

Antibodies to Conformational Epitopes of Soluble Liver Antigen Define a Severe Form of Autoimmune Liver Disease

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Prevalence and clinical relevance of antibodies to soluble liver antigen (tRNP^{(Ser)Sec}/SLA) in autoimmune hepatitis (AIH) have been investigated using partially purified or prokaryotically expressed antigen. The aim of this study was to improve the detection of anti-tRNP^{(Ser)Sec}/SLA by establishing an immunoassay that was able to identify antibodies directed to conformational epitopes and to investigate the clinical implication of this autoantibody in autoimmune liver disease. By using eukaryotically expressed tRNP^{(Ser)Sec}/SLA as target in a radioligand assay (RLA), 81 patients with autoimmune liver disease (AILD) (33 type 1 AIH, 31 type 2 AIH, and 17 autoimmune sclerosing cholangitis [ASC]), 147 pathologic, and 56 healthy controls were investigated. RLA results were compared with those obtained using a commercial enzyme-linked immunosorbent assay (ELISA) and immunoblot. Reactivity to tRNP^{(Ser)Sec}/SLA was present in 58% of patients with type 1 and type 2 AIH, 41% with ASC, but in only 3 pathologic controls. RLA was similarly disease-specific but remarkably more sensitive than ELISA and immunoblot. A prospective study showed that anti-tRNP^{(Ser)Sec}/SLA-positive patients run a severe clinical course, having worse histology, needing longer to achieve remission, relapsing and requiring liver transplantation or dying more frequently than anti-tRNP^{(Ser)Sec}/SLA negative patients. Anti-tRNP^{(Ser)Sec}/SLA production was favored by the possession of DR3 and A1-B8-DR3 in AIH type 1 and ASC, and prevented by the possession of A2 in all 3 types of AILD, particularly in type 2 AIH. In conclusion, anticonformational tRNP^{(Ser)Sec}/SLA reactivity is frequent in type 1 and type 2 AIH and ASC, defining patients with a worse prognosis. (HEPATOLOGY 2002;35:658-664.)

Autoimmune hepatitis (AIH) is a progressive inflammatory disorder of the liver, characterized by the presence of circulating autoantibodies, histologic evidence of chronic hepatitis, hypergammaglobulinaemia, and response to immuno-

suppression. It is classified into 2 main groups¹: type 1 AIH positive for antinuclear antibodies (ANA) and/or anti-smooth muscle (SMA) antibodies, and type 2 AIH positive for liver kidney microsomal antibody type 1 (LKM1).² A third group of AIH has been proposed in which autoantibodies to soluble liver antigen (SLA) are detected, often in the absence of conventional autoantibodies,³ but there are contrary views as to its distinct identity, and it has not been accepted by the International Autoimmune Hepatitis Group.¹ The target of anti-SLA has been in turn described to be a thermolabile cytosolic protein of pI 5.0,³ liver cytokeratins 8 and 18,⁴ and glutathione S-transferase.⁵ Recently, through the screening of a human lymphoma cDNA library with sera containing high concentration of anti-SLA antibodies, a cDNA clone encoding an enzymatic protein of 50 kd was isolated.⁶ The exact function of this protein is unknown, although there is evidence to suggest its role in serine selenocysteine metabolism.⁷⁻⁹ Sera reacting with this newly isolated clone reacted also with LP (liver/pancreas), another autoantibody target reported in AIH,¹⁰ suggesting that SLA and LP are the same.⁶ Using a human liver cDNA library, a second clone encoding a 35 kd polypeptide fragment of the same protein was immunoisolated.¹¹ In 1992, Gelpí et al. reported that UGA suppressor tRNA-associated antigenic protein (tRNP^{(Ser)Sec}) is an autoantibody target in a particularly severe form of ANA/SMA-positive AIH.⁷ More recently, the same group, immunoscreening a human liver cDNA library, showed that tRNP^{(Ser)Sec} shares 99% identity with the clone identified by Wies et al.,⁶ showing that this

Abbreviations: AIH, autoimmune hepatitis; ANA, anti-nuclear antibody; SMA, anti-smooth muscle antibody; LKM1, anti-liver kidney microsomal antibodies type 1; SLA, soluble liver antigen; LP, liver pancreas antigen; tRNP^{(Ser)Sec}, UGA tRNA suppressor associated antigenic protein; AILD, autoimmune liver disease; ASC, autoimmune sclerosing cholangitis; AST, aspartate aminotransferase; IgG, immunoglobulin G; HAI, histological inflammation activity index; PCR, polymerase chain reaction; RLA, radioligand assay; ELISA, enzyme-linked immunosorbent assay; SLE, systemic lupus erythematosus; RU, relative units; IB, immunoblot.

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protein is the target of SLA.⁸ The investigators of the above studies^{6,8,11} concur that the establishment of assays where the antigen is eukaryotically expressed would be a major advance, because antibodies react preferentially with conformational epitopes.

In this article we report (1) the establishment of a sensitive immunoassay to detect antibodies directed to conformational epitopes on eukaryotically expressed tRNP(Ser)^{Sec}; (2) the comparison between the new eukaryotic system, a prokaryotic system based on the DNA sequence reported by Wies et al.,⁶ and an immunoblot using human liver cytosol¹²; and (3) the clinical implications of the presence of anti-tRNP(Ser)^{Sec} in autoimmune liver disease (AILD).

Materials and Methods

Patients and Controls. Eighty-one patients with AILD were studied (Table 1). All fulfilled diagnostic criteria for "definite" or "probable" AIH.¹ Thirty-three had type 1 and 31 type 2 AIH, including 2 patients, who were autoantibody negative at presentation, but became positive for SMA (1/10) and LKM1 (1/640) 25 and 3 months later, respectively. The remaining 17 patients had autoimmune sclerosing cholangitis (ASC) with characteristic cholangiographic changes¹³ and were positive for ANA and/or SMA. Seventy-three patients had autoantibody titres $\geq 1/80$ at diagnosis. Fifty-five presented with a history of prolonged acute hepatitis, 26 with chronic malaise with or without signs of portal hypertension. Other causes of liver disease were excluded by appropriate investigations.¹⁴ At the time of diagnosis, the median aspartate aminotransferase (AST) level was 327 IU/L (36 to 1,956

IU/L); median bilirubin 58 μ mol/L (5 to 616 μ mol/L) and median immunoglobulin G (IgG) 22.6 g/L (7.4 to 81.5 g/L).

All patients were treated with immunosuppression (prednisolone with or without azathioprine).¹⁴ Forty-seven patients (21 type 1 AIH, 18 type 2 AIH, and 8 ASC) were studied before starting treatment (group A). In 44 of these patients 211 serum samples were tested over a median follow-up time of 22.5 months (21 days to 10 years and 3 months) on a median of 4 occasions (2-18) per patient. A further 34 patients, on immunosuppression at the time of testing (12 type 1 AIH, 13 type 2 AIH, and 9 ASC) represent group B. A total of 120 sera from these 34 patients was obtained between 38 days to 7 years (median 7 months) after diagnosis.

Forty (20 type 1 AIH, 14 type 2 AIH, 6 ASC) of the 44 (91%) children in group A achieved sustained remission (persistently normal transaminases for at least 3 months) after a median of 7 months of treatment (1 to 50 months). Eighteen of these 40 patients (3 type 1 AIH, 10 type 2 AIH, 5 ASC) relapsed 1-4 times (median 1) during follow-up. Nine patients (5 type 2 AIH and 4 ASC) required liver transplantation 4 days to 15 years (median 4 years) after presentation; 1 patient with type 2 AIH died of sepsis 4 months after diagnosis and 1 patient with type 1 AIH died of lymphoma 10 years after diagnosis.

Histologic data were available for 79 patients: 69 underwent the first liver biopsy before starting immunosuppressive treatment, whereas 10 with coagulopathy had a biopsy from 1 to 9 months (median 2.5 months) after starting treatment. Sixty-three of them had moderate or severe, and 16 mild or minimal necroinflammation. A total of 52 follow-up liver biopsies were performed in 29 patients (13 in group A and 16 in group B) over a median of 45 months (4 months to 10 years) from diagnosis. The severity of the necroinflammatory process was semiquantitatively graded on a scale of 0 to 4 (0 = absent; 1 = minimal; 2 = moderate; 4 = severe) and separate scores were obtained for each of the following: portal inflammation, lobular/nodular activity, and interface hepatitis, with the latter defined as biliary and/or lymphocytic type.¹⁵ A histologic inflammatory activity index (HAI) was derived from the sum of the 3 separate scores for the different components of the necroinflammatory lesions (maximum score = 12).¹⁵

As pathologic controls (Table 1) we tested 147 patients with other hepatic or extrahepatic autoimmune disease, virus-induced liver disease, and other non-AILDs. Fifty-six healthy subjects were also investigated. The study was approved by the Ethical Committee of King's College Hospital.

Reverse Transcription Polymerase Chain Reaction. After isolating and reverse transcribing total RNA from HepG2 cells by a QIAGEN RNeasy (QIAGEN Ltd., Crawley, UK), the sequence encoding the tRNP(Ser)^{Sec} (Accession No. AJ238617, henceforth referred to as tRNP(Ser)^{Sec}/SLA) was amplified by polymerase chain reaction (PCR) with the use of the EcoRI-sense and KpnI-antisense primers (5'-CAG AGA ATG GCT GGG ATG AAA₁₁₉₋₁₃₉-3' and 5'-AGA CCA AAG TCT GCT CCT TGAC₁₆₁₆₋₁₆₃₇-3'). The PCR consisted of 35 cycles of denaturation for 1 minute at 94°C, annealing for 1 minute at 55°C, and extension at 72°C for 2 minutes in an automated thermal cycler (Roche Diagnostics Ltd., Lewes, UK) with the use of Super TAQ polymerase (HT Biotech-

Table 1. Demographic Data and Frequency of Reactivity to tRNP(Ser)^{Sec}/SLA in Patients With Autoimmune Liver Disease and Controls

Disease Category	Number of Patients F/M	Age Range (Median) Years	Number of Positive (%)
Autoimmune liver disease	81		44 (54)*
ANA/SMA-positive AIH	22/11	3-17 (13)	19 (58)*
LKM1-positive AIH	26/5	3-20 (8)	18 (58)*
ASC	10/7	3-17 (11)	7 (41)*
Other autoimmune liver disease	20		0
PBC	19/1	26-84 (56)	0
Virus-induced liver disease	40		0
HBV infection	5/15	4-14 (9)	0
HCV infection	8/12	2-15 (8)	0
Nonautoimmune liver disease	41		1 (2)
α 1-antitrypsin deficiency	3/5	2 months-12 (3)	0
Alagille's syndrome	4/6	4-16 (10)	0
Biliary atresia	8/7	1-17 (10)	1
Wilson's disease	2/6	8-16 (11.5)	0
Nonhepatic autoimmune disease	46		2 (4)
Coeliac disease	3/5	2-16 (7)	0
Diabetes	7/3	12-28 (22)	0
Polymyositis	8/4	20-67 (50)	0
SLE	14/2	21-77 (46)	2 (12.5)
Healthy controls	56		0
Children	19/17	5 months-15 (10)	0
Adults	8/12	20-60 (42)	0

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus.

*Significantly higher than control groups, $P < .05$ to $<.00001$.

nology Ltd., Cambridge, UK) and TaqStart antibody (Clontech Laboratories UK Ltd., Basingstoke, UK).

Expression of tRNP^{(Ser)Sec}/SLA in a Eukaryotic System. The PCR product was ligated into the *in vitro* transcription vector pSP72 (Promega Ltd., Southampton, UK). Competent cells (DH5 α , Life Technologies Ltd., Paisley, UK) were transformed with ligation mixture (Rapid Ligation kit, Roche Diagnostics). Following extraction of DNA with a miniprep kit (QIAGEN Ltd.), the nucleotide sequence of the clone was verified by sequencing on an ABI377 sequencer (Perkin-Elmer Corp, Applied Biosystems Div, Foster City, CA). The protein was subsequently expressed in a eukaryotic system (TNT coupled reticulocyte lysate system; Promega Ltd.) and was labelled with 35 S-methionine (10 Ci/mL) (Amersham International, Little Chalfont, UK) during translation. The radioactivity was measured in a MicroBeta counter (EG&G Wallac, Milton Keynes, UK) to evaluate the efficiency of the reaction.

Radioligand Assay for Detection of Antibodies to tRNP^{(Ser)Sec}/SLA. Radioligand Assay (RLA) and characterization of immunoprecipitates were performed by the methods described previously.^{16,17} Patients' sera were diluted 1/50 and incubated with a 10,000 cpm aliquot of recombinant protein. Antibody-bound protein was separated from free antigen by the addition of protein A Sepharose (Pharmacia Biotech, St. Albans, UK). The total immunoprecipitation mixture was then transferred into 96-well filtration plates (Multiscreen Durapore HVPP membranes, Millipore UK Ltd., Watford, UK). The immunocomplex bound Sepharose beads were washed and then fixed to dried multiwell filters by MeltiLex (Millipore UK Ltd.). Radioactivity of the precipitated protein was counted on multiwell filters in a MicroBeta counter (EG&G Wallac).

To characterize the immunoprecipitates, 2 μ L of serum from a patient positive for anti-tRNP^{(Ser)Sec}/SLA were incubated with 35 S-labelled recombinant protein (40,000 cpm) and the immunocomplexes were loaded on a 12% polyacrylamide mini gel (Bio-Rad Laboratories, Hemel Hempstead, UK). Proteins were electrophoretically transferred onto a nitrocellulose filter with a semidry transfer cell (Bio-Rad Laboratories), and the dried membrane was then autoradiographed for 24 hours. A total of 537 sera (334 from 81 patients with AILD and 203 sera from controls) were tested by RLA.

Enzyme-Linked Immunosorbent Assay to Detect Anti-SLA/LP Antibodies. Three hundred-ninety sera tested by RLA (109 positive and 281 negative, see Results) were also tested by a commercial enzyme-linked immunosorbent assay (ELISA) detecting anti-SLA/LP of the IgG class (EUROIMMUN UK Ltd., Cardiff, UK), where the SLA protein prokaryotically expressed using the DNA sequence described by Wies et al.¹⁸ is coated on the wells of a microplate. The samples comprised all the 334 sera from the 81 patients with AILD and 56 control subjects (20 primary biliary cirrhosis, 16 systemic lupus erythematosus [SLE] and 20 normal subjects). According to the manufacturer's instructions, positive values were considered those exceeding 20 relative units per milliliter (RU/mL).

Immunoblot. One hundred fifty-seven sera, tested by RLA and ELISA, were also tested by immunoblot (IB) by using human liver cytosolic fraction as antigen. These comprised 101 sera from

patients with AILD and the 56 control sera tested by ELISA. The cytosolic fraction was prepared according to de Duve et al.¹⁹ as previously described.²⁰ After adjusting the concentration to 4 mg/mL, 250 μ g liver cytosolic protein were separated on a 8-cm 12% polyacrylamide gel and transferred onto a nitrocellulose filter, which was cut into strips and incubated for 2 hours with test sera at a dilution of 1/300 in TNT buffer (10 mmol/L Tris HCl, 150 mmol/L NaCl, 0.05% Tween 20), washed, and then incubated with peroxidase-conjugated rabbit anti-human IgG (DAKO, Copenhagen, Denmark) diluted 1/750 in TNT buffer for 1 hour. Bound antibodies were revealed by adding 50 mg diaminobenzidine, 70 μ L 6% H_2O_2 in 100 mL 0.1 mol/L Tris-HCl, pH 7.5, and the reaction was terminated with excess distilled water.

HLA Typing. HLA typing using standard techniques^{21,22} was performed in 75 patients with AILD (31 with type 1 AIH, 29 with type 2 AIH, and 15 with ASC).

Statistical Analysis. χ^2 and the 1-tailed Fisher's exact tests were used to compare antibody frequencies observed in different groups. The normality of variable distributions was tested by using the Kolmogorov-Smirnov goodness of fit test. Differences in AST, IgG, and bilirubin levels between groups were analyzed by Wilcoxon's rank sum test. Correlation between the titers of anti-tRNP^{(Ser)Sec}/SLA antibodies obtained by the different methods was determined by using Pearson's correlation coefficient on log-transformed normalized data. A *P* value less than .05 was considered significant.

Results

Expression of tRNP^{(Ser)Sec}/SLA in a Eukaryotic System. A 1,518-bp product reverse transcribed from HepG2 cell RNA and encoding a 50 kd protein (Fig. 1) was shown by sequence analysis to be identical to the published sequence of AJ238617 from 119 to 1,637 apart from an uninformative base substitution at position 354 (adenosine instead of thymidine). A total of $44,682 \pm 5,473$ cpm of trichloroacetic acid precipitable protein/ μ L was obtained from

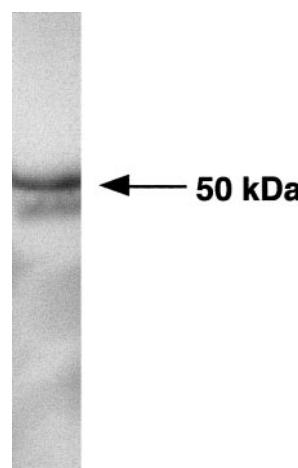


Fig. 1. Immunoprecipitation of *in vitro* translated tRNP^{(Ser)Sec}/SLA. Immunoprecipitation with a serum containing high titer of anti-tRNP^{(Ser)Sec}/SLA antibody (cpm 3575) from a patient with type 1 AIH. The protein (band pointed by an arrow) translated from the tRNP^{(Ser)Sec} cDNA has the predicted size of 50 kd.

the transcription/translation reaction, corresponding to 5.56 ± 0.73 percentage incorporation of ^{35}S -methionine. A pooled translation product was used in the radioligand assay. Values were considered positive when exceeding 1,085 cpm, representing 3 SD (51) above the mean (932) of 56 healthy controls. Of 537 sera tested, 110 were positive, 107 from patients with AILDs (mean cpm 3,125, SD 1,033) and 3 from pathologic controls (cpm 2,818, 2,146, and 2,843 respectively). The levels of cpm in positive cases were significantly higher than those in anti-tRNP^{(Ser)Sec}/SLA-negative AIH patients (mean 904, SD 108) and in pathologic controls (mean 914, SD 110, $P < .001$ for all) (Table 2).

Frequency of Anti-tRNP^{(Ser)Sec}/SLA by RLA. Forty-four (54%) of the 81 patients with AILD were positive for anti-tRNP^{(Ser)Sec}/SLA, the frequency of positivity being similar in type 1 AIH, type 2 AIH, and ASC (Table 1), whereas 37 patients were constantly negative up to 146 months of follow-up. Anti-tRNP^{(Ser)Sec}/SLA antibody-positive patients had significantly higher baseline HAI (median 8, 2-12) than those seronegative (7, 1-12; $P = .005$), but there was no correlation between baseline HAI and titer of anti-tRNP^{(Ser)Sec}/SLA (cpm). At diagnosis there was no significant difference in age, sex distribution, mode of presentation, associated autoimmune disorders, or biochemical abnormalities at the time of antibody testing, between anti-tRNP^{(Ser)Sec}/SLA-positive or -negative patients. Applying the scoring system proposed by the International Autoimmune Hepatitis Group,¹ anti-tRNP^{(Ser)Sec}/SLA positivity was more frequent among patients with "definite" AIH (aggregate scores exceeding 15) than among those with a score less than 15 (27/44 [61%] vs. 13/28 [46%], $P = .01$), the antibody levels correlating with the score ($r = 0.54$, $P < .005$).

In patients tested more than once, 2 patterns of positivity were observed: (1) persistently positive (8 patients on all 39 occasions) and (2) intermittently positive (30 patients on 73/196 occasions). Persistently positive patients tended to have higher HAI scores (4, 2-8) than intermittently positive (2, 0-10, $P = .09$) patients during follow-up, although there was no difference in biochemical abnormality of liver function or in frequency of relapse.

In group A, 21 of 44 patients were anti-tRNP^{(Ser)Sec}/SLA positive at presentation and 11 (52%) became negative during immunosuppressive treatment after a median of 10 months (1.5-12

months). HAI scores were significantly higher in the 21 (48%) seropositive patients both at baseline (9, 3-12) and during follow-up (8, 2-8) when compared with the 23 seronegative (7, 3-10; $P = .01$; and 2, 0-7; $P = .01$, respectively). Time to sustained remission was longer in anti-tRNP^{(Ser)Sec}/SLA antibody positive (16 months, 1-50 months) than negative (5 months, 1-12 months) patients ($P < .001$). Fourteen of the 21 anti-tRNP^{(Ser)Sec}/SLA-positive patients relapsed 1-4 times (median 1) during follow-up, including 5 who had become negative, compared with 4 of the 23 seronegative patients ($P < .001$). Throughout the observation period, titers of anti-tRNP^{(Ser)Sec}/SLA antibodies were correlated with AST, bilirubin, and IgG levels ($r = 0.3$, $P = .02$; $r = 0.35$, $P = .05$; and $r = 0.34$, $P = .05$, respectively).

One of the 2 children with AIH who were negative for autoantibodies at presentation became positive for anti-tRNP^{(Ser)Sec}/SLA antibody at the same time he became positive for LKM1. Four of 6 children who had conventional autoantibody titres $\leq 1/80$ at presentation tested positive for anti-tRNP^{(Ser)Sec}/SLA.

Of the 44 patients positive for anti-tRNP^{(Ser)Sec}/SLA on at least one occasion, 10 died or required liver transplantation compared with 1 of the 37 persistently seronegative patients ($P < .01$). The difference in survival/requirement for transplantation remains significant if the 2 children who died of liver-unrelated conditions (sepsis and lymphoma) are removed ($P = .02$).

Among the 203 controls, only 3 were positive including 1 patient with biliary atresia and 2 with SLE (Table 1).

Correlation Between Anti-tRNP^{(Ser)Sec}/SLA and Other Autoantibodies. During follow-up, ANA/SMA became negative in 22 patients and decreased in titer in 18 of the 50 patients with AIH type 1 or ASC, whereas LKM1 became negative in 5 patients and decreased in titer in 4 of the 31 patients with AIH type 2. Among patients positive for anti-tRNP^{(Ser)Sec}/SLA the titer of this antibody correlated with ANA ($r = 0.173$, $P < .05$) but not with SMA and LKM1.

Anti-SLA/LP by ELISA. Of the 390 sera also tested by commercial ELISA (334 from AILD patients and 56 from controls), 281 were RLA seronegative and 109 RLA seropositive (107 from AILD and 2 from SLE patients). Ten of the RLA-positive sera were also positive by ELISA (9 sera from 4 patients with type 1 AIH, 1 with ASC, and 1 with SLE). Four additional RLA-positive sera (from 3 patients with type 2 AIH) had values (10, 11, 11, and 13 RU/mL) below the manufacturer's cutoff point (20 RU/mL) but clearly distinct from the background noise of the assay (mean \pm SD: 4.1 ± 2 RU). Of the 281 seronegative cases, 280 were also negative by ELISA, whereas 1 from a patient with type 1 AIH was positive with a value of 33 RU/mL. Three additional sera from the same patient, including 1 obtained 20 days after the positive result, tested negative by both ELISA and RLA. There was a high degree of association between the results of the two immunoassays ($\chi^2 = 35$, $P < .0001$), and the cpm of RLA was strongly correlated with RU of the ELISA ($r = 0.88$, $P < .001$).

Immunoblot. Of 157 sera also tested by IB, (101 from AILD patients and 56 from controls), 100 were RLA seronegative and 57 RLA seropositive (55 from AILD and 2 from SLE patients). Of the 57 RLA-positive sera, 28 were positive by IB for a 50 kd cytosolic band (Fig. 2); of the 100 RLA-negative sera, 98 were negative whereas 2 reacted with the 50 kd band ($\chi^2 = 52$, $P < .0001$).

Table 2. Comparison of the Three Immunoassays for Detecting Anti-tRNP^{(Ser)Sec}/SLA Antibodies in Patients With Autoimmune Liver Disease and Controls

Category	Number of Sera: Positive/Tested		
	Radioligand Assay	ELISA	Immunoblot
Autoimmune liver disease	107/334	13/334	28/101
ANA/SMA-positive AIH	34/115	8/115	13/40
LKM1-positive AIH	61/166	*4/166	10/42
ASC	12/53	1/53	5/19
Pathologic controls	3/147	1/36	3/36
20 PBC	0/20	0/20	1/20
15 biliary atresia	1/15		
16 SLE	2/16	1/16	2/16
Other pathologic controls	0/96		
Normal controls	0/56	0/20	0/20

Abbreviation: PBC, primary biliary cirrhosis.

*Four sera with anti-SLA titer between 10 and 20 relative units.

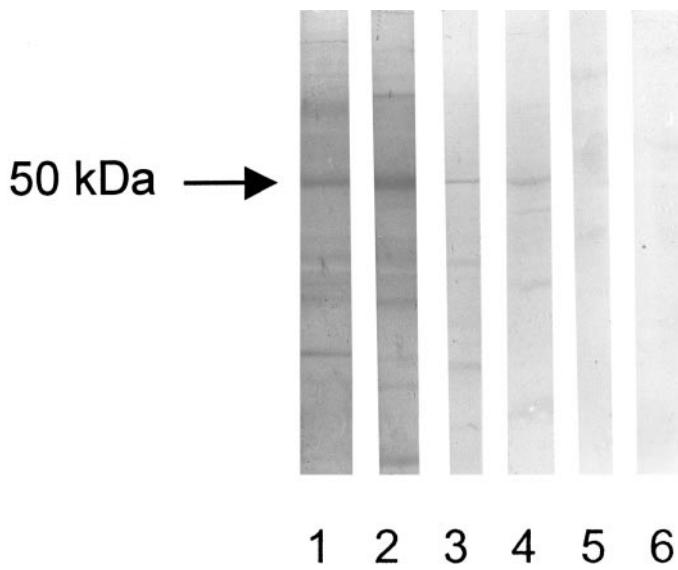


Fig. 2. Immunoblot of human liver cytosol probed with patients' sera. A 50 kd band is seen in lane 1-4 and absent in lanes 5 and 6. **Lane 1 and 2:** patients with autoimmune hepatitis type 1; **Lane 3:** patient with systemic lupus erythematosus. These 3 sera were also positive by radioligand assay and ELISA. **Lane 4:** patient with autoimmune hepatitis type 1 also positive by radioligand assay, but not by ELISA. **Lane 5:** the only patient positive by ELISA and negative by radioligand assay; **Lane 6:** healthy control.

Of 9 sera positive by both RLA and ELISA, 7 were also positive by IB. Of the 100 sera negative by RLA and ELISA, 2 were positive by IB ($\chi^2 = 55, P < .0001$).

Association Between HLA and Anti-tRNP^{(Ser)Sec}/SLA. Seventy-five patients with AILD had HLA A1, A2, B7, B8, DR3, and DR4 typed. Age and sex distribution was similar among patients with different HLA. Of the 75 patients, 42 (19 type 1 AIH, 17 type 2 AIH, and 6 ASC) were positive for anti-tRNP^{(Ser)Sec}/SLA on at least one occasion and 33 (12 type 1 AIH, 12 type 2 AIH, and 9 ASC) were negative. Comparing anti-tRNP^{(Ser)Sec}/SLA-positive with -negative patients, DR3 was more frequent and A2 less frequent within the undivided population (21/38 vs. 10/31, $P = .056$; 13/42 vs. 16/31, $P = .07$, respectively); A1-B8-DR3 more frequent in type 1 AIH (11/18 vs. 3/11, $P = .07$); A2 less frequent in type 2 AIH (5/17 vs. 9/11, $P = .007$); DR3 and A1-B8-DR3 more frequent in type 1 AIH and ASC grouped together in view of their identical autoimmune serology (15/23 vs. 5/18, $P = .017$; 11/23 vs. 3/20, $P = .02$, respectively).

Discussion

Having established a new radioligand assay, we show that reactivity to eukaryotically expressed tRNP^{(Ser)Sec}/SLA is present in over half of the patients with type 1 and type 2 AIH and in 41% of those with ASC, but is rare in other disorders. Moreover, we show that anti-tRNP^{(Ser)Sec}/SLA positivity at presentation predicts a more severe clinical course.

Anti-tRNP^{(Ser)Sec}/SLA in AIH type 1 has been reported before, though at a lower frequency than in our series, whereas its presence in AIH type 2 and ASC is a novel finding. All but 2 of the previous reports investigating anti-tRNP^{(Ser)Sec}/SLA focused on type 1 and

autoantibody-negative AIH.^{6,8,23,24} In type 1 AIH anti-tRNP^{(Ser)Sec}/SLA reactivity is 58% in our series, 47% using an immunoprecipitation assay based on HeLa cell extracts,⁸ and down to 13.4%²³ and 12%²⁴ using a modification of the original inhibition ELISA.³ In other studies the frequency within type 1 AIH is not specified.^{6,11} The higher prevalence of anti-tRNP^{(Ser)Sec}/SLA in our series can be attributed to timing of testing, characteristics of the detection assay, and the age of the patients. Although most of our patients were tested at diagnosis, all or the majority^{3,24} of those in previous studies were tested during immunosuppression. This, as we show, leads to disappearance of anti-tRNP^{(Ser)Sec}/SLA in about half of the positive patients within 1 year from starting treatment. Another possible reason is the use of eukaryotically expressed human protein as target of anti-tRNP^{(Ser)Sec}/SLA, which, as anticipated by other investigators,^{6,8,11} accounts for a higher specificity and sensitivity, because antibodies react preferentially with conformational epitopes. Lastly, in a cross-sectional study including patients from 3 to 72 years of age, 70% of those anti-tRNP^{(Ser)Sec}/SLA positive were below the third decade²⁴: all our patients were younger than 30 years of age when first tested.

The only 2 previous studies including LKM1-positive patients could not detect anti-SLA in any of them by inhibition ELISA.^{3,24} In the first study, however, sera had been heat inactivated before testing and the target antigen was not a recombinant protein but was derived from the supernatant of rabbit liver homogenate following ultracentrifugation.³ Similarly, in the second study, the target antigen was neither recombinant nor of human origin but was derived from rat liver.²⁴ Four sera from our patients with type 2 AIH positive by RLA, had ELISA readings below the manufacturer's cutoff point, although clearly distinct from the background noise of the assay, suggesting that LKM1-positive patients may be detected in a prokaryotically-based ELISA, if the arbitrary upper limit of normal is redefined, but that they react more readily with conformational epitopes.

We were unable to confirm that anti-SLA is a marker of autoantibody negative AIH or cryptogenic cirrhosis.^{6,24} Among our 64 patients with AIH, all those who were anti-tRNP^{(Ser)Sec}/SLA positive were also positive for conventional autoantibodies, whereas the only 2 autoantibody-negative patients at diagnosis were also negative for anti-tRNP^{(Ser)Sec}/SLA. Interestingly, one of them became positive for both LKM1 and tRNP^{(Ser)Sec}/SLA during follow-up. One explanation for the low number of autoantibody-negative patients in our series is that they presented in childhood, where low titers of autoantibodies are significant for the diagnosis of AIH.¹ In previous reports on anti-tRNP^{(Ser)Sec}/SLA reactivity in AIH, even when children were included,²⁴ conventional autoantibodies were variably considered negative if the titer was less than 1/40 or less than 1/80^{23,24} in breach of the recommendations of the International Autoimmune Hepatitis Group.¹ Had we chosen an arbitrary cutoff point of 1/80, 4 of our patients would have been only anti-tRNP^{(Ser)Sec}/SLA positive at presentation.

Besides AIH, we have found a high frequency of anti-tRNP^{(Ser)Sec}/SLA positivity among patients with ASC. This is not surprising, because it has been recently shown that AIH and ASC, at least in young patients, share clinical, biochemical, immunologic, histologic, and response to treatment features, the differential diagnosis between the 2 conditions often being possible only if a cholangio-

gram is performed at presentation.¹³ It is therefore conceivable that at least some of the anti-tRNP^{(Ser)Sec}/SLA-positive patients included in previous studies did in fact have ASC. Interestingly, positivity for anti-tRNP^{(Ser)Sec}/SLA has been reported in some patients with primary biliary cirrhosis/AIH overlap syndrome.²⁵

Presence of anti-tRNP^{(Ser)Sec}/SLA is influenced by the patient's HLA, its production being favored by the possession of DR3 and A1-B8-DR3 in AIH type 1 and ASC, and prevented by the possession of A2 in all 3 types of AILD, particularly in type 2 AIH. These results are in partial agreement with Kanzler et al.,²³ who noted an increased frequency of A1 and B8, but not DR3 in anti-SLA-positive patients.²³

To evaluate the performance of the newly established RLA, we compared it with a commercial ELISA, where the antigen is prokaryotically expressed from the clone isolated by Wies et al. from a cDNA lymphoma cell line.¹⁸ Only 10 of 109 sera positive by RLA gave results above the cutoff point provided by the manufacturer. Even considering positive the 4 additional RLA-positive sera giving ELISA readings clearly above the background, the sensitivity of ELISA remains vastly lower than that of RLA. Only one of 281 RLA-negative sera was positive by ELISA, indicating that the 2 tests have the same specificity. It is possible that the only patient positive by ELISA and negative by RLA was a false-positive patient, because 3 follow-up sera were negative, including one obtained 20 days after the positive sample, by which time negativity would not be expected in view of the 21- to 28-day half-life of IgG antibodies. In a recent study Volkmann et al.,¹¹ using sera selected for anti-SLA positivity by the original inhibition assay,³ show that the commercial ELISA fails to detect 25% of the patients. The sensitivity gap between the commercial ELISA and RLA is even greater, with ELISA being able to detect only 1 in 10 RLA-positive samples.

By immunoblot, half of the RLA-positive sera reacted with a 50 kd protein contained in the human cytosolic liver fraction, whereas only 2 of 100 RLA-negative sera did so. This finding shows, first, that, in agreement with the original report,³ an antigen of 50 kd is the major target of anti-tRNP^{(Ser)Sec}/SLA, and, second, that RLA is more sensitive than IB, the latter technique including a conformation-altering denaturation step.

Our prospective study shows the clinical relevance of anti-tRNP^{(Ser)Sec}/SLA positivity. While anti-tRNP^{(Ser)Sec}/SLA does not define a third distinct type of AIH, it does identify patients who have a severe clinical course, irrespectively of the underlying autoimmune liver disease. Not only did anti-tRNP^{(Ser)Sec}/SLA-positive patients have a histologically more active disease, but also needed longer to achieve remission, relapsed, and required liver transplantation or died more frequently than anti-tRNP^{(Ser)Sec}/SLA-negative patients. This, together with the observation that anti-tRNP^{(Ser)Sec}/SLA titers correlate with laboratory evidence of disease activity, indicates that anti-tRNP^{(Ser)Sec}/SLA estimation might be a useful marker of liver damage in AILD.

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