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Research Article

Sera from patients with dihydralazine-induced hepatitis were shown to contain anti-liver microsomal autoantibodies (anti-LM) by indirect immunofluorescence. These anti-LM antibodies were different from anti-liver/kidney microsomes (anti-LKM) 1 or 2 autoantibodies which have been previously described. Sera recognized a single 53,000 = Mr polypeptide in human liver microsomes as judged by immunoblotting, and the target antigen was identified as cytochrome P-450IA2 (P-450IA2) by (a) comparison of immunoblotting patterns with anti-human P-450IA2 and anti-rat P-450IA2 and with five anti-LM sera, and (b) specific immunoinhibition of microsomal ethoxyresorufin and phenacetin O-deethylation activities (both P-450IA2 supported reactions) by anti-LM antibodies. Finally, purified human P-450IA2 was recognized by these anti-LM sera. The anti-LM antibodies are specific for the disease because none of the other antisera tested behaved in the same manner as anti-LM, even those from patients treated with dihydralazine and without hepatic disease. A possible role of P-450IA2 in the metabolism of dihydralazine was suggested by competitive inhibition of ethoxyresorufin-O-deethylase observed in microsomal incubations. Thus, a new example is presented in which a cytochrome P-450 may be a target for autoantibodies in drug-induced hepatitis.

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Anti-Liver Endoplasmic Reticulum Autoantibodies Are Directed against Human Cytochrome P-450IA2

A Specific Marker of Dihydralazine-induced Hepatitis

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Abstract

Sera from patients with dihydralazine-induced hepatitis were shown to contain anti-liver microsomal autoantibodies (anti-LM) by indirect immunofluorescence. These anti-LM antibodies were different from anti-liver/kidney microsomes (anti-LKM) 1 or 2 autoantibodies which have been previously described. Sera recognized a single $53,000 = M_r$ polypeptide in human liver microsomes as judged by immunoblotting, and the target antigen was identified as cytochrome P-450IA2 (P-450IA2) by (a) comparison of immunoblotting patterns with anti-human P-450IA2 and anti-rat P-450IA2 and with five anti-LM sera, and (b) specific immunoinhibition of microsomal ethoxyresorufin and phenacetin O-deethylation activities (both P-450IA2 supported reactions) by anti-LM antibodies. Finally, purified human P-450IA2 was recognized by these anti-LM sera. The anti-LM antibodies are specific for the disease because none of the other antisera tested behaved in the same manner as anti-LM, even those from patients treated with dihydralazine and without hepatic disease. A possible role of P-450IA2 in the metabolism of dihydralazine was suggested by competitive inhibition of ethoxyresorufin-O-deethylase observed in microsomal incubations. Thus, a new example is presented in which a cytochrome P-450 may be a target for autoantibodies in drug-induced hepatitis. (J. Clin. Invest. 1990. 85:1967-1973.) drug metabolism • immunoblots • immunoinhibition • target antigen

Introduction

Autoantibodies have been found in drug-induced liver diseases such as hepatitis caused by halothane (anti-liver/kidney microsomes 1; [anti-LKM1]¹) or by tienilic acid (Ticrynafen) (anti-LKM2) (1, 2 and references therein). Anti-LKM1 antibodies were also found in children with an idiopathic type of chronic active hepatitis where a drug could not be identified in

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1. Abbreviations used in this paper: anti-LM, anti-liver microsomes; anti-LKM, anti-liver and kidney microsomes.

the etiology of the disease (1, 2, 3). The microsomal drug-metabolizing enzymes, P-450IID6 (for anti-LKM1), P-450IIC8/ 9/10 (for anti-LKM2), trifluoroacetylated carboxylesterase, or several unidentified microsomal proteins (4-15) were found to be recognized by the various autoantibodies. In drug-induced hepatitis the events shown in Fig. 1 have been proposed to explain initial steps of the diseases. Most of these steps were clearly established for tienilic acid-induced hepatitis (11-13) and a drug-metabolizing enzyme was shown to be a target for autoantibodies. Covalent binding to drug-metabolizing enzymes has been shown in some other instances; the targets for autoantibodies seem to be either modified or native proteins (5-15). This triggering mechanism for drug-induced autoimmune diseases could be more general and found in other cases. Dihydralazine has been shown to induce a hepatitis that can be characterized by the presence of autoantibodies in the patient serum (anti-liver microsomes; termed anti-LM) (16-18). Here we demonstrate that these autoantibodies are specifically directed against human P-450IA22 (19-21) which may be responsible for dihydralazine metabolism. Thus, the hypothesis depicted in Fig. 1 could be quite general in immunoallergic drug-induced hepatitis.

2. The evidence that P-450_{PA} (described in reference 19 and used in this paper), the phenacetin O-deethylase, is the human P-450IA2 gene product may be summarized as follows. Antibodies raised to rat P-450_{ISF-G} (P-450IA2) or human P-450_{PA} recognize a single polypeptide in human liver microsomes and inhibit microsomal phenacetin O-deethylase activity; the level of detectable protein is highly correlated with phenacetin O-deethylase (22, 23) but not with aryl hydrocarbon hydroxylase activity (24). Both rat and human P-450IA gene families appear to encode only two products, the IA1 and IA2 proteins (20). P-450IA1 is thought to be the aryl hydrocarbon hydroxylase. An antibody prepared to rat P-450_d (P-450IA2), which did not recognize P-450_c (P-450IA1), was used to isolate a catalytically inactive protein which had an NH2-terminal sequence (21) matching that predicted by the human P₃-450 cDNA (P-450IA2) sequence (25). Our own human P-450_{PA} preparation, which shows phenacetin O-deethylase activity, did not yield an unambiguous NH2-terminal sequence because of impurities that were present but principal peaks were observed for ala, leu, gln, val, and pro at cycles 1, 2, 4, 6, and 7, as predicted (19, 25) (positions 3 and 5 are predicted to contain ser, which has a low recovery). Hepatic protein immunoreactive with anti-rat P-450IA2 and microsomal phenacetin O-deethylase activity are both increased in cigarette smokers but aryl hydrocarbon hydroxylase is not (23, 24). The drug furafylline inhibits human liver microsomal phenacetin Odethylation but stimulates aryl hydrocarbon hydroxylase (24). Although the evidence appears to be indirect, collectively there is a strong basis for the view that the human enzyme P-450_{PA} used in this paper (19) corresponds to the P-450IA2 gene product.

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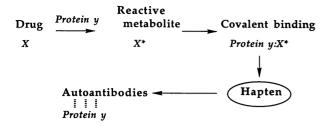


Figure 1. Postulated initial events in autoimmune hepatitis.

Methods

Chemicals. Electrophoresis reagents were from Serva Fine Biochemicals (Heidelberg, FRG), nitrocellulose sheets were from Bio-Rad Laboratories (Richmond, CA), peroxidase-conjugated and fluorescein isothiocyanate-conjugated immunoglobulins were from DAKO-PATTS (Copenhagen), and resorufin was from Aldrich-Chimie (Strasbourg, France). NADPH, 7-ethoxyresorufin, and 7-pentoxyresorufin were obtained from Boehringer Mannheim GmbH (Mannheim, FRG). Other reagents were of the highest quality available and purchased either from Prolabo (Paris) or from Sigma Chemical Co. (St. Louis, MO). Isosafrole was a generous gift of Dr. H. Hoellinger (Centre National de la Recherche Scientifique U A 400, Paris). Dihydralazine was a generous gift from CIBA-Geigy (Basel).

Animals. Male Sprague-Dawley rats (150-200 g) were treated with β -naphthoflavone (50 mg/kg) or isosafrole (150 mg/kg) daily for 3 d (i.p.); rats had free access to water and a standard diet (UAR, Villemoisson, France).

Patients. Sera were obtained from five patients (designated NAL, DAC, WOL, POU, and MOU) with dihydralazine-induced acute hepatitis. In all cases, diagnosis was considered as definite or probable on the basis of the following criteria: (a) hepatitis developed 2-6 mo after the onset of treatment; (b) patients received no hepatotoxic drugs other than dihydralazine; (c) clinical and histological data were suggestive of or consistent with drug (dihydralazine)-induced hepatitis (16-18); (d) no other cause of hepatitis was found, in particular, serological tests for recent infection with hepatitis A and B viruses, cytomegalovirus, and Epstein-Barr virus were negative; and (e) after rechallenge hepatitis occurred again more rapidly than the first time. Clinical information regarding these patients has been published elsewhere (16-18).

Patient sera. Sera were obtained from the above described patients during the acute phase of the disease.

Control sera. Control sera were as follows: six from patients with hepatitis A virus infection, five from patients with hepatitis B virus infection, five from patients suffering from both hepatitis B and delta virus infection, 12 from patients with hepatitis caused by drugs other than dihydralazine including two anti-LKM2 sera; all these patients had an acute hepatitis, a high serum bilirubin (> 100 \(mu\)mol/liter) and aminotransferases level (> 10 times the upper limit of the normal). In addition six sera from patient suffering from chronic liver disease including an anti-LKM1 (a generous gift from L. Kiffel, Institut National de la Santé et de la Recherche Médicale U 75, Paris, and Dr. J. C. Homberg, Hopital St. Antoine, Paris) were used as control. Two control sera were obtained from patients without any hepatic disease with normal serum aminotransferase and bilirubin levels, and without dihydralazine treatment. In addition control sera from two patients. who had received dihydralazine for 1-6 mo without any hepatic disease, were used. Anti-LKM1 and anti-LKM2 sera were described previously (1, 10, 11). These autoantibodies are directed against human liver P-450IID6 (P-450_{DB}) and IIC8/9/10 (P-450_{MP}), respectively (1, 5-13, 20).

Human livers. 13 human livers were obtained from donors for kidney transplantation. Livers were removed within 30 min after circulatory arrest and frozen at -80° C as small cubes (26). Microsomes were prepared as previously described (26). Fetal human liver micro-

somes were a generous gift of Dr. T. Cresteil, (INSERM U 75, Paris) (27).

P-450s and antibodies. The nomenclature of Nebert et al. (20) is used here, along with previous nomenclature for human and rat P-450s (28, 29). The characteristics of human P-450IIC8/9/10 (P-450_{MP}) (11, 19) (the precise assignment of protein and DNA sequences in this subfamily is not clear), human P-450IIIA4 (P-450_{NF}) (30), anti-human P-450₉ (31), human liver microsomal epoxide hydrolase (32), rat P-450IA2 (P-450_{ISF-G}) (29), and human P-450IA2 (P-450_{PA}) (19) and the corresponding polyclonal antibodies have been described, as well as monoclonal anti-human P-450IIIA4 (30, 33); rabbit anti-rat P-450IIE1, a generous gift from Dr. C. S. Yang (Rutgers University, Piscataway, NJ) (34), cross-reacted with the human P-450IIE1. Human liver microsomal P-450₁₀ was purified in this laboratory; it is different from all other known P-450s but has not been yet fully characterized (28).

Assays. Immunoblotting analysis was performed as previously described (11, 29, 31, 35). Protein concentrations were estimated by the method of Lowry et al. using BSA as standard (36). 7-Ethoxyresorufin and 7-pentoxyresorufin O-dealkylase activities were performed as described (37) except that 200–600 μ g of protein was used. Phenacetin O-deethylase activity was assayed as described elsewhere (19). In immunoinhibition studies, 3.5–70 μ l of sera was added per milligram of microsomal protein and the monooxygenase activity was then measured and compared to the activity observed in the absence of serum. In competitive inhibition studies, 200 μ M dihydralazine (final concentration) was added to the incubation medium and the final 7-ethoxyresorufin concentration was varied from 0.016 to 0.12 μ M.

Immunoblots were performed with 13 different preparations of human liver microsomes and were probed with the five anti-LM anti-bodies, with anti-human P-450IA2, with anti-rat P-450IA2, and with anti-human P-450IIIA4. The intensity of each band was measured by densitometry.

Cross correlations. These 13 human liver microsomal preparations were used to measure monooxygenase activities and P-450 content by immunoblotting. Monooxygenase activities were expressed as nano-

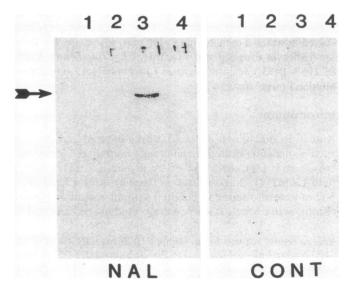


Figure 2. Immunoblots of human liver microsomes, P-450, and epoxide hydrolase. The individual lanes contained: P-450IIIA4, 5 pmol (lane 1); P-450IIC8/9/10, 5 pmol (lane 2); human liver microsomal sample HL97, 8 μ g protein (lane 3); and epoxide hydrolase, 4 pmol (lane 4). Blots were developed with either NAL an anti-LM serum (diluted 1/100) or CONT serum from a patient without hepatic disease and not treated with dihydralazine (diluted 1/100). The upper staining in the NAL immunoblot was an artifact often seen with human sera; the band indicated by the arrow has $M_r = 53,000$.

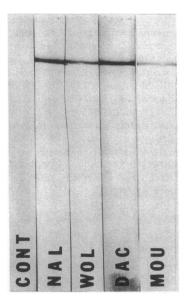


Figure 3. Immunoblot of a single human liver microsomal preparation (HL 96, 50 μg microsomal protein per lane) with different antisera. Sera were diluted 1/350. CONT, Sera from a control patient (taking dihydralazine but without disease); NAL, WOL, DAC, and MOU, anti-LM sera from the corresponding patients.

moles per minute and per milligram of protein and P-450 content as densitometric arbitrary units per milligram of proteins. The liver with the highest value (HL 96) was chosen as the reference and all measurements (monooxygenase activities and P-450 content) are expressed relative to this liver sample (relative to 100%). These relative values were cross-correlated using linear regression analysis; Student's t test was used and correlations were considered to be statistically significant when P was < 0.05.

A Weller and Coons indirect immunofluorescence method was performed on 4- μ m-thick liver and kidney slices from untreated and isosafrole or β -naphthoflavone-treated rats (1).

Results

Immunoblotting. Sera from patients with dihydralazine-induced hepatitis were shown by immunofluorescence techniques to contain autoantibodies (anti-LM) that recognize antigens in liver microsomes of untreated rats (16-18). One anti-LM serum (NAL) recognized a single band in human liver microsomes ($M_r = 53,000$; Fig. 2). All four other sera behaved similarly (Fig. 3 shows four of the five sera); this band was absent in human fetal liver and adult human kidney microsomes (data not shown). Two control sera (patients without liver disease) did not recognize any band in human liver microsomes (Figs. 2 and 3). The protein recognized in these blots had a relative molecular mass typical of P-450s, and further studies were concentrated on this family of xenobiotic-metabolizing enzymes. This band was present in all human liver microsomes examined (Fig. 4); the high variability of the concentration of the recognized protein in human liver microsomes is apparent in this experiment. The mobility of this band was compared to those of known P-450s and epoxide hydrolase in the same microsomal preparation, with the use of antibodies. Anti-rat P-450IA2 recognized a band with the same relative molecular mass as the anti-LM/NAL band while all of the other anti-sera tested recognized different bands (Fig. 5). These results are consonant with those presented in Fig. 2,

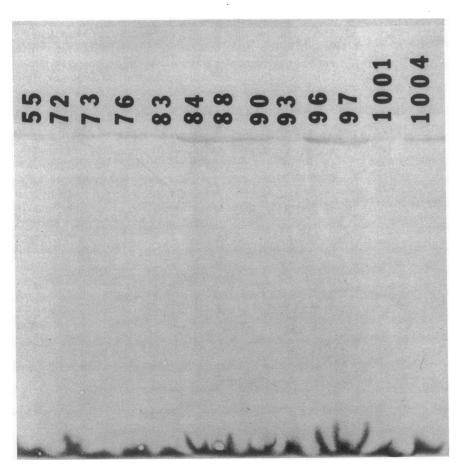


Figure 4. Immunoblot of 13 different human microsomal liver preparations with anti-LM NAL (diluted 1/350). Numbers correspond to human livers, and 50 µg of microsomal protein were loaded on each lane.







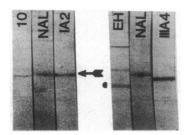


Figure 5. Immunoblots of a single human liver microsomal preparation (HL 96) with different antisera. Each series of three lanes corresponds to a single gel transfer: NAL, anti-LM serum, diluted 1/100; IIE1, anti-rat P-450IIE1 immunoglobulin (0.8 µg/ml); 9, anti-P-450₉ serum, diluted 1/100; LKM1, anti-LKM1 serum, diluted 1/200; Cont., serum from patient without hepatic disease and not treated with dihydralazine, diluted 1/100; IIC9, anti-P-

450IIC8/9/10 serum, diluted 1/200; *LKM2*, anti-LKM2 serum, diluted 1/200; *10*, anti-human P-450₁₀, diluted 1/100; *IA2*, anti-rat P-450IA2, diluted 1/200; *EH*, anti-human epoxide hydrolase serum, diluted 1/200 (dot indicates purified epoxide hydrolase); *IIIA4*, monoclonal antibody anti-P-450IIA4, diluted 1/200. The arrow shows the comigration of the bands recognized by NAL and anti-rat P-450IA2.

where purified P-450IIC8/9/10 and P-450IIIA4 were not recognized by anti-LM/NAL, and indicated that P-450IA2 may be the antigen corresponding to these autoantibodies.

Purified rat and human P-450IA2 preparations were examined to address this hypothesis. None of the anti-LM sera recognized rat P-450IA2 (data not shown) but all of them recognized human P-450IA2 in immunoblotting experiments. The results with anti-LM/NAL (Fig. 6) demonstrate that the band recognized by anti-LM in human liver microsomes comigrates with purified human P-450IA2. Denaturation of the cross-reactive epitopes during the preparation of the samples for electrophoresis might explain why anti-LM failed to recognize rat P-450IA2 in immunoblotting analysis and why anti-LM/NAL recognized both purified rat and human P-450IA2 in dot blots (data not shown). Similar observations have been reported for immunoblotting analysis with anti-LKM1. Indeed, anti-LKM1 recognized rat liver P-450 db1 (IID1) in dot

mil A 2

Figure 6. Immunoblots of human cytochrome P-450IA2 and human liver microsomes. The individual lanes contained: IA2, purified human P-450IA2 (5 μg); and Mi, human liver microsomes (HL96, 50 μg protein). They were developed with anti–LM (NAL) serum, diluted 1/400. The arrow indicates the beginning of the gel

blot and not in immunoblot and recognized human P-450 db1 (IID6) in immunoblot (6). Proteins are denaturated during Western blotting and not during dot blotting procedure; it was postulated that rat P-450 has only a few common epitopes with human enzyme; these epitopes are probably destroyed during denaturation while human enzyme keeps some epitopes recognized by anti-LKM1.

38 control sera were tested and none of them was able to exhibit, in immunoblots, a band comigrating with the band recognized by anti-LM sera. They include sera with high bilirubin and high serum aminotransferases (16), sera from patients with chronic liver disease (six and among them one anti-LKM1), sera from patients with drug-induced hepatitis (12 and among them two anti-LKM2), two sera from patients without liver disease and with normal aminotransferases and bilirubin, and finally two sera from patients taking dihydralazine without any liver disease.

Correlation studies. To further address the identity of the protein recognized in human liver microsomes by the various anti-LM preparations, further immunoblotting experiments were done with 13 different preparations of human liver microsomes and the five anti-LM sera; an example with anti-LM NAL is shown in Fig. 4. Relative staining intensities with the five different anti-LM sera were cross-correlated; the correlation coefficient (r) was always > 0.85 (P < 0.001), suggesting very strongly that each anti-LM preparation recognizes the same protein. To provide further evidence that this protein is P-450IA2 the same analyses were performed with antirat P-450IA2, anti-human P-450IA2, and anti-human P-450IIIA4 on the same 13 human liver microsomes. In addition 7-ethoxyresorufin and phenacetin O-deethylase activities (supported by P-450IA2) (19, 28, 37, 39) and 7-pentoxyresorufin O-dealkylation (not supported by P-450IA2) (37, 39) were measured in the same microsomal preparations. Results obtained with anti-LM (mean of the results obtained with the five anti-LM sera), anti-rat P-450IA2, and anti-human P-450IA2 blotting and 7-ethoxyresorufin and phenacetin Odeethylase activities were all highly correlated with each other (Table I; r always > 0.75, P < 0.01), indicating that all are linked to human P-450IA2. 7-Pentoxyresorufin O-dealkylation and P-450IIIA4 concentrations were not correlated to the other results (Table I).

Immunoinhibition. All anti-LM sera tested were able to inhibit > 90% of P-450IA2-supported monooxygenase activities, namely ethoxyresorufin and phenacetin O-deethylation (Figs. 7 and 8); they did not inhibit pentoxyresorufin O-deal-kylation (which is not supported by P-450IA2) while, as a

Table I. Correlations of Estimated Levels of Individual P-450s from Immunoblots Probed with Several Antibodies and Monooxygenase Activities in 13 Human Liver Microsomal Samples

	Correlation coefficient (r)					
	Anti-LM	Rat P-450IA2	Human P-450IA2	EROD	POD	PROD
Rat P-450IA2	0.91*					
Human P-450IA2	0.86*	0.86*				
EROD	0.93*	0.89*	0.78 [†]			
POD	0.89*	0.84*	0.75 [†]	0.99*		
PROD	0.03	-0.09	-0.12	0.23	0.22	
Human P-450IIIA4	0.07	-0.25	-0.31	0.20	-0.10	0.39

Anti-LM, mean of immunoblotting values obtained with five anti-LM autoantibodies; rat P-450IA2, immunoblotting values obtained with rabbit anti-rat P-450IA2; human P-450IA2, immunoblotting values obtained with rabbit anti-human P-450IA2; P-450IIIA4, immunoblotting values obtained with mouse monoclonal anti-P-450IIIA4; P-450IIIA4; P-450IIA4; P-450IIA4;

control, anti-human P-450IIIA4 clearly inhibited this activity (data not shown). Anti-LM sera were even better inhibitors than rabbit anti-rat P-450IA2 or anti-human P-450IA2 preparations (Fig. 7 B). These findings are consistent with the results found with anti-LKM2 antibodies, which are stronger inhibitors than rabbit anti-human P-450IIC8/9/10 sera (11). None of the several control sera used were significant inhibitors (< 20% inhibition for the highest concentration, 70 μ l

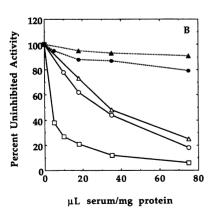


Figure 7. Immunoinhibition of 7-ethoxyresorufin O-deethylase activity. The open symbols represent results obtained with anti-LM and anti-P-450IA2 sera and the closed symbols represent results obtained with control sera. (A) The uninhibited activity of the microsomes (sample HL84) was 25 pmol product formed per minute per milligram of protein: NAL serum (11), WOL serum (\triangle), DAC serum (\bigcirc), MOU serum (11), serum from a patient taking dihydralazine and without hepatic disease (.), anti-LKM2 (1), and preimmune rabbit serum (\triangle). (B) The uninhibited activity of the microsomes (sample HL38) was 19 pmol product formed per minute per milligram of

protein: NAL serum (\square), anti-human P-450IA2 (\triangle), anti-rat P-450IA2 (\bigcirc), serum from a patient with a high level of bilirubin (> 500 μ M) (\bullet), and preimmune rabbit serum (\triangle). Each point represents a single experiment except for NAL and DAC where each point represents a triplicate and a duplicate, respectively.

serum per milligram microsomal protein); these controls include two sera from patients without hepatic disease but taking dihydralazine, two sera with levels of bilirubin as high as in one of the anti-LM (NAL, serum bilirubin > 500 μ M), rabbit preimmune serum, anti-human P-450IIIA4, and anti-LKM2. Finally, these inhibitions were reproduced with three different human liver microsomal preparations tested (HL38, HL84, and HL124; Figs. 7 and 8). Thus, it appears that anti-LM autoantibodies are directed against some part of the active site of P-450IA2.

Induction. In preceding papers (16–18), it had been shown by immunofluorescence techniques that anti-LM recognizes antigens in the liver (and not kidney) endoplasmic reticulum of untreated rats. This observation is consistent with the presence of P-450IA2 in rat liver and not in rat kidney (38). As P-450IA2 is inducible by β -naphthoflavone and isosafrole (29), immunofluorescence studies were performed in liver and kidney slices prepared from either untreated, β -naphthoflavone-treated, or isosafrole-treated rats. No immunofluorescence was seen with a control serum regardless of which organ or treatment was considered. Immunofluorescence was present in liver and absent in kidneys of untreated rats; it increased in liver and appeared in kidneys upon treatment with β -

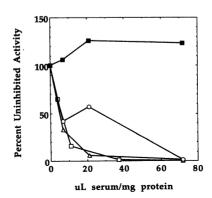


Figure 8. Immunoinhibition of phenacetin Odeethylase activity. The open symbols represent results with anti-LM sera and the closed symbols represent results with control sera. The uninhibited activity of microsomes (sample HL124) was 276 pmol product formed per minute per milligram of protein: anti-LKM2 serum (a), NAL serum (\square), MOU serum (\triangle), DAC serum (o). Each point represents a single experiment.

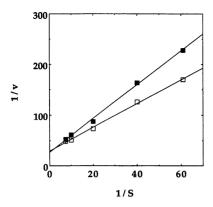


Figure 9. Inhibition of 7-ethoxyresorufin O-deethylase activity by dihydralazine. Catalytic activity was measured with microsomes prepared from human liver HL97 in presence (□) of 200 μM dihydralazine.

naphthoflavone or isosafrole (data not presented). The levels of immunoreactive material appeared to roughly parallel the concentration of P-450IA2 in livers of treated and untreated animals (29, 38). Moreover, isosafrole is a selective inducer of P-450IA2 in liver (29), although it is possible that anti-LM may also recognize the related P-450IA1 under these conditions. These results further reinforce the view that P-450IA2 is the antigen recognized by anti-LM.

Dihydralazine inhibition. Dihydralazine metabolism is poorly understood in humans (40). However, a number of other aromatic amines are oxidized by P-450IA2 (23, 41), and to obtain some indication regarding the possibility of oxidation of dihydralazine by P-450IA2 we examined the inhibition of ethoxyresorufin O-deethylation with dihydralazine. Dihydralazine was inhibitory at 200 μ M; in a competitive manner (Fig. 9), these results suggest that P-450IA2 might metabolize dihydralazine to reactive metabolite that binds to the enzyme. However, this hypothesis needs to be further substantiated by measurements of dihydralazine metabolism and covalent binding in human liver microsomes.

Discussion

In this study we demonstrate by means of cross-correlation, immunoblotting, and immunoinhibition studies that human autoantibodies found in dihydralazine-induced hepatitis are specifically directed against P-450IA2. The purification of the protein by immunoaffinity techniques has also been done (results not shown) but only minute amounts of protein were obtained and were insufficient for further analysis. All five available sera behaved similarly, indicating that these autoantibodies may be relatively specific to the disease. This view is reinforced by the observation that control sera were not inhibitory and always failed to recognize P-450IA2 either in microsomes or in isolated form. Thus, P-450IA2 may metabolize dihydralazine and could produce a reactive metabolite capable of binding to P-450IA2, subsequently behaving as a hapten (Fig. 1). This is the first time that this P-450, P-450IA2, has been implicated in such an autoimmune disease. This new example shows that this kind of drug-induced hepatitis could be more common than thought and that systematic research of specific autoantibodies in such diseases could be useful in detecting the onset of the disease and in the comprehension of its mechanism. However, many other questions remain to be solved: (a) Why do so few people develop the disease? (b) Is there a relationship between the level of expression of a P-450 and the development of the disease? The level of P-450IA2 is known to vary at least 40-fold in people and is inducible by cigarette smoking and ingestion of charred food (22, 23, 28). Since P-450IA2 is inducible, it might be possible to develop an animal model by treating animals with dihydralazine and an inducer such as β -naphthoflavone or isosafrole. (c) Is P-450 present at the surface of the membrane, and do the autoantibodies recognize P-450 at the membrane surface? (d) What is the pathogenic role of these autoantibodies? (e) Are the antibodies the consequence or the cause of the disease? Some of these questions are now under investigation in our laboratories

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