction was then dialyzed and applied to a DE51, DE52, and DE53 column series, as previously described (13). The column series was washed with 300 ml of dialysis buffer (13) and then 500 ml of buffer B (5 mM potassium phosphate buffer, pH 7.7, containing 20% glycerol, 0.1 mM EDTA, and 0.2% Tergitol NP-10). Immunoblot analyses of the 417-nm-absorbing material eluted by buffer B indicated that P450BE was present. Those fractions containing P450BE with a high relative purity, determined by silver-stained SDS-polyacrylamide gels, were combined.

The fraction from the DEAE columns was then dialyzed against 2 liters of 10 mM potassium phosphate buffer (pH 6.5) containing 20% glycerol, 0.1 mM EDTA, and 0.2% Tergitol NP-10, with two changes over 48 hr. The dialyzed fraction was applied to a CM-cellulose column (1.6 × 5 cm) equilibrated with 100 ml of dialysis buffer, at 1 ml/min. The column was then washed with 125 ml of dialysis buffer and 417-nm-absorbing material was eluted through the use of a 250-ml 0–150 mM NaCl gradient in dialysis buffer. One peak of 417-nm-absorbing material eluted with the gradient, and it was found to contain P450BE by immunoblot analyses and silver-stained SDS-polyacrylamide gels. However, by using immunoblot analyses, this fraction was also shown to contain trace amounts of IIIA3/4.

In order to remove the trace amounts of IIIA3/4 in the combined fractions from the CM-cellulose column, immunoabsorption chromatography was performed. The column material was prepared by the method of Thomas *et al.* (14), starting with 180 mg of rabbit anti-IIIA3/4 IgG. The CM-cellulose fraction was dialyzed overnight against 1 liter of 10 mM potassium phosphate buffer containing 20% glycerol, 0.1 mM EDTA, and 0.2% Tergitol NP-10. Solid KCl was then added to a final concentration of 0.2 M. The sample was then applied at room temperature to the immunoaffinity column (1.6 × 8 cm), which had been equilibrated with 3 column volumes of dialysis buffer containing 0.2 M KCl, at 0.1 ml/min. The resultant flow-through fraction was found to contain only P450BE, as determined by immunoblots developed with both BE26 and rabbit anti-IIIA3/4 antibodies and silver-stained SDS-polyacrylamide gels. The detergent was removed from P450BE through the use of a small hydroxylapatite column (12).

Enzyme assays. The rate of *N*-demethylation of *N*-nitrosodimethylamine (1 mM final concentration) was determined using the Nash reaction (15). Ethoxycoumarin *O*-deethylase activity was determined by the method of Greenlee and Poland (16) and pentoxyresorufin *O*-dealkylase activity was determined as described by Lubet *et al.* (17).

Protein biochemistry. For automated amino-terminal amino acid analyses, P450BE was electroblotted onto a polyvinylidene difluoride membrane, as described by Matsudaira (18). Sequence analyses were performed on a gas-phase sequencer (Applied Biosystems Model 470A). Limited proteolytic digestion of the P450s was performed by the method of Cleveland *et al.* (19). SDS-polyacrylamide gel (10%) electrophoresis was performed as described by Laemmli (20), and gels were stained according to the manufacturer's directions (Bio-Rad). Protein concentrations were determined colorimetrically, as described (21).

Sequence alignments and computational procedures. The sequences of the P450s were retrieved from the Protein Information Resource database (22), release 28.0 (30,367 entries). The accession codes are as follows: rat IIB1(O4RTPB), rat IIB2 (A21162), rat IIA1 (A29560, A34272), rat IIE1 (A25341, A28145), and human IIE1 (A31949, B25341). Alignments of putative epitope peptides were accomplished with the aid of the Pearson and Lipman algorithm, FASTA version 1.4c (23), scanning the entire Protein Information Resource database. Alignments were scored with respect to identities and conservative substitutions and with reference to the potential antigenicity of the peptide segment. Antigenicity analyses were carried out with MSEQ software (24, 25) and the antigenicity parameters of Welling *et al.* (26).

Results and Discussion

In an attempt to identify a human P450(s) related to rat IIB1, murine monoclonal antibodies recognizing seven different epitopes (BE26, BEA33, BE28, BEF29, BE46, B50, and B51) on rat IIB1 (8) were used to develop immunoblots of human liver microsomes. Only two of these monoclonal antibodies, BE26 and BEA33, were found to recognize proteins in human liver microsomes. As shown in Fig. 1, monoclonal antibody BE26 recognized a single protein and BEA33 reacted with two proteins in human liver microsomes. The slower migrating protein recognized by BEA33 migrated in SDS-polyacrylamide

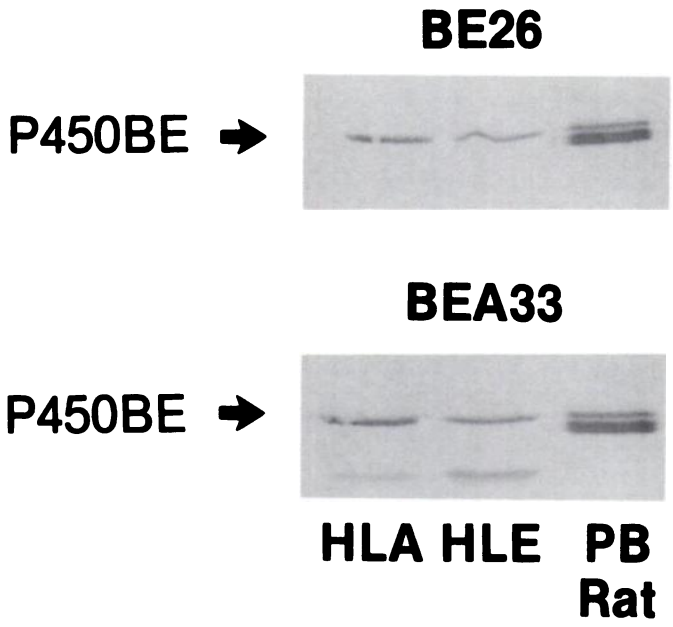

Fig. 1. Immunoblots of liver microsomes developed with monoclonal antibodies BE26 and BEA33. Immunoblot analyses were performed as described in Experimental Procedures, using microsomes from human livers A or E or livers from phenobarbital-treated rats. The immunoblots were developed with monoclonal antibodies BE26 (*top*) or BEA33 (*bottom*).

gels at the same apparent molecular weight as the protein recognized by BE26 (Fig. 1), suggesting that these antibodies were recognizing the same protein. Two polyclonal antibody preparations against rat IIB1 were also used to develop immunoblots of human liver microsomes. These polyclonal preparations recognized several proteins in the human liver microsomes, despite recognizing only IIB1 and IIB2 in microsomes from phenobarbital-treated rats (data not shown). The protein containing two epitopes similar to rat IIB1, as determined by its reactivity with both BE26 and BEA33, was tentatively termed P450BE. The faster migrating protein recognized by BEA33 (Fig. 1) appears to be related to IIA6.[2]

The relative levels of P450BE in microsomes isolated from 14 human livers were determined by video-imaging densitometry of immunoblots developed with the BE26 monoclonal antibody. The relative levels of P450BE were found to vary by about 8-fold in these human microsomal samples (Table 1). The levels of P450BE could not be associated with the age, gender, smoking habits, or drug history of the patients (Table 1). This is of particular interest because patients E and I were administered barbiturates, which are known inducers of the rat IIB subfamily. The levels of phenobarbital-inducible human IIIA3/4 were found to be elevated in the two microsomal specimens obtained from the patients administered barbiturates, in comparison with the levels of IIIA3/4 in the remaining 12 specimens (Table 1).

The relative levels of P450BE were also compared with the levels of several other human P450s, including IA2, IIC8, IIC9, IID6, IIE1, and total IIIA (Table 1). Surprisingly, an excellent correlation (r = 0.87) was observed between the microsomal levels of P450BE and IIE1 (Table 1). This same strong correlation was also observed between P450BE and IIE1 when monoclonal antibody BEA33 was used to determine microsomal levels of P450BE (data not shown). These data suggest that the human P450 recognized by the monoclonal antibodies to two different epitopes on rat IIB1 is highly related to human IIE1. Alternatively, the expression of the human P450 related to the phenobarbital-inducible rat IIB1 is regulated in a fashion very similar to that of human IIE1.

In order to further investigate the relationships between P450BE, human IIE1, and rat IIB1, metabolic assays indicative of IIE1 (*N*-nitrosodimethylamine *N*-demethylation) and rat IIB1 (pentoxyresorufin *O*-dealkylation) were performed using the various human liver microsomal samples. As has been previously demonstrated (27), the relative levels of IIE1 were found to correlate well (r = 0.88) with the ability of the microsomes to *N*-demethylate *N*-nitrosodimethylamine (Tables 1 and 2). In addition, the levels of P450BE correlated well (r = 0.91) with the ability of microsomes to *N*-demethylate *N*-nitrosodimethylamine. Rat IIB1 has been shown to be the form of P450 responsible for the *O*-dealkylation of pentoxyresorufin (17). However, the ability of the various human liver microsomal samples to *O*-dealkylate pentoxyresorufin did not correlate with their levels of P450BE or any of the other human P450s quantified in Table 1.

Yamano *et al.* (5) screened a human liver cDNA library with a rat IIB1 cDNA and isolated a human cDNA that was found to be 76% related to rat IIB1 by deduced amino acid sequence. When the human IIB cDNA was cloned into an expression system, the expressed P450 was found to *O*-deethylate ethoxycoumarin (5). Wrighton *et al.* (27) have shown that purified human IIE1 and rat IIE1 in reconstituted systems actively *O*-deethylate ethoxycoumarin. Human liver microsomes were found to *O*-deethylate ethoxycoumarin (Table 2), and this activity correlated well with the levels of both P450BE (r = 0.87) and human IIE1 (r = 0.85). Furthermore, the ethoxycoumarin *O*-deethylase activity of microsomes isolated from human liver was inhibited in a dose-dependent manner, to a maximum of about 70% inhibition, by rabbit anti-human IIE1 antibodies (data not shown). These observations indicate that, in human liver microsomes, IIE1 catalyzes not only the *N*-demethylation of *N*-nitrosodimethylamine but also the majority of the *O*-deethylation of ethoxycoumarin. In addition, the P450 recognized in human liver microsomes by the two monoclonal antibodies against rat IIB1, P450BE, appears to be highly functionally related to human IIE1.

In an effort to identify definitively the form of P450 recognized by the BE26 and BEA33 monoclonal antibodies, P450BE was purified from human liver microsomes prepared from specimen D. Sequential chromatography of cholate-solubilized microsomes on octylamino-Sepharose 4B-CL, DEAE-cellulose, and CM-cellulose columns was used to purify P450BE. The various fractions containing 417-nm-absorbing material generated by these procedures were monitored by immunoblot analyses developed with the BE26 monoclonal antibody and for relative purity by silver-stained SDS-polyacrylamide gels. The immunoblot analyses demonstrated that the combined fraction after CM-cellulose chromatography was composed primarily of P450BE, with IIIA3/4 being a minor contaminant. Preliminary experiments using combinations of various classical chromatographic media failed to resolve the P450BE from the contaminating IIIA3/4. Therefore, immunoabsorption chromatography was performed using rabbit anti-IIIA3/4 IgG

[2] S. Wrighton, unpublished observations.

TABLE 1

Patient histories and immunoquantification of human P450BE

Immunoquantifications of the human liver P450BE and other P450s were performed as described in Experimental Procedures. The densitometric value obtained for specimen A was arbitrarily set at 100%.

Patient code	Relative levels							Gender	Age	Smoking habits	Drug history
	P450BE	IIE1	IA2	IIC8	IIC9	IID6	IIIA[a]				
A	100	100	100	100	100	100	100	M	25	75[b]	None
B	28	80	74	106	123	371	110	M	50	35[b]	None
C	133	154	72	209	130	338	118	M	22	UK[c]	Ethanol (0.25%) (unspecified drug abuse)
D	151	173	30	84	133	431	73	M	31	NS[d]	None
E	66	74	38	196	72	341	262	M	14	NS	Pentobarbital (coma induced 1 week before death), pancuronium bromide, dopamine, furosemide, mannitol, heparin, cefazolin
F	90	64	64	167	77	325	192	F	50	Heavy	Alcoholic; insulin
G	39	29	80	87	101	361	142	F	48	UK	Teldrin
H	85	73	94	46	93	184	73	F	28	UK	None
I	154	147	53	149	217	302	315	M	43	NS	Phenobarbital, phenytoin, propranolol
J	19	54	65	230	265	291	121	F	55	UK	None
K	85	115	57	BLQ[e]	124	BLQ	92	M	23	UK	Ethanol (0.056%), dopamine
L	42	61	45	BLQ	55	127	66	F	58	UK	Dopamine, mannitol, vasopressin, acetominophen
M	62	88	33	BLQ	88	325	86	M	18	UK	Ethanol (0.273%)
N	90	87	96	127	81	BLQ	98	M	21	UK	Dopamine, vasopressin, dobutamine, vecuronium, ethanol (0.087%)

[a] Total level of P450IIIA3, IIIA4, AND IIIA5.
[b] Values denotes pack-years, which are defined as the number of packages of cigarettes per day × years smoked.
[c] UK, unknown.
[d] NS, nonsmoker.
[e] BLQ, below limit of quantification.

TABLE 2

***N*-Nitrosodimethylamine (NDMA) *N*-demethylase, ethoxycoumarin *O*-dealkylase, and pentoxyresorufin *O*-dealkylase activities of human liver microsomes**

Catalytic activities were determined as described in Experimental Procedures.

Patient code	NDMA *N*-demethylase	Ethoxycoumarin *O*-dealkylase	Pentoxyresorufin *O*-dealkylase
	nmol of product/mg of protein/min	*nmol of product/mg of protein/min*	*pmol of product/mg of protein/min*
A	0.78	0.50	0.81
B	0.48	0.40	0.60
C	1.04	0.81	1.01
D	1.12	0.86	0.87
E	0.51	0.73	1.10
F	0.53	0.53	1.00
G	0.33	0.26	0.55
H	0.64	0.50	0.82
I	1.37	0.91	0.79
J	0.31	0.28	0.87
K	0.53	0.58	0.96
L	0.50	0.25	0.51
M	0.61	0.42	0.42
N	0.52	0.42	1.10

bound to CNBr-activated Sepharose 4B. The CM-cellulose fraction was applied to the immunoaffinity resin, and the resulting flow-through fraction was found to be homogeneous, as determined by silver-stained SDS-polyacrylamide gels (Fig. 2) and its reaction with BE26 or BEA33 and not anti-IIIA3/4 in immunoblot analyses (Fig. 3). Thus, the highly specific measures used to select the fractions containing P450BE generated by the various chromatographic procedures resulted in a preparation of P450BE that was homogeneous (Fig. 2), albeit with very poor overall yield (4.6 nmol of P450).

As shown in Fig. 2, purified P450BE migrated in SDS-polyacrylamide gels at the same apparent molecular mass, 54 kDa, as did human IIE1, which was purified as previously described (27). In addition, the monoclonal antibodies BE26 and BEA33 to rat IIB1 recognized both purified P450BE and human IIE1 (Fig. 4). Equally important is the finding that neither of these antibodies recognized rat IIE1 in immunoblot (Fig. 4) or enzyme-linked immunosorbant assays (8). Furthermore, the same preparation of anti-rat IIE1 previously used in the immunoaffinity purification of human IIE1 (28) recognized not only human and rat IIE1 but also P450BE (Fig. 4). Amino-terminal amino acid sequence analysis is one of the most widely accepted methods used to determine the identity of closely related P450s. The amino-terminal sequence obtained for P450BE (S-A-L-G-V-T-V-A-L-X-V-W-A-A-F-L-L-L-X-S) was found to match exactly that previously reported by Wrighton *et al.* (27) for the same positions in human IIE1. Another method often used to determine whether the structures of proteins are similar is peptide mapping after limited proteolysis. As shown in Fig. 5, the proteolytic maps of P450BE and human IIE1 generated with two proteases and detected by immunoblots developed with BE26 or rabbit anti-human IIE1 are identical. Therefore, these protein biochemical characterizations demonstrate that the P450 tentatively identified as P450BE is human IIE1.

The observed immunochemical findings were rather unexpected, but these data do indicate clearly that two epitopes in rat IIB1 and IIB2 (and one in IIA1 for BEA33) must be shared with human IIE1 but not rat IIE1. Thus, specific regions of the primary structures of the IIB and IIA forms must be essentially

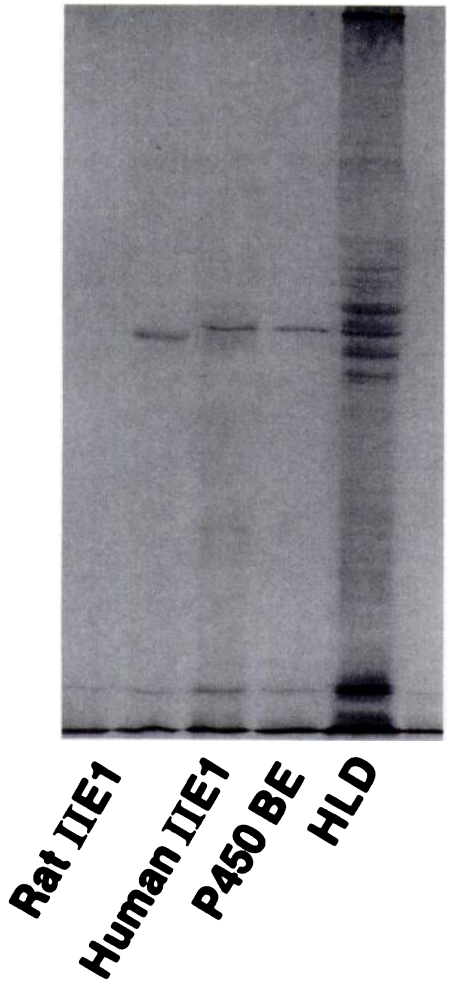

Fig. 2. Electrophoretic profile of rat IIE1, human IIE1, P450BE, and microsomes from human liver specimen D (*HLD*). The purified proteins (0.5 μg each) and microsomes from human liver specimen D (5 μg) were prepared, subjected to SDS-polyarylamide gel electrophoresis, and visualized by silver staining, as described in Experimental Procedures.

identical to the heterologous human IIE1 but dissimilar to rat IIE1. Sequence alignments of these P450s showed that three regions satisfied these general criteria, thereby suggesting that the epitopes in question may be of a linear character. According to the sequence and numbering of IIB1, these three regions are located at T_{255}LDPSAPR (region A), F_{377}RGYLLPK (region B), and P_{428}FSTGKR (region C). Antigenicity calculations showed these regions to be the first, ninth, and sixth most antigenic in the primary structure, respectively. Regions A and B should be exposed loops in the tertiary structure, whereas region C should be buried near the heme[3]; this observation would favor regions A and B as the epitopes of the monoclonal antibodies BE26 and BEA33. However, assignment of sequence to specific epitopes is premature as yet. Future competitive enzyme-linked immunosorbant assays with the appropriate synthetic peptides should readily establish the specific location of the epitopes. In this connection, note that three regions at L_{157}RKSQGAP, F_{195}LRL, and F_{223}LKY were weak matches for epitopes but will, nonetheless, be examined.

In conclusion, using two monoclonal antibodies that recognize different epitopes on rat IIB1 (8), a human microsomal

[3] S. D. Black and X. Wu, unpublished observations.

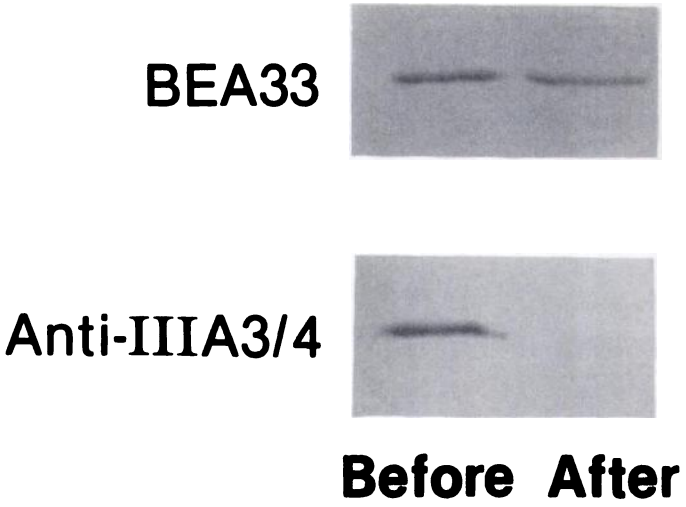

Fig. 3. Immunoblots of the CM52 fraction containing P450BE, before and after immunoabsorption. Immunoblot analyses of the CM52 fraction containing P450BE, before (*left lane*) (2 μg) and after (*right lane*) (2 μg) immunoabsorption, were performed and developed with monoclonal antibody BEA33 (*top*) or rabbit anti-IIIA3/4 (*bottom*), as described in Experimental Procedures.

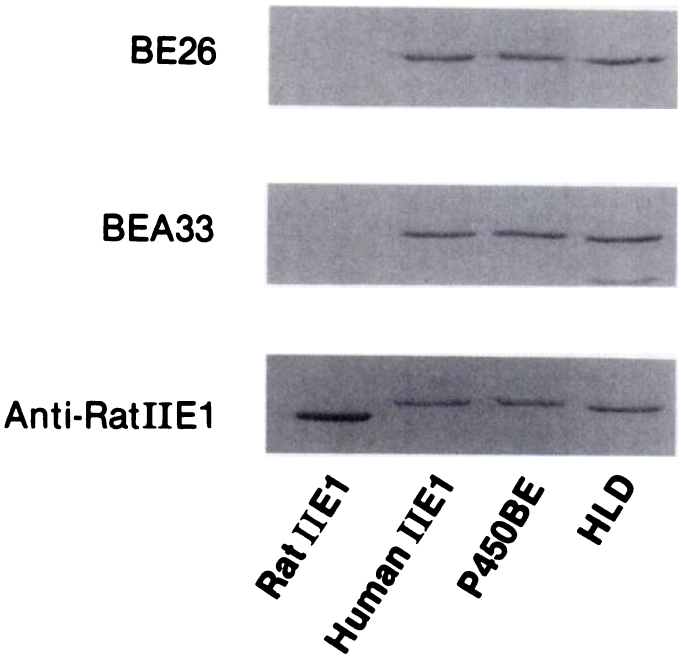

Fig. 4. Immunoblots of rat IIE1, human IIE1, P450BE, and microsomes from human liver D (*HLD*). Immunoblot analyses of the purified proteins (1 μg each) and microsomes from human liver sample D (35 μg) were performed and developed with monoclonal antibodies BE26 (*top*) or BEA33 (*middle*) or rabbit anti-rat IIE1 IgG (*bottom*), as described in Experimental Procedures.

P450 was identified and characterized. The data on the relative levels of the P450 recognized by these antibodies, its catalytic activities, and its structural characteristics combine to demonstrate that P450BE is not a member of the human CYP2B subfamily but instead is human IIE1. One possible explanation for this observation is that, during the evolution of the CYP2 family from a common ancestor, the genes for human CYP2E1 and rat CYP2B1 have conserved a structural unit(s) that is recognized by monoclonal antibodies BE26 and BEA33, whereas the other members of the family have not. An extensive

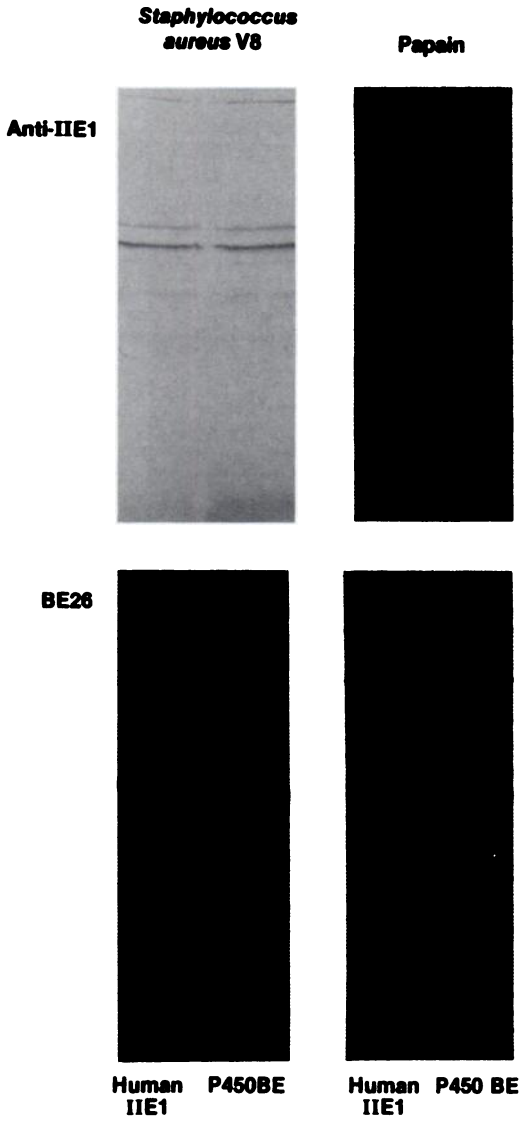

Fig. 5. Proteolytic peptide maps of human liver IIE1 and P450BE. Human IIE1 (*left lane of each panel*) and P450BE (*right lane of each panel*) were digested with either *S. aureus* V8 (*left*) or papain (*right*), and the peptides were separated as previously described (21). The peptides were visualized by immunoblotting with either rabbit anti-human IIE1 IgG (*top*) or monoclonal antibody BE26 (*bottom*).

survey of >50 human liver microsomal specimens with 12 different antibody preparations directed against IIB subfamily members in the rat or the rabbit failed to identify an expressed P450 in the human IIB subfamily (data not shown). The results of this study clearly demonstrate that extreme caution should be used when interpreting the results of immunochemical techniques used to phenotype the P450s (29), when species lines are crossed.

Acknowledgments

The authors wish to thank Wayne Levin and Dene Ryan for supplying rat IIE1 and Mel Johnson for performing the amino acid sequence analyses.

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