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Cytochrome P450 1A2 Is a Hepatic Autoantigen in Autoimmune Polyglandular Syndrome Type 1*

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ABSTRACT

Autoantibodies directed against proteins of the adrenal cortex and the liver were studied in 88 subjects of Sardinian descent, namely six patients with autoimmune polyendocrine syndrome type 1 (APS1), 22 relatives of APS1 patients, 40 controls with other autoimmune diseases, and 20 healthy controls. Indirect immunofluorescence, using tissue sections of the adrenal cortex, revealed a cytoplasmatic staining pattern in 4 of 6 patients with APS1. Western blotting with adrenal mitochondria identified autoantigens of 54 kDa and 57 kDa, Western blotting with placental mitochondria revealed a 54-kDa autoantigen. The 54-kDa protein was recognized by 4 of 6 patients with APS1 both in placental and adrenal tissue, whereas the 57-kDa protein was detected only by one serum. Using recombinant preparations of cytochrome P450 proteins, the autoantigens were identified as P450 scc and P450 c17.

One of six APS1 patients suffered from chronic hepatitis. In this patient, immunofluorescence revealed a centrolobular liver and a proximal renal tubule staining pattern. Western blots using microsomal preparations of human liver revealed a protein band of 52 kDa. The autoantigen was identified as cytochrome P450 1A2 by use of recombinant protein preparations. P450 1A2 represents the first hepatic autoantigen reported in APS1. P450 1A2 usually is not detected by sera of patients with isolated autoimmune liver disease and might be a hepatic marker autoantigen for patients with APS1. (*J Clin Endocrinol Metab* **82:** 1353–1361, 1997)

AUTOIMMUNE polyendocrine syndrome type 1 (APS1) is a rare autosomal recessive disorder characterized by a variable combination of disease components (1–3). The first clinical manifestation of APS1 usually occurs in childhood, and progressively new components appear throughout life, the majority (63%) of the patients suffering from three to five of them (2). The most frequent components in APS1 are chronic mucocutaneous candidiasis, hypoparathyroidism, adrenocortical failure, and gonadal failure in females (2, 3). Hepatitis is a serious, but less frequent, disease component (1–3). Defects in immunoregulation were suggested to play a role in the disease mechanism, and the presence of organ-specific autoantibodies was demonstrated repeatedly (4–12; also see Ref. 14)

Recently, progress was made in the study of APS1-related Addison's disease, which affects more than 60% of APS1 patients (1–3). Adrenal autoantigens in APS1 are cytochromes P450 c17, P450 scc, and P450 c21, which are all enzymes involved in steroidogenesis (5–13). P450 c21 is reported to be present in the adrenal cortex; expression of P450 c17 is found in adrenal tissue and steroid-producing cells of testis and ovary; and P450 scc is expressed in adrenals, gonads, and placenta (8). It was shown previously that autoantibodies directed against the adrenal cortex alone correlate with a high risk of adrenocortical failure, and antibodies directed against steroidal cells in females, in addition, correlate with a high risk of ovarian failure (14).

Chronic hepatitis is a serious disease component present in 10–18% of patients with APS1 (1–3), and occasional deaths related to hepatitis are reported in APS1 (2, 15). However, hepatitis as a disease component of APS1 still is poorly investigated. Autoantibodies associated with autoimmune hepatitis as part of APS1 were not described before, and the corresponding antigens accordingly remained unidentified.

Here we report the characterization of six patients with APS1 and confirm P450 scc and P450 c17 as adrenal autoantigens in APS1 in patients from Sardinia. We present the first characterization of hepatic autoantibodies in patients with APS1 and the molecular identification of a hepatic autoantigen in APS 1. The molecular target, cytochrome P450 1A2, is different from the targets of LKM1 and LKM3 autoantibodies that are found in autoimmune hepatitis type 2. Potential diagnostic consequences of this finding are discussed.

Subjects and Methods

Patients

We studied six patients with APS1 (Table 1). Patient 1 suffered from liver disease. Patient 1 is female and lacks markers for hepatitis B, C, and

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D viruses and HIV. Serum ceruloplasmin and α-1 antitrypsin levels are normal. She presented at the clinic at the age of 6 yr with acute hepatitis that subsequently turned chronic. Four years after the onset of hepatitis, she developed adrenal failure and chronic mucocutaneous candidiasis. A percutaneous liver biopsy revealed the histologic picture of chronic active hepatitis.

We also studied sera of 22 first-degree relatives of APS1 patients, of 40 patients with other autoimmune diseases (namely 1 with isolated Addison's disease, 10 with Hashimoto disease, 10 with insulin-dependent diabetes mellitus (IDDM), 3 with IDDM and celiac disease, 4 with autoimmune hepatitis type 1 and type 2, 10 with chronic hepatitis C virus, 1 with primary billiary cirrhosis, 1 with systemic lupus erythematosus) and of 20 healthy controls (Table 2). All subjects were of Sardinian descent. As shown in Fig. 1, the 6 APS1 patients belong to 5 different families. In family 3 and in family 5, 1 sister affected by APS1 died of acute adrenal failure (at 8 yr old and 15 yr old, respectively). None of the other 22 relatives had evidence of an autoimmune disorder.

Materials

Human liver tissue was obtained during liver transplantation from a patient's liver that was removed because of hepatocellular carcinoma. The tissue otherwise would have been discarded.

For the immunofluorescence studies, a goat antihuman IgM, IgG, IgA polyclonal FITC-conjugated antiserum was used (Dianova, Hamburg, Germany). The antibody used for Western blots was an alkaline phosphatase conjugated anti-IgG, IgA, and IgM antiserum (Dianova). The complementary DNA (cDNA) construct pBS/1A2, used for subcloning the cDNA of P450 1A2, was provided by one of us (R. H. Tukey, UC San Diego). The cDNA constructs, pUC18-scc and PUC 18-c17, containing the cDNAs of P450 scc and P450 c17, were a kind gift of Walter Miller, UC San Francisco (16, 17). The DH5α FIQ cells were from GIBCO-BRL (Eggenstein, Germany).

Nitro blue tetrazolium chloride/5-bromo-chloro-3 indolyl phosphate substrates and isopropylthiogalactoside (IPTG) for bacterial induction were from Promega (Madison, WI). Vectors for the expression of bacterial proteins were from the pQE 30 series of Quiagen (Hilden, Ger-

TABLE 1. Disease components and analytical results of six patients with APS1

Patients	Disease components in APS1	IF Adrenals (Human)	WB Adrenal mitoch. (Human)	WB Placental mitoch. (Human)	WB P450 scc	WB P450 c17	IF Liver (Rat)	IF Kidney (Rat)	WB Liver micros. (Human)	WB P4501A2
Patient 1	M. candidiasis Addison's disease (11) Chronic hepatitis (15) Pernicious anemia	1:40	54 kD	54 kD	+		1:1000	1:320	52 kD	+
Patient 2	M. candidiasis Hypoparathyroidism Addison's disease (8)	1:1000	54 kD 57 kD	54 kD	+	+				
Patient 3	Hypoparathyroidism									
Patient 4	M. candidiasis Hypoparathyroidism Addison's disease (7) Fat malabsorption Polyneuropathy	1:1000	54 kD	54 kD	+					
Patient 5	M. candidiasis Hypoparathyroidism Addison's disease (19) Pernicious anemia	1:40	54 kD	54 kD	+					
Patient 6	M. candidiasis Hypoparathyroidism Addison's disease (7)									

Listed in parentheses in the column of disease components is the time interval between the first occurrence of chronic active hepatitis or Addison's disease and the time point of blood sampling. M. candidiasis, mucocutaneous candidiasis; IF, indirect immunofluorescence; WB, Western blot.

TABLE 2. Diseases and analytical results of the control subjects

	Addison's disease	IDDM	IDDM & celiac disease	Hashimoto's disease	AIH	HCV	PBC	SLE	APS1 Relatives	Healthy controls
No. subjects	1	10	3	10	4	10	1	1	22	20
IF/WB human adrenals										
IF rat liver/kidney										
LKM					2	2				
SMA (F-actin)					2					
ANA				8	1	5		1		
AMA							1			
WB human liver microsomes:										
LKM (50 kDa)					1	2				
LKM (50 kDa + 68 kDa)					1					
WB—P450 c17										
WB—P450 scc										
WB—P450 1A2										

IDDM, Insulin-dependent diabetes mellitus; AIH, autoimmune hepatitis; HCV, hepatitis C virus; PBC, primary biliary cirrhosis; SLE, systemic lupus erythematosus; IF, indirect immunofluorescence; WB, Western blot; LKM, liver kidney microsomal autoantibodies; SMA, smooth muscle antibodies; ANA, antinuclear antibodies; AMA, antimitochondrial antibodies.

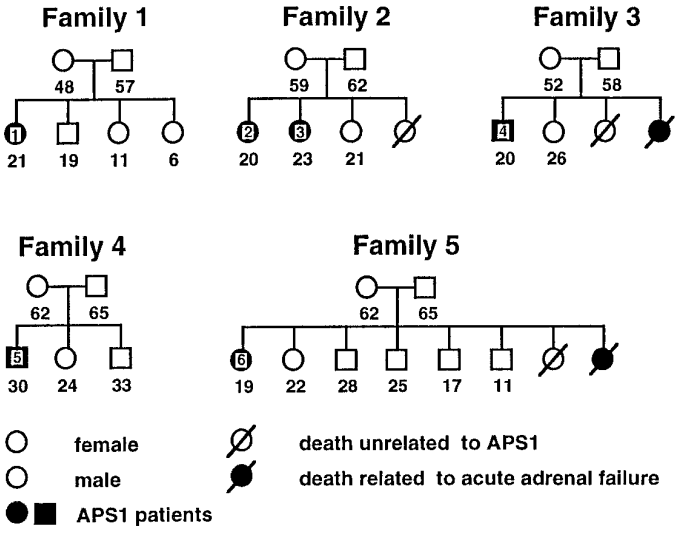

FIG. 1. Families of the APS1 patients studied. The *number below* each *symbol* indicates the age in years. *Numbers in* a *black square* or *circle* indicate the number for this APS1 patient used in this paper. The dead relatives of APS1 patients are not included in this study.

many). Restriction enzymes were purchased from New England Biolabs (Schwalbach/Taunus, Germany), and the sequencing kit was from Pharmacia (Freiburg, Germany). All other chemicals used were of the highest degree available and purchased from Sigma (Heidelberg, Germany).

Methods

Indirect immunofluorescence. Frozen sections of rat liver and kidney and of human adrenals were incubated at room temperature for 30 min with patient sera at dilutions of 1:40, 1:80, 1:160, 1:320, 1:640, and 1:1280 in phosphate buffered saline (PBS). The sections were washed twice in PBS and incubated with a goat antihuman anti-IgM, IgA, and IgG FITC-conjugated antiserum at a dilution of 1:100 in PBS for 30 min at room temperature. The sections were washed twice in PBS and embedded in 90% vol/vol glycerol in PBS. For analysis of the results, we used an Olympus IMT2 microscope (Olympus, Hamburg, Germany) fitted with an Olympus SC 35 type 12 camera.

Antigen preparations. One gram of frozen tissue was homogenized with 20 strokes of a homogenisator in 3 mL ice-cold solution of 0.25 mol/L sucrose containing 0.1 mmol/L phenylmethylsulfonylfluoride. Cellular debris and nuclei were removed by centrifugation (Sigma SK15 centrifuge, 1000 × *g*, 4 C, 15 min). The supernatant was fractionated by centrifugation (Sigma SK15 centrifuge, 3000 × *g*, 4 C, 15 min) into a mitochondrial pellet and the supernatant containing microsomes and the soluble liver proteins.

Mitochondrial preparation. For mitochondrial preparations, the pellet was resuspended in the sucrose solution, and the mitochondria were washed three times, as described above. The washed pellet was frozen on dry ice and stored at −80 C. The resulting fraction is enriched in mitochondria.

Microsomal preparation. The supernatant containing microsomes and soluble liver proteins was subjected to ultracentrifugation (Beckman ultracentrifuge, TLA 100 rotor, 100,000 × *g*, 4 C, 1 h). The supernatant was discarded, and the microsomal pellet was resuspended in 0.5 ml sucrose solution. The subcellular fractions were frozen on dry ice and stored at −80 C. The resulting preparation is enriched in microsomal proteins.

Western blotting. Fifty micrograms of tissue antigens and bacterial extracts containing recombinant proteins were separated on a 10% polyacrylamide gel and transferred to nitrocellulose (18, 19). The blots were blocked in PBS containing 0.1% Tween 20 and 5% nonfat dry milk and incubated for 1 h with a 1:100 dilution of the patient's or control sera in PBS-Tween. The blots were then washed three times for 10 min with PBS-Tween and incubated for 1 h with a 1:1000 dilution of an alkaline phosphatase conjugated antihuman IgM, IgA, IgG antiserum in PBS-Tween. After washing three times with PBS-Tween and two times in alkaline phosphatase buffer, the blots were developed using the nitro blue tetrazolium chloride/5-bromo-chloro-3 indolyl phosphate detection system (20).

Cloning and expression of recombinant cytochromes P450s. The primers used for amplification and modification of cytochromes P450 according to Waterman (21) had the following sequences: P450 scc-primers: 5′ GTGAGGTACCGCTCTGTTATTAGCAGTTTTTCTGTCAGTCCTGGTCAAAGGCTAC 3′ and 5′ GCTCAAGCTTTGATCACTGCTGGGTTGCTTC 3′; P450 c17-primers: 5′ GTGAGGTACCGCTCTGTTATTAGCAGTTTTTCTGCTTACCCTAGCTTATTTGTTT 3′ and 5′ GCTCAAGCTTTGATCACTGCTGGGTTGCTTC 3′; P450 1A2-primers: 5′ GTGAGGTACCGCTCTGTTATTAGCAGTTTTTCTGTTCTGCCTGGTATTCTGGGTG 3′ and 5′ GCTCAAGCTTCAATTGATGGAGAACG 3′.

The cDNAs were amplified by the Vent polymerase according to the manufacturers recommendations (New England Biolabs) (Fig. 2A). Denaturation was performed for 1 min at 94 C, annealing for 2 min at 62 C, and the elongation for 3 min at 72 C. Twenty cycles were applied for the amplification of the cDNA, with a final elongation step of 7 min. After PCR, the modified cDNAs were purified by the Quiagen PCR-purification kit, digested with *Hin*dIII and *Kpn*I, and inserted into the respective restriction sites of the pQE 30 vector. All constructs were confirmed by several restriction digests and by sequence analysis of the N- and C-termini, using the T7 sequencing kit, according to the manufacturer's recommendations. For expression of the recombinant cytochrome P450 proteins, all cDNA-expression vectors were transfected into DH5αFIQ cells. The expression of the recombinant proteins was induced by the addition of IPTG to a final concentration of 2 mmol/L for 3 h after the cells were grown to late-log phase. The cells were harvested by centrifugation, lysed in SDS-sample buffer, and the proteins were analyzed by SDS-PAGE and Western blotting. The expression of cytochrome P450 1A2 was further confirmed by Western blotting using a polyclonal rabbit antihuman P4501A2 antibody that was a kind gift from Prof. P. Beaune of the University of Paris.

Absorption studies. For absorption experiments, all sera were incubated with total proteins from *Escherichia coli (E. coli)* cells, carrying the expression vector alone or expressing the recombinant cytochromes P450 c17, P450 scc, or P450 1A2, according to the method described by Uibo *et al.* (8). Experiments of indirect immunofluorescence and immunoblotting (of human adrenal, human liver antigen preparations, and recombinant P450 enzymes) were performed with nonadsorbed and absorbed sera.

Results

Indirect immunofluorescence on human adrenal sections

Indirect immunofluorescence studies of the sera were performed on cryosections of human adrenal tissue (Table 1). Four of six APS1 sera detected tissue antigens in all three layers (zona glomerulosa, the zona fasciculata, and the zona reticularis) of the human adrenal cortex. None of the 82 other sera, including the serum from a patient with idiopathic Addison's disease, recognized adrenal antigens.

Immunoblotting with human adrenal antigens

To further characterize the adrenal autoantigens involved, we prepared mitochondrial subfractions of human adrenal and placental tissues. Western blotting, with patient sera 1, 2, 4, and 5, revealed a protein band at 54 kDa in both tissues (Fig. 3, A and B). In addition to the 54-kDa protein, patient serum no. 2 recognized a second band at 57 kDa in adrenal but not in placental tissue. In contrast, none of the sera from the patients' first-degree relatives, from patients with other autoimmune diseases, or from healthy controls showed pro-

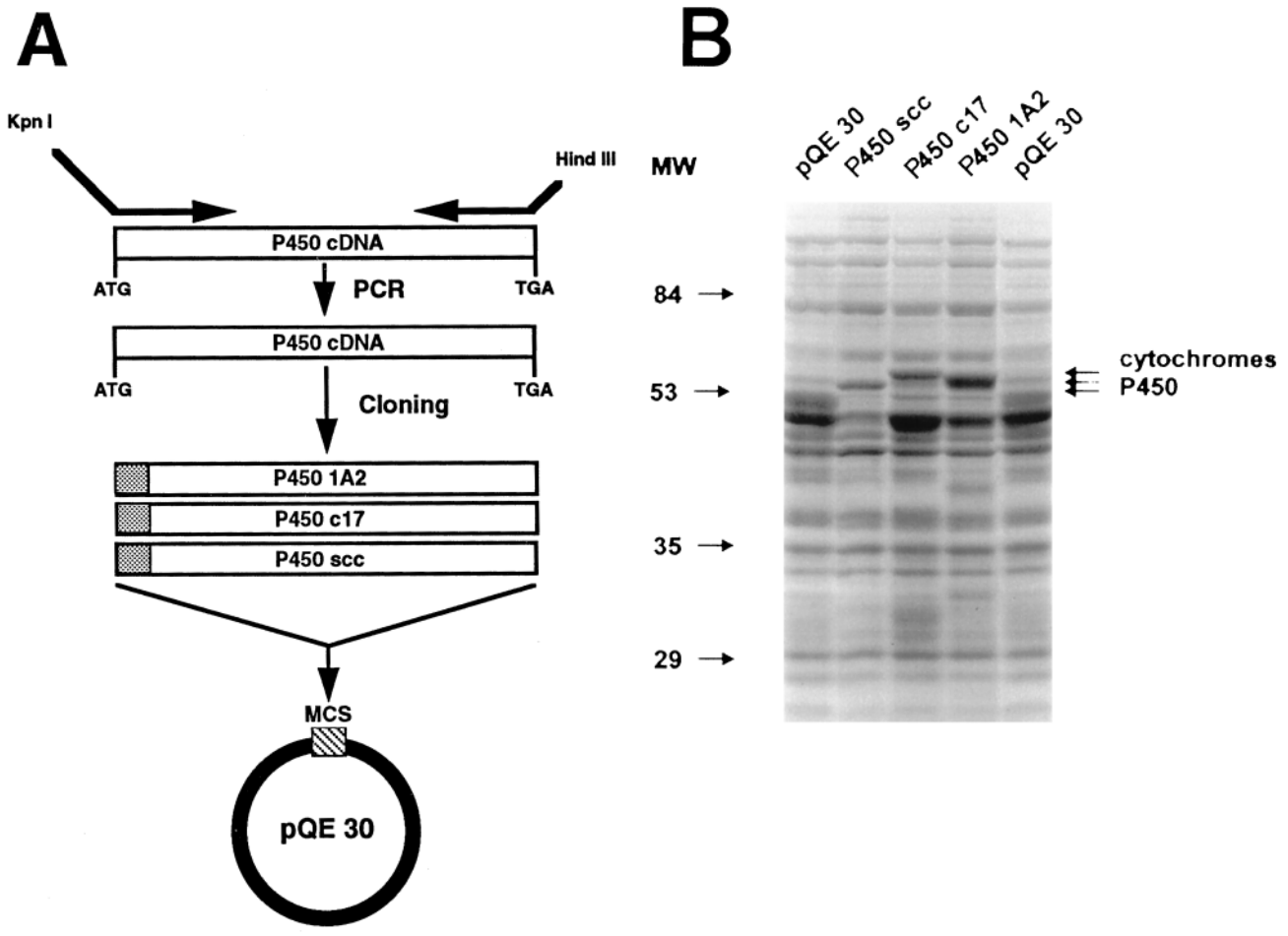

FIG. 2. Expression of recombinant cytochromes P450 scc, P450 c17, and P450 1A2 in *E. coli*. A, Construction of the expression vectors; B, the figure shows a typical result after Coomassie Blue staining of *E. coli* lysates expressing cytochromes P450 1A2, P450 scc, and P450 c17. *Arrows* on the *left* indicate the position of the molecular weight standards, *arrows* on the *right* mark the positions of the recombinant cytochrome P450 proteins.

tein bands at the levels of 54 or 57 kDa (Fig. 3, A and B, Table 2). Also, the serum of the patient with isolated Addison's disease failed to recognize any of these autoantigens. This result is in accordance with the working hypothesis that the autoantigenes recognized in immunofluorescence are the 54-kDa and 57-kDa protein bands found in Western blotting.

Immunoblotting with recombinant cytochromes P450 scc and P450 c17

Bacterial lysates of clones expressing cytochromes P450 scc and P450 c17 were used to test for the presence of autoantibodies directed against these two proteins. Sera 1, 2, 4, and 5, which also reacted with the 54-kDa adrenal and placental autoantigens, recognized the recombinant P450 scc (Fig. 3C). Serum 2, which in addition detected a 57-kDa adrenal antigen, recognized P450 c17 (Fig. 3D). In contrast, none of 82 sera from the patients' first-degree relatives, patients with other autoimmune diseases, and healthy controls recognized P450 scc or P450 c17 (Fig. 3, C and D and Table 2).

Control experiments with lysates from bacteria expressing the empty pQE vector were performed with all patient and control sera, demonstrating the specificity of the reaction by the absence of the specific 54-kDa and 57-kDa bands (data not shown).

Absorption studies were performed with recombinant preparations of P450 scc and P450 c17 (data not shown). As expected, recombinant P450 scc absorbed the bands at 54 kDa and P450 c17 the band at 57 kDa, in Western blots with human adrenal mitochondria, demonstrating the identity of the molecular targets. Interestingly, using P450 scc for absorption, no other signal was left in blots with patient sera 1, 4, and 5, whereas only the 57-kDa band remained in blots using serum 2.

Also, the signals in immunofluorescence could be absorbed by the recombinant preparations of P450 scc and P450 c17, if P450 scc were used for sera 1, 4, and 5 and a combination of P450 scc and P450 c17 was used for patient serum 2 (data not shown). These results clearly demonstrate that the immunofluorescence is caused by autoantibodies directed against cytochromes P450 scc and P450 c17. They further show that no autoantibodies directed against cytochrome P450 c21 are present that would not have been absorbed by the recombinant antigens.

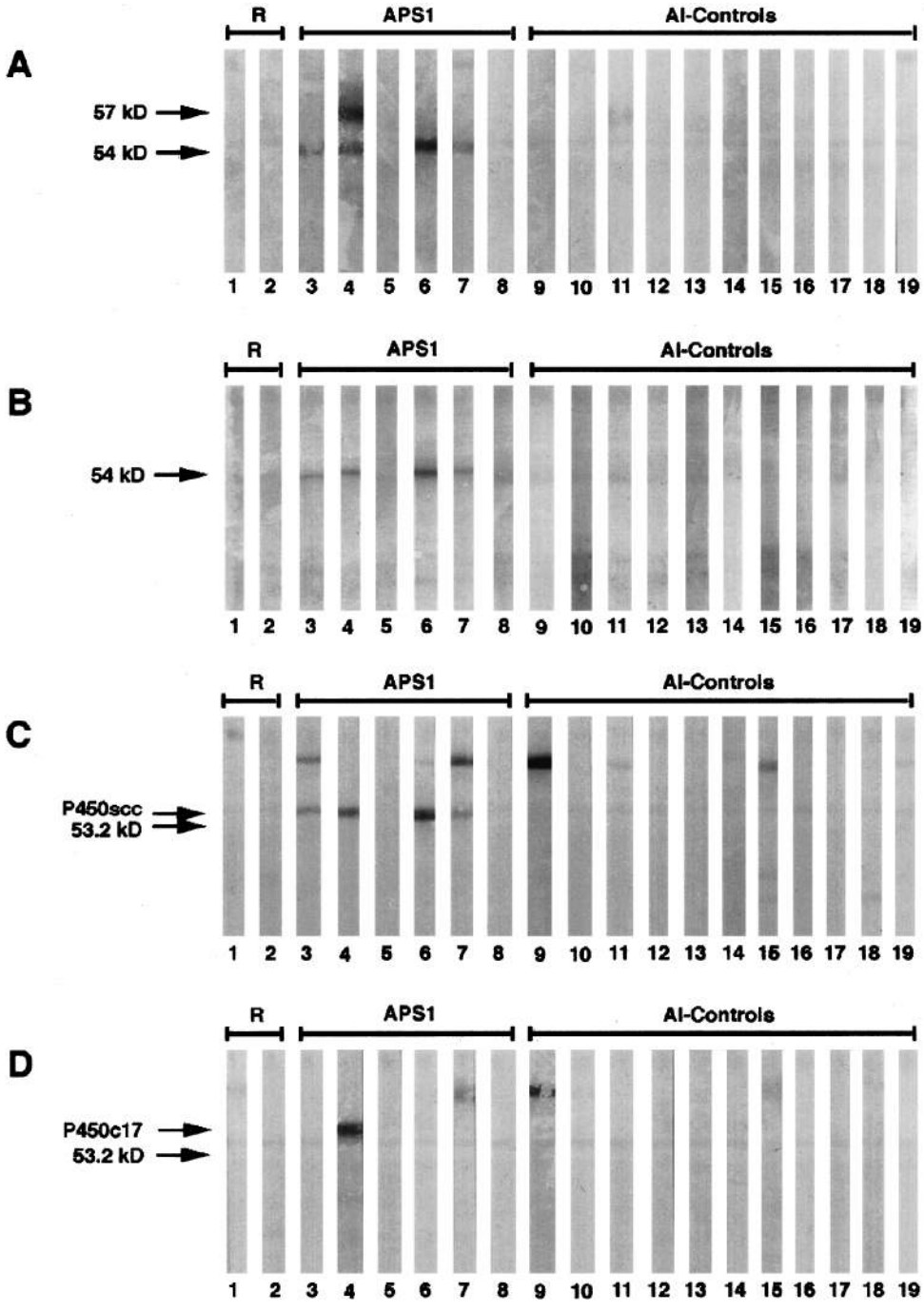

FIG. 3. Western blots with adrenal antigens, placental antigens, and recombinant cytochromes P450 scc and P450 c17. A, Western blots with mitochondrial antigens of human adrenals; B, Western blots with mitochondrial antigens of human placenta; C, Western blots with P450 scc expressed in *E. coli*; D, Western blots with P450 c17 expressed in *E. coli*; Lanes 1 and 2, first-degree APS1 patients' relatives; lanes 3–8, APS1 patients 1–6; lane 9, patient with isolated Addison's disease; lanes 10–12, patients with Hashimoto disease; lane 13, LKM1-positive patient with autoimmune hepatitis; lane 14, LKM1-positive patient with hepatitis C virus; lane 15, patient with primary billiary cirrhosis; lanes 16–18, patients with IDDM and celiac disease; lane 19, patient with systemic lupus eythematosus.

Indirect immunofluorescence on rat liver and kidney sections

To investigate the hepatic autoantigens involved in chronic autoimmune hepatitis associated with APS1, indirect immunofluorescence was performed (Tables 1 and 2). Six out of 88 sera tested revealed immunostaining of liver and kidney sections. These sera were the serum of patient 1, who suffered from chronic hepatitis and APS1, two sera from patients with LKM-1 positive autoimmune hepatitis, two sera from patients with LKM-1 positive hepatitis C, and one serum from a patient with primary biliary cirrhosis, who was positive for antimitochondrial antibodies. The staining pattern of the serum of patient 1 (Fig. 4) was characterized by a predominant staining of the perivenous hepatocytes (titer $>$ 1:1000) and of the proximal renal tubules (titer 1:320). This pattern differs from the homogeneous staining pattern found in patients with isolated autoimmune hepatitis, suggesting that the serum of patient 2 recognizes an autoantigen that is different from LKM1 (anticytochrome P450 2D6) and LKM3 (anti-UDP-glucuronosyltransferase) autoantigenes that were described earlier to be targets for autoimmunity in patients with autoimmune and virus hepatitis (22, 23).

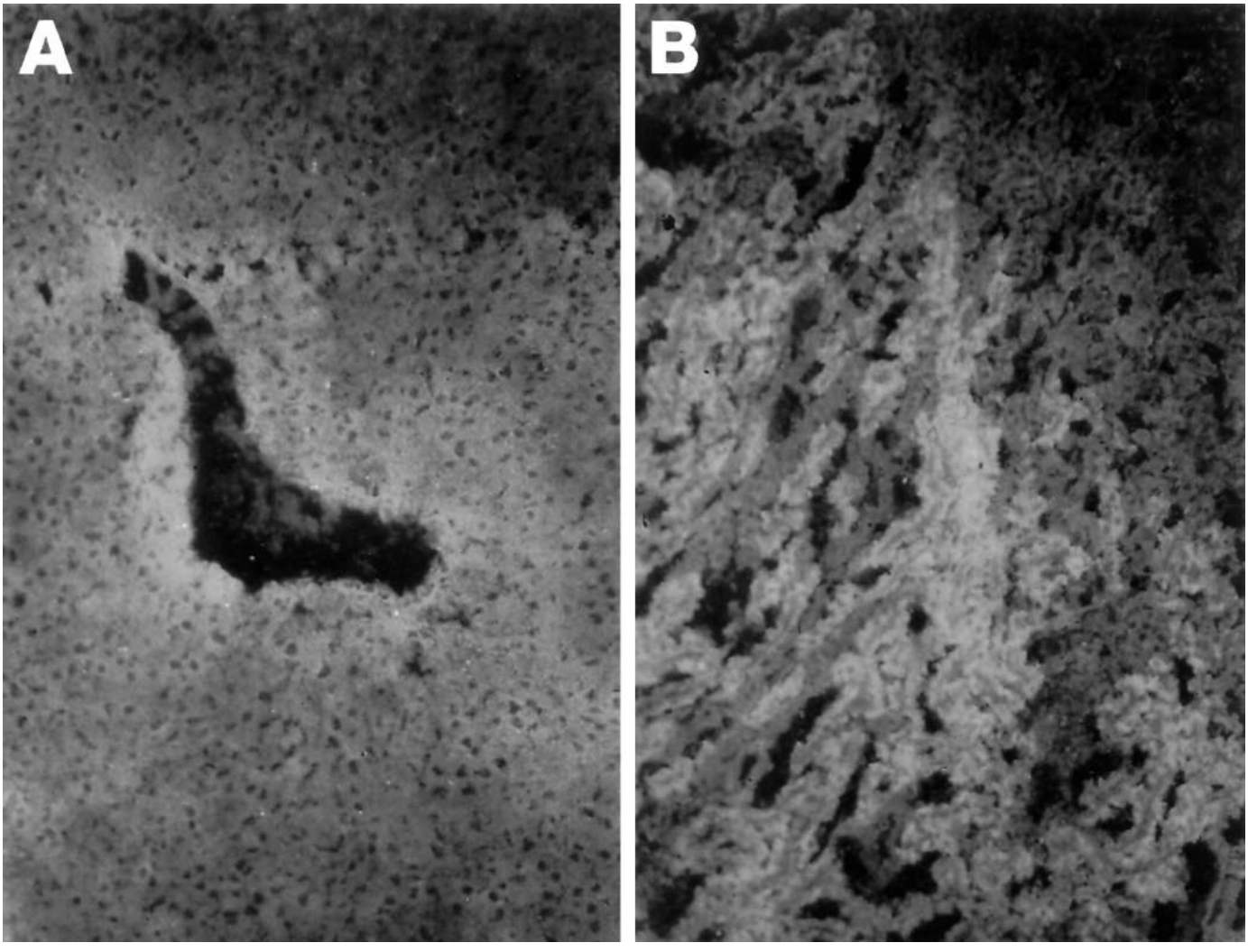

FIG. 4. Indirect immunofluorescence of rat liver and rat kidney sections. Patient serum 2 was diluted 1:320 in PBS, and indirect immunofluorescence was performed on cryosections on rat liver (A) and rat kidney (B). For immunostaining, a goat antihuman anti-IgM, IgA, and IgG FTC-conjugated antiserum was used at a dilution of 1:100.

Immunoblotting with human liver antigens

To further characterize the hepatic antigens involved, the microsomal fraction of human liver tissue was handled under nonreducing conditions and tested by immunoblotting. As shown in Fig. 5A, a protein band of approximately 52 kDa was detected by the serum of patient 1. Bands of 50 kDa and 68 kDa were detected by the LKM1-positive patients, the 50-kDa band representing an autoantibody directed against cytochrome P450 2D6 (22). The patient with PBC shows a band at about 70 kDa, which is caused by a high-titer antimitochondrial antibody produced by this patient.

Immunoblotting with P450 1A2

To identify the hepatic autoantigen recognized by patient 1, we expressed cytochrome P450 1A2 in *E. coli* (Fig. 2). Preparations of the recombinant cytochrome P450 1A2 were used in Western blotting experiments. All of the 88 sera were tested; however, only the serum of patient 1 was positive (Fig. 5B). This result demonstrates that P450 1A2 is an autoantigen in autoimmune hepatitis associated with APS1. To demonstrate the specificity of the reaction, a Western blot of serum 1 was performed, using *E. coli* extracts of clones carrying the empty vector alone, in parallel with preparations containing recombinant P450 1A2. No band appeared in the control extract, whereas a clear signal was visible in the lane containing P450 1A2 (Fig. 5C).

P450 1A2 is expressed in liver, but not in kidney, and therefore, antibodies directed against P450 1A2 were described before as LM autoantibody in patients with dihydralazine-induced hepatitis (24). There are two potential explanations for the renal immunofluorescence pattern. The first explanation consists of a cross-reaction of the autoantibody with a kidney antigen. A cross-reaction also would explain why the signal is only detected at lower serum dilutions. The second explanation is the presence of an unrelated second autoantibody present at a lower titer. To distinguish between these possibilities, absorption studies were performed.

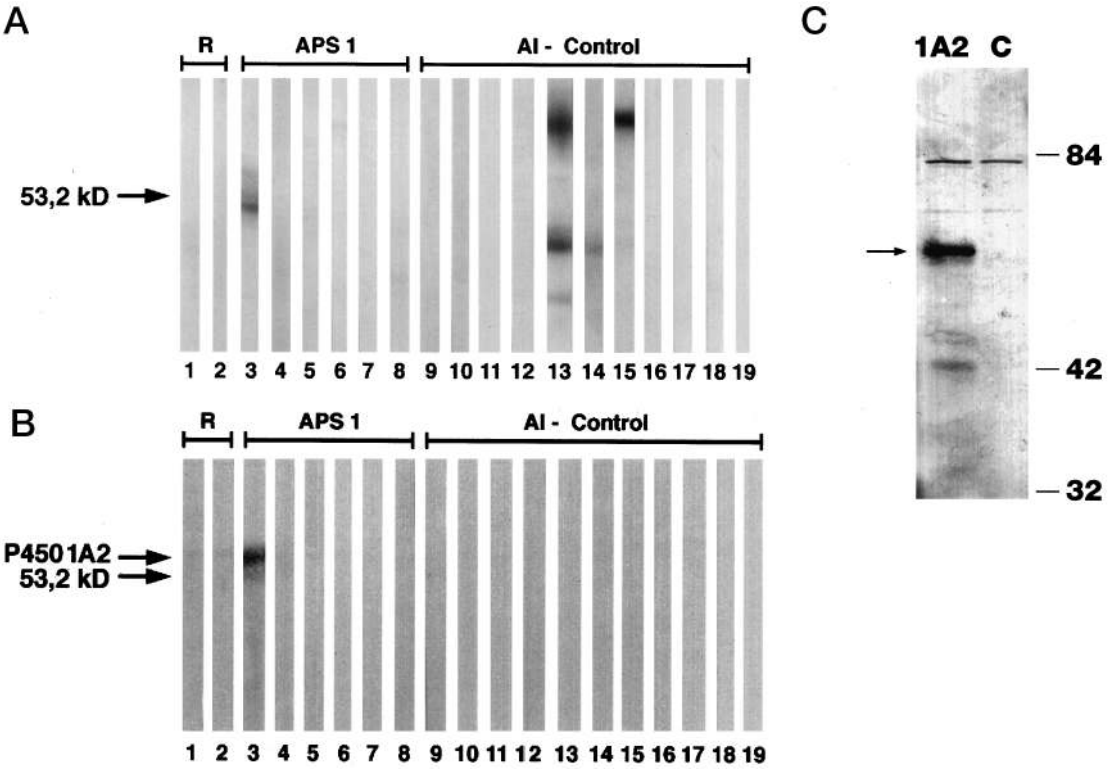

FIG. 5. Western blotting of microsomal antigens of human liver and recombinant P450 1A2. The numbering of patient sera is the same as in Fig. 3, A–C. A, Western blotting of microsomal antigens of human liver; B, Western blotting of a recombinant preparation of P450 1A2. The *arrow* on the *left* indicates the position of the recombinant cytochrome P450 1A2. C, Lysates from cells induced by IPTG carrying the empty vector (control) and expression vector for cytochrome P450 1A2 were analyzed by Western blot using the serum of patient 1, who is suffering fom APS1-related autoimmune hepatitis. The *arrow* on the *left* indicates the position of the recombinant cytochrome P450 1A2, the *arrows* on the *right* indicate the molecular mass in kDa.

Absorption studies

After absorption with preparations of recombinant P450 1A2, the immunofluorescence studies shown in Fig. 4 were repeated (data not shown). In three independent experiments, the immunofluorescence disappeared in liver and in kidney. In control experiments performed with LKM1 positive control sera, the immunofluorescence patterns persisted after absorption with the recombinant P450 1A2.

Figure 6A shows a result after Western blotting with liver microsomes. The serum of patient 1 revealed a clear signal at 52 kDa in a Western blot with liver microsomes before absorption (Fig. 6A, lane 1) the signal almost completely disappeared after absorption with P450 1A2 (Fig. 6A, lane 2). The third lane of Fig. 6A shows that the signals of an LKM1-positive serum that are not related to P450 1A2 persist after absorption with P450 1A2. Figure 6B shows a result after Western blotting using recombinant P450 1A2.

Based on these results, we conclude that the 52-kDa band detected in Western blotting experiments with liver microsomes is caused by an autoantibody directed against P450 1A2. The fact that the immunostaining of kidney sections that was found at lower serum dilutions could be absorbed sug-

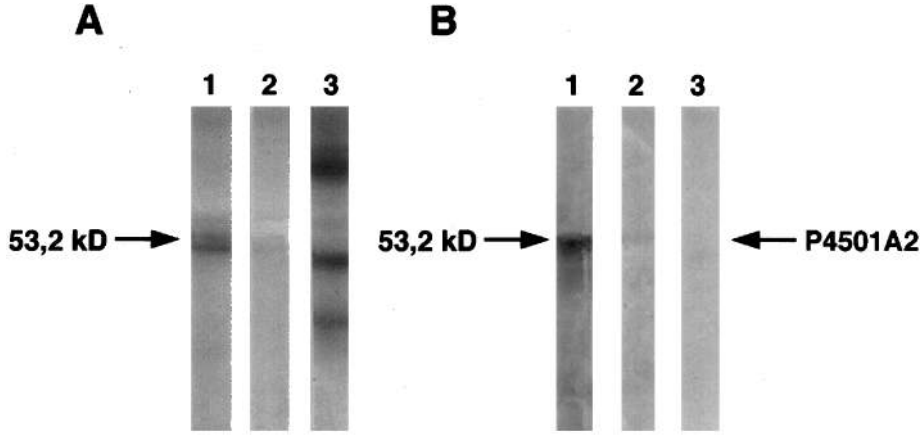

FIG. 6. Absorption experiments with recombinant P450 1A2. A, Immunoblotting using liver microsomes; B, immunoblotting using recombinant P450 1A2; Lane 1, serum of patient 1 before absorption with cytochrome P450 1A2; lane 2, serum of patient 1 after absorption with cytochrome P450 1A2; lane 3, LKM1-positive control serum after absorption with P4501A2.

gests that the autoantibody directed against P450 1A2 shows some cross-reactivity with renal autoantigens.

Discussion

APS1 is characterized by the progressive manifestation of a multitude of endocrine and nonendocrine disease components (1, 2, 3). Frequent disease components, Addison's disease, and premature ovarian failure in females are linked to the presence of adrenal and steroidal cell autoantibodies (9, 14, 25–27). Autoantigens detected by these autoantibodies in APS1 patients are P450 c21, P450 scc, and P450 c17 (5–12). In our study, four of six Sardinian patients with APS1 recognized cytochrome P450 scc, and one patient's serum detected cytochrome P450 c17. This result is in accordance with studies showing that autoantibodies directed against cytochrome P450 scc are produced with a high frequency in Scandinavian patients with APS1 (7, 8, 12); however, other investigators failed to detect autoantibodies directed against P450 scc (10). Differing results also are reported concerning the frequency of detection of P450 c17. Whereas Krohn and his colleges reported the presence of P450 c17 autoantibodies in a substantial fraction of their patients (5, 8, 9), others failed to detect reactivity directed against cytochrome P450 c17 (7, 10, 12). In our study, we confirm that cytochrome P450 c17 is recognized by sera from patients with APS1, albeit at a lower frequency than P450 scc. The identification of the autoantigens was based on apparent molecular weight in SDS-PAGE and on the use of recombinant preparations expressed in *E. coli* (Fig. 3, A–D). There also is some disagreement as to whether P450 c21 is an autoantigen in APS1. Although Uibo *et al.* (8) report P450 c21 being an autoantigen in 15/50 patients with APS1, Winqvist *et al.* (7, 12) failed to detect this autoantibody in APS1. In our group of six patients with APS1, we also did not detect other adrenal autoantigens than P450 scc and P450 c17.

The most important aspect of this paper, however, is the detection and identification of a hepatic autoantigen in patients with APS1. Ten to eighteen percent of APS1 patients develop chronic hepatitis (1–3), and occasionally, patients are reported to have died within only a few days from hepatitis, which occurred unexpectedly and without signs of prewarning (2, 15). However, in contrast to the wealth of information collected by the researchers working on adrenal and gonadal failure in APS1, very little is known about hepatitis as a disease component in APS1. Here we report for the first time the characterization of hepatic autoantigens and the identification of cytochrome P450 1A2 as hepatic autoantigen in APS1. The identification of the autoantigen was facilitated by an immunofluorescence pattern that was different from the patterns resulting from LKM1 and LKM3 autoantibodies. When Western blots were performed with rat microsomes, we failed to detect a specific protein recognized by the antiserum (data not shown). However, using human liver microsomes, a protein band of 52 kDa appeared. This is in accordance with observations published by P. Beaune and his colleges (24), who identified cytochrome P450 1A2 as hepatic autoantigen in dihydralazine hepatitis. In addition, control experiments with intestinal microsomes were performed (data not shown), demonstrating by the absence of the 52-kDa signal that an organ specific autoantigen is detected by the serum of patient 1. To prove that cytochrome P450 1A2 also is an autoantigen in APS1, P450 1A2 was expressed in *E. coli*. The serum not only specifically recognized cytochrome P450 1A2 in Western blot experiments, but absorption with recombinant P450 1A2 resulted in a complete disappearance of the immunofluorescence pattern and the reactivity in Western blots with human liver microsomes. At lower dilutions of the serum, immunofluorescence also was detected in kidney sections, in spite of the fact that P450 1A2 is not expressed in kidney. This result seems to be caused by a cross-reaction of the antibody, because the renal immunofluorescence also could be absorbed with the recombinant cytochrome P450 1A2.

Cytochrome P450 1A2 was described earlier by our group as autoantigen in an unusual case of autoimmune hepatitis (28, 29). This patient suffered from vitiligo, alopecia, and nail dystrophy and had a brother who had died from Addison's disease at the age of 8 yr. We now believe that in accordance with the criteria for diagnosis of APS1 from Neufeld (1), this patient also suffered from APS1. Adding the present patient to that previous one, we now have two patients on record suffering from autoimmune hepatitis in APS1 with cytochrome P450 1A2 as hepatic autoantigen.

Extensive control studies were performed. A total of 40 patient sera with other autoimmune diseases and 22 first-degree relatives of the patients were tested for autoantibodies directed against hepatic antigens and cytochrome P450 1A2. However, only patient serum 1 and 4 sera from patients with other liver diseases reacted with human hepatic microsomes (Fig. 5, Tables 1, 2). When extracts of expression clones containing the recombinant P450 1A2 were used, reaction of only patient serum 1 was detected, showing that the detection of the hepatic autoantibodies and P450 1A2 did not represent false positive results caused by elevated IgG-levels that are typical for patients with autoimmune diseases. Most patients with autoimmune hepatitis type 2 recognize P450 2D6, and about 10% recognize UGT 1 proteins. Of 15 German patients with autoimmune hepatitis type 2, none was found to recognize cytochrome P450 1A2 (data not shown). Determination of the exact molecular target of autoimmunity in patients with autoimmune hepatitis could help to distinguish patients with autoimmune hepatitis-2 from patients with hepatitis in APS1. The later patients should be closely monitored for the development of further disease manifestations.

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