Gut, 1980, **21**, 955–961

Autoantibodies against liver-specific membrane lipoprotein in acute and chronic liver diseases: studies on organ-, species-, and disease-specificity

M MANNS, K-H MEYER ZUM BÜSCHENFELDE,* AND G HESS

From the Freie Universität Berlin—Klinikum Charlottenburg, Department of Internal Medicine, Berlin, Federal Republic of Germany

SUMMARY A double antibody radioimmunoprecipitation test was used to detect anti-human LSP, anti-rabbit LSP, and autoantibodies against the human kidney equivalent of LSP (anti-HKP) in patients' serum. Anti-human LSP was found in 27/62 cases with chronic active liver disease (CALD), 9/16 cases with chronic persistent hepatitis (CPH), and 14/33 patients with acute viral hepatitis (AVH), 2/10 patients with 'inactive' cirrhosis of the liver (Ci), 4/14 patients with alcohol induced liver disease (ALD), 1/7 patients with miscellaneous liver diseases (MLD), and in 6/58 patients with primary non-hepatic autoimmune disease (PNHA). Frequencies of anti-LSP did not depend on HBsAg status. Anti-rabbit LSP was detected in only 9% of patients with AVH as compared with 42% for anti-human LSP. No such difference was observed in the other groups of patients. Anti-HKP was found in 6/62 patients with CALD, 1/7 patients with MLD, and 2/58 patients with PNHA; no anti-HKP occurred in patients with CPH, AVH, ALD, and Ci. The frequency of anti-LSP was not correlated with the presence of non-organ-specific autoantibodies in patients with CALD; furthermore, no correlation with sex-distribution, age, gammaglobulin levels, and SGOT occurred in this group of patients. No correlation existed between anti-LSP and liver membrane autoantibodies detected by indirect immunofluorescence on isolated rabbit hepatocytes (LMA). The reported data show that naturally occurring anti-LSP, characteristic for acute and chronic inflammatory liver diseases, are mostly directed against organ-specific determinants of the LSP complex. It is suggested that the occurrence of antibodies to species-specific determinants of LSP reflects a transient state of autoimmunity. The LMA immunofluorescence test seems to detect antibodies against other liver membrane antigens as well as LSP.

Indirect evidence suggests that an antibody dependent cell mediated immunity is involved in the immunopathogenesis of acute and chronic liver diseases.[1–4] As the cytotoxicity was blocked by the addition of small amounts of liver specific membrane lipoprotein (LSP),[1,2] it was suggested that the damage to the hepatocytes might be caused by an immunological reaction against LSP. Recently, Jensen *et al.*[5] and Kakumu *et al.*[6] described the detection of circulating autoantibodies to LSP by radioimmunoprecipitation (RIP). Anti-LSP was frequently found in sera of patients suffering from acute and chronic inflammatory liver diseases independent of their HBsAg status. Hopf *et al.*[7] detected circulating liver membrane autoantibodies in human sera by an indirect immunofluorescence technique (LMA). In a subsequent study[8] LMA were closely related to HBsAg negative chronic inflammatory liver diseases.

In the present study we used a modified version of the RIP described by Kakumu *et al.*[6] to detect circulating autoantibodies to LSP in patients' sera. The aim of this study was to further evaluate the disease-specificity, species-specificity, and organ-specificity of anti-LSP and to compare these data with the results obtained by the LMA immunofluorescence test.

*Address for correspondence: Professor Dr K-H Meyer zum Büschenfelde. Freie Universität Berlin—Klinikum Charlottenburg, Abteilung für Innere Medizin und Poliklinik, Spandauer Damm 130, 1000 Berlin 19/Fed. Rep. Germany.

Received for publication 26 June 1980

Methods

PATIENTS

Sera were obtained from 31 healthy subjects, 142 patients with various liver diseases, and 58 patients with primary non-hepatic autoimmune diseases. Among the patients with liver diseases 33 had acute viral hepatitis, 16 had chronic persistent hepatitis (CPH), 55 patients had chronic active hepatitis (CAH), and seven patients had non-alcoholic cirrhosis of the liver with piecemeal necrosis. These patients with 'active' cirrhosis and the 55 patients with CAH were included in the group of chronic active liver diseases (CALD). Patients with chronic hepatitis were submitted to biopsy and classified according to the criteria of De Groote *et al.*[8a] Ten patients had histological signs of 'inactive' non-alcoholic cirrhosis of the liver, 14 had alcoholic liver disease, and among the seven patients with miscellaneous liver disease three had fatty livers, two bacterial cholangitis, one had halothane hepatitis, and one suffered from hepatocellular carcinoma; the latter disease coexisted with non-alcoholic HBsAg negative cirrhosis. Among the 58 patients with primary non-hepatic autoimmune diseases 10 had Crohn's disease, four had ulcerative colitis, four had rheumatoid arthritis, 13 had Grave's disease, 13 patients had juvenile onset diabetes mellitus, five had systemic lupus erythematosus, six had Hashimoto-thyroiditis, one panarteriitis nodosa (this patient was an HBsAg-carrier), one patient had polymyalgia rheumatica, and one had primary Sjøgren-syndrome. Human sera were heat inactivated at 56°C for 30 minutes and stored at -20°C until used.

ASSAY

Antibodies to LSP were determined according to the method of Kakumu *et al.*[6] using some modifications. Briefly, human liver specific protein (HLSP), rabbit liver specific protein (RLSP), and the equivalent protein preparation from human kidney (HKP) were prepared according to the procedure described by McFarlane *et al.*[7a] using the first peak of Sepharose 6 B chromatography as antigen preparation. Twenty-five microlitres each of HLSP, RLSP, or HKP (protein concentration 0·3 mg/ml) were iodinated with 1 mCi of I^{125} by the chloramine T-method.[9] The reaction mixture was subjected to gel-chromatography on Sephadex G 100 (gel bed 0,5 × 10 cm). The column was coated with 3% bovine serum albumin in Tris EDTA buffer, the column was eluted with Tris EDTA buffer, pH 8; 250 µl fractions were collected in plastic tubes containing 250 µl Tris EDTA buffer, containing 3% BSA. The fractions of the first peak, representing protein bound iodine that contained the highest I^{125} reactivity, were pooled and used as test antigen in the radioimmunoprecipitation test. Based on a calculation of the average proportion of protein bound iodine eluted from the gel, the specific activity of labelled LSP was approximately 60 mCi/mg.

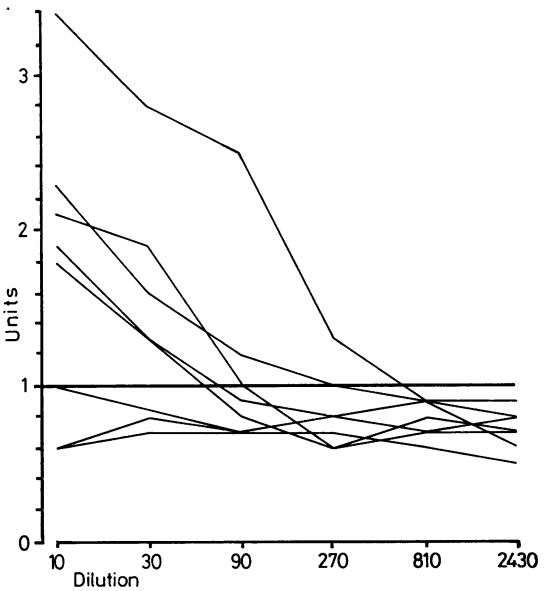

Fig. 1 *Titration curves for five anti-LSP positive sera from patients with CALD and for three healthy controls. Human LSP served as antigen. Each point represents the mean of three different experiments. Horizontal line represents the upper limit of normal range.*

The assay was done in triplicate with disposible plastic tubes. To 500 µl of Tris EDTA buffer, pH 8, containing 0·25% BSA 50 µl of test serum diluted 1:10 in Tris EDTA buffer and 100 µl of labelled antigen (32 000 cpm), containing approximately 1 ng LSP, were added. The mixture was left for 72 hours at 4°C, then 100 µl of anti-human IgG prepared in rabbits (Hyland Travenol Laboratory, Munich, Germany), diluted 1:10 in Tris EDTA, were added as second antibody. After another 48 hours at 4°C tubes were centrifuged for 20 minutes at 3000 rpm in a Sorvall centrifuge. The supernatants were removed and the precipitate was counted in a Beckman Gamma-counter. Titration curves of representative sera from patients with CAH and healthy controls diluted in normal human serum (1:10) are presented in Fig. 1. Each value represents the mean of three different experiments. LSP was labelled within four weeks after preparation, labelled LSP was used within four weeks after iodination.

Units were calculated to allow comparison of

results from different experiments and to give a semiquantitative measurement

$$\text{Units} = \frac{\text{cpm of serum sample tested}}{\text{mean of healthy controls} + 2 \text{ standard deviations}}$$

Seven to 10 healthy subjects were used as control sera in each test procedure. Data from normal persons used as controls in different assays for anti LSP show a range from 308–650 cpm. Positive internal controls were included in each test procedure. When fresh LSP were labelled as described above and used within four weeks after iodination, units of internal controls did not vary by more than 10%. In separate experiments the IgG-association of anti LSP was proved, when anti-human IgG serum was replaced by normal rabbit serum as second antibody. Units 1·1 and above were regarded positive.

SEROLOGICAL TESTS

Antinuclear antibodies (ANA), antimitochondrial antibodies (AMA) and smooth muscle antibodies (SMA) were tested by indirect immunofluorescence and examined under a Leitz-Orthoplan fluorescent microscope.[7 7a] Antigammaglobulin factors (AGF) were tested by latex-fixation test (Hyland Laboratories, Munich, Germany). HBsAg was tested using a commercial radioimmunoassay (AUSRIA II from Abbott Laboratories, Chicago, USA). Liver membrane autoantibodies were determined by indirect immunofluorescence using isolated rabbit hepatocytes as described earlier.[7]

Statistical methods

Student's t-test, chi-square test and Wilcoxon rank test, where appropriate, were used to analyse data.

Results

ANTIBODIES TO HUMAN LSP:

Antibodies to human LSP were detected in 27/62 (44%) of patients with CALD, 9/16 (56%) patients with CPH, 14/33 (42%) patients with acute viral hepatitis, and 2/10 patients (20%) with 'inactive' cirrhosis of the liver. The incidence of anti-LSP was similar for HBsAg positive and HBsAg negative cases (Table 1, Fig. 2). Anti-LSP was also found in 4/14 patients (28%) with alcohol-induced liver diseases. These four anti-LSP positive cases all showed periportal inflammation on liver biopsy. Another two patients with periportal inflammation were anti-LSP negative. The rest, eight cases, were anti-LSP negative and had no periportal inflammation. One of seven patients with miscellaneous liver diseases who suffered from hepatocellular carcinoma accompanied by non-alcoholic cirrhosis of the liver was anti-LSP positive as well as 6/58 (10%) patients with primary non-hepatic autoimmune diseases. From these six anti-LSP positive patients with non-hepatic autoimmune disorders one had rheumatoid arthritis, one had Crohn's disease, two suffered from ulcerative colitis, and two from Grave's disease. None of 13 patients with juvenile onset diabetes mellitus and of 31 healthy controls had anti-LSP. Patients with primary non-hepatic autoimmune disorders had no clinical or biochemical signs of liver disease. Units of anti-human LSP in various hepatic and non-hepatic diseases are demonstrated in Fig. 3.

Table 1 *Frequency of autoantibodies against human and rabbit liver specific protein (anti-H-LSP and anti-R-LSP)*

Diagnosis	*No. of cases*	*Cases with*			
		Anti-H-LSP		*Anti-R-LSP*	
		(no.)	*(%)*	*(no.)*	*(%)*
Chronic liver disease	62	27	44	18	29
HBsAg positive	36	13		8	
HBsAg negative	26	14		10	
Chronic persistent hepatitis	16	9	56	8	50
HBsAg positive	10	6		5	
HBsAg negative	6	3		3	
Acute viral hepatitis	33	14	42	3	9
HBsAg positive	18	7		0	
HBsAg negative	15	7		3	
Cirrhosis of liver	10	2	20	1	10
HBsAg positive	2	0		0	
HBsAg negative	8	2		1	
Alcohol-induced liver diseases	14	4	29	2	14
Miscellaneous liver diseases	7	1	14	0	0
Primary non-hepatic autoimmune diseases	58	6	10	2	3
Juvenile onset diabetes mellitus	13	0		0	
Crohn's disease	10	1		0	
Ulcerative colitis	4	2		0	
Chronic thyroiditis	19	2		1	
Miscellaneous autoimmune diseases	12	1		1	
Healthy blood donors	31	0	0	0	0

Within the group of CALD anti-LSP was not correlated with sex distribution and the presence of non-organ-specific autoantibodies (ANA, AMA, SMA) when tested by chi-squared test ($P > 0.05$). Furthermore, no significant differences were apparent in serum gammaglobulin or glutamic-oxaloacetate transaminase (SGOT) levels in those with and those without detectable anti-LSP ($P > 0.05$) by Wilcoxon's rank test (Table 2).

Eleven of 26 patients with HBsAg negative CALD were receiving immunosuppressive therapy at the time when blood samples were taken. Of these cases, six were anti-LSP positive and five were anti-LSP negative. In all cases treatment had been given for

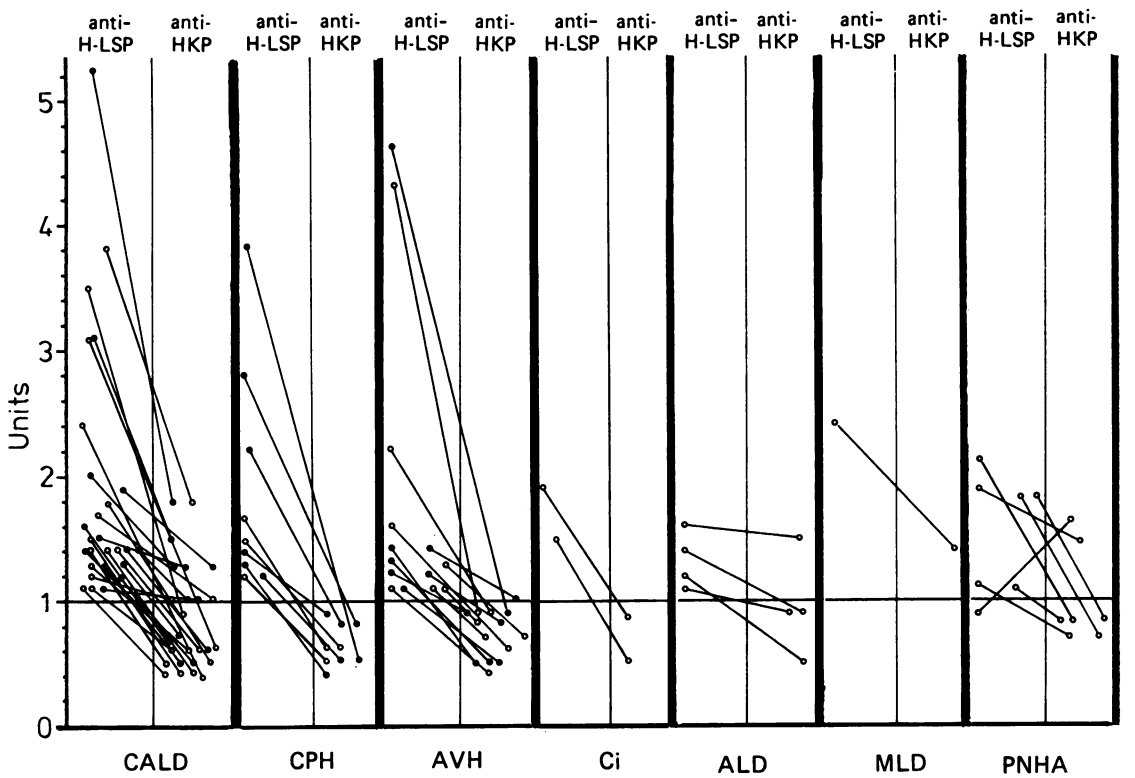

Fig. 2 *Comparison of the results of anti-human LSP (left) and those obtained with the equivalent kidney protein fraction HKP (right) in the different groups of patients. Only anti-LSP or anti-HKP positive cases were selected. AVH: acute viral hepatitis, Ci: cirrhosis of the liver, ALD: alcohol induced liver disease, MLD: miscellaneous iver diseases, PNHA: primary non-hepatic autoimmune diseases; ●: HBsAg positive, ○: HBsAg negative cases.*

not longer than six months. No patients from other groups of liver diseases were treated with immunosuppressive drugs.

ANTIBODIES TO RABBIT LSP

Antibodies to rabbit LSP were detected in 18 of 62 patients (29%) with CALD, 8/16 patients (50%) with CPH, 3/33 patients with acute viral hepatitis (9%), 1/10 patients with cirrhosis (10%), and 2/14 patients (14%) with alcoholic liver disease; none of the patients with miscellaneous liver diseases had anti-LSP, whereas 2/58 (3%) patients with primary non-hepatic autoimmune diseases had anti-rabbit LSP (Table 1). All 31 healthy controls were negative for anti-rabbit LSP. The occurrence of anti-rabbit LSP did not depend on HBsAg status. All patients positive for anti-rabbit LSP were positive for anti-human LSP as well.

ANTIBODIES TO HKP: KIDNEY EQUIVALENT OF LSP

When radioimmunoprecipitation was performed with labelled HKP as antigen, antibodies were detected in 6/62 (10%) patients with CAH, 1/7 (14%) patients with miscellaneous liver diseases, and 1/14 (7%) patients with alcoholic liver disease. Of the 58 patients with primary non-hepatic autoimmune disorders two (3%) had anti-HKP, one of the two suffered from rheumatoid arthritis and one from systemic lupus erythematosus; the latter was the only patient reacting with HKP who did not cross-react with liver specific protein. No anti-HKP was detectable in sera from patients with CPH, acute viral hepatitis, 'inactive' cirrhosis of the liver, and in healthy controls. Figure 2 demonstrates the comparison of results for anti-H-LSP and anti-HKP in patient sera reacting with human LSP or HKP.

LIVER MEMBRANE AUTOANTIBODIES DETECTED BY LMA IMMUNOFLUORESCENCE TEST

LMA was found in 12/26 (46%) patients with HBsAg negative CALD and in 3/8 (38%) patients with HBsAg negative cirrhosis of the liver (Fig. 3). One of 10 patients with Crohn's disease and one of

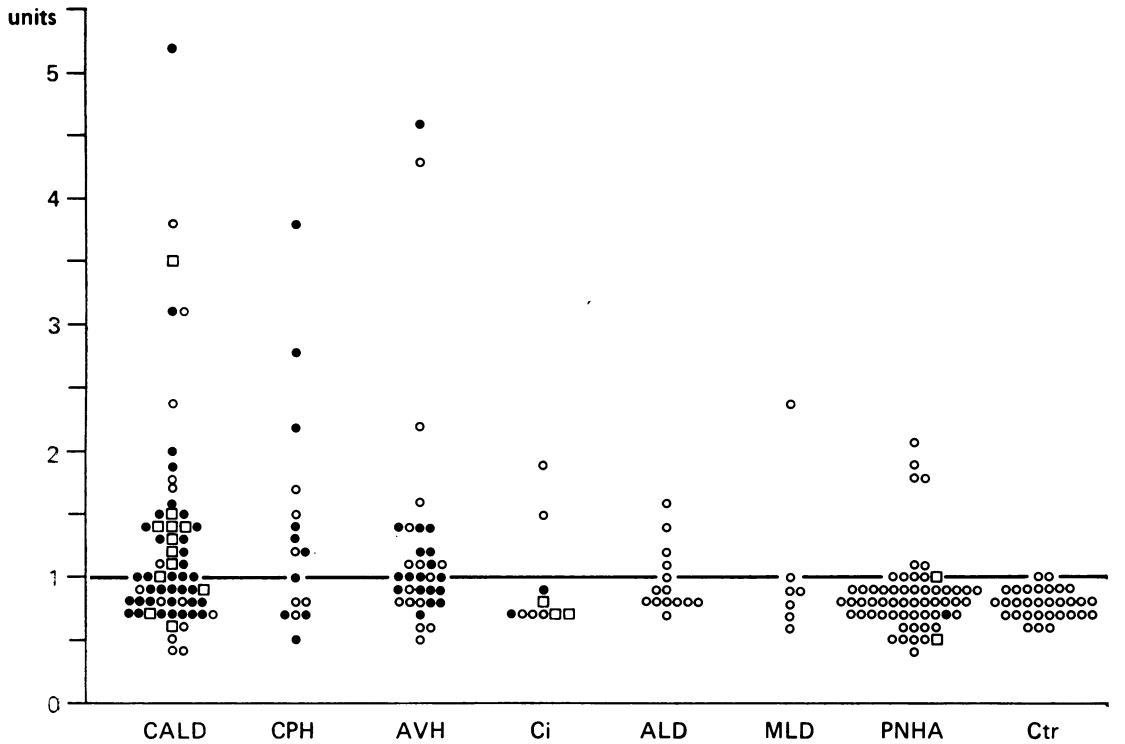

Fig. 3 *Units of anti-human LSP in sera of patients with various liver diseases, primary non-hepatic autoimmune diseases (PNHA) and healthy controls (Ctr), including a comparison of anti-LSP with the results obtained by the LMA-immunofluorescence test.* ●*: HBsAg positive, LMA negative;* ○*: HBsAg negative, LMA negative;* □*: HBsAg negative, LMA positive. Horizontal line represents upper limit of normal range. For abbreviations of liver diseases see legend to Fig.2.*

Table 2 *Clinical and serological data on patients with chronic active liver disease*

	Patients with anti-LSP (no.=27)	*Patients without anti-LSP (no.=35)*
Sex (F:M)	17:10	20:15
Mean age (yr)†	54,3±15,7	45,7±17,7
HBsAg positive	48%	64%
Gammaglobulin (g/l)†	20,4±9,3	18,7±5,4
SGOT (U/l)†	103±257	37±31
Autoantibodies present*	72%	54%

*One or more of the non-organ-specific autoantibodies ANA, AMA or SMA were detectable in serum of patients.
†Mean ± standard deviation.

Table 3 *Comparison of LMA-immunofluorescence test with radioimmunoprecipitation-tests for anti-human LSP and anti-rabbit LSP*

	LMA+	*LMA-*
Anti-human LSP positive	8	55
Anti-human LSP negative	9	159
Anti-rabbit LSP positive	1	33
Anti-rabbit LSP negative	16	181

five patients suffering from systemic lupus erythematosus had LMA as well, but no anti-H-LSP, anti-R-LSP, or anti HKP was detectable in these sera. No correlation was observed between the LMA immunofluorescence test and anti-rabbit LSP or anti-human LSP (Table 3).

Discussion

The data on anti-LSP in acute and chronic hepatitis reported in this paper are in agreement with those presented by Jensen *et al.*[5] and Kakumu *et al.*[6] The incidence for anti-LSP was similar for HBsAg positive and HBsAg negative cases (Table 1). No correlation was observed between the incidence of anti-LSP and age, sex, gamma globulin levels and the presence of non-organ-specific autoantibodies (ANA, AMA, SMA) for CALD. Furthermore, there was no statistically significant difference in SGOT levels between the groups of anti-LSP positive and anti-LSP negative cases, although SGOT levels tended to be higher in the anti-LSP

positive group (Table 2). From the data presented it cannot be determined whether immunosuppressive therapy affects anti-LSP. This has to be evaluated in a future controlled prospective trial. In agreement with Perperas *et al.*[10] we observed a low incidence of anti-LSP in alcoholic liver disease. Anti-LSP were rarely found in patients with primary non-hepatic autoimmune diseases (6/58 patients). Although these six patients did not show clinical or biochemical evidence of liver disease, accompanying liver diseases do occur in some of these diseases—that is, ulcerative colitis, Crohn's disease, systemic lupus erythematosus.

Recently we reported on the detection of species-specific and non-species-specific determinants of the LSP-complex.[11] These determinants could be demonstrated by a sheep anti-human LSP serum and by sera from rabbits with experimentally induced CAH. Therefore rabbit LSP was included as test antigen in this study to evaluate whether species-specific and/or non-species-specific determinants of LSP are targets in human liver diseases. The highest incidence of anti-rabbit LSP was observed in CAH, CPH, and Ci, whereas in AVH only 9% were positive compared with 42% for anti-human LSP.

This is an interesting observation, as autoimmunity to LSP was found to be transient in uncomplicated AVH.[5 6] We suggest that humoral immunity to non-species-specific determinants of LSP reflect self-perpetuating autoimmunity, whereas antibodies to the species-specific determinant of LSP reflect a transient state of auto-immunity. This observation needs further clarification.

The present preparation of LSP is far from being a pure antigen, and additional purification seems necessary. It has to be evaluated whether organ-specific, non-organ-specific, species-specific, and non-species-specific determinants are located on a single molecule, on different molecules, or whether we are dealing with a real antigen complex.

Kakumu *et al.*[6] reported a lack of absorption of anti-LSP by kidney proteins in five sera. Investigations on the organ-specificity of anti-LSP were not reported by Jensen *et al.*[5] Only a few sera reacted with the LSP equivalent prepared from human kidneys (HKP), whereas most of the sera positive for anti-HKP were positive for anti-LSP as well; one patient with SLE had antibodies only to HKP (Fig. 2). These data show that naturally occurring autoantibodies to LSP are mostly directed against organ-specific determinants of LSP and not against non-organ-specific components of the LSP complex, as could be concluded from studies done by Behrens and Paronetto.[12] Nevertheless, at the present stage of LSP purification, studies on humoral and cell-mediated immunity to LSP in liver diseases should include appropriate controls with HKP. This has often been omitted in previous investigations when authors did not distinguish between LSP, the molecule, and the antigen preparation of LSP. Future investigations should therefore concentrate on further characterisation and purification of the organ-specific moiety of the 'antigen complex' LSP.

It has been suggested previously that liver membrane autoantibodies demonstrated by the LMA immunofluorescence test may represent anti-LSP.[5 6 13] This could be excluded by the data presented in this paper. Two different investigations support these findings and show that other membrane antigens beside LSP are targets for circulating autoantibodies in chronic inflammatory liver diseases.[14 15]

This work was supported by the Deutsche Forschungsgemeinschaft, Bonn, Me 244/11. We are indebted to Miss U Dang for excellent technical assistance. We thank Mr Dipl Math Gerhard G Thomas from the Institut für Medizinische Statistik und Dokumentation, Freie Universität Berlin, for his advice on the statistical analysis.

References

[1]Thomson AO, Cochrane AMG, McFarlane IG, Eddleston ALWF, Williams R. Lymphocyte cytotoxicity to isolated hepatocytes in chronic active hepatitis. *Nature* 1974; **252**: 721–2.

[2]Cochrane AMG, Moussouros A, Thomson AD, Eddleston ALW, Williams R. Antibody-dependent cell-mediated (K cell) cytotoxicity against isolated hepatocytes in chronic active hepatitis. *Lancet* 1976; **1**: 441–4.

[3]Kawanishi H, MacDermott RP. K-cell-mediated antibody-dependent cellular cytoxicity in chronic active liver disease. *Gastroenterology* 1979; **76**: 151–8.

[4]Hütteroth TH. Lebermembranantigene bei entzündlichen Lebererkrankungen: Charakterisierung, Expression und Bedeutung als Targetantigene. *Habilitationsschrift* 1979, Berlin.

[5]Jensen DM, McFarlane IG, Portmann BS, Eddleston DM, Williams R. Detection of antibodies directed against a liver-specific membrane lipoprotein in patients with acute and chronic active hepatitis. *N Engl J Med* 1978; **299**: 1–7.

[6]Kakumu S, Arakawa Y, Goji H, Kashio T, Yata K. Occurrence and significance of antibody to liver-specific membrane lipoprotein by double-antibody immunoprecipitation method in sera of patients with acute and chronic liver diseases. *Gastroenterology* 1979; **76**: 665–72.

[7]Hopf U, Meyer zum Büschenfelde KH, Arnold W. Detection of a liver membrane autoantibody in HBsAg negative chronic active hepatitis. *N Engl J Med* 1976; **294**: 578–82.

[7a]McFarlane IG, Wojcicka B, Zucker CM, *et al.* Purification and characterization of human liver-specific

membrane lipoptotein (LSP). *Clin Exp Immunol* 1977; **27**: 381-90.

[8]Tage-Jensen U, Arnold W, Dietrichson O *et al.* Liver-cell-membrane autoantibody specific for inflammatory liver disease. *Br Med J* 1977; **1**: 206–8.

[8a]De Groote J, Desmet VJ, Gedigh KP *et al.* A classification of chronic hepatitis. *Lancet* 1968; 626.

[9]Hunter WM, Greenwood FC. Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature* 1962; **194**: 495–6.

[10]Perperas A, Tsantoulas DC, Portmann B, Eddleston ALWF, Williams R. Antibodies to a liver membrane antigen in alcoholic liver disease. Abstract. *14th Meeting of the European Association for the Study of the Liver 1979, Düsseldorf.*

[11]Manns M, Meyer zum Büschenfelde KH, Hütteroth TH, Hopf U. The liver specific protein: Evidence for species-specific and non-species-specific determinants. *J Clin Lab Immunol* 1980; **3**: 9–13.

[12]Behrens UJ, Paronetto F. Studies on 'liver-specific' antigens. I. Evaluation of the liver specificity of 'LSP' and 'LP-2'. *Gastroenterology* 1979; **77**: 1045–52.

[13]Hopf U, Meyer zum Büschenfelde KH, Arnold W. Detection of a cytophilic autoantibody in chronic active hepatitis. *Lancet* 1975; **1**: 690.

[14]Meyer zum Büschenfelde KH, Manns M, Hütteroth TH, Hopf U, Arnold W. LM-Ag and LSP—two different target antigens involved in the immunopathogenesis of chronic active hepatitis? *Clin Exp Immunol* 1979; **37**: 205–12.

[15]Manns M, Meyer zum Büschenfelde KH, Hütteroth TH, Hess G. Detection and characterization of liver membrane autoantibodies in chronic active hepatitis by a solid-phase radioimmunoassay. *Clin Exp Immunol* 1980; **42**: 263-72.