Different types of smooth muscle antibodies in chronic active hepatitis and primary biliary cirrhosis: their diagnostic and prognostic significance

P KURKI,* A MIETTINEN, E LINDER, P PIKKARAINEN, M VUORISTO, AND M P SALASPURO

From the Department of Bacteriology and Immunology, University of Helsinki, and Gastroenterological Unit of the Second Department of Medicine, University of Helsinki, Helsinki, Finland

SUMMARY The diagnostic and prognostic significance of the different types of serum smooth muscle antibodies (SMA) were investigated in sera of 24 patients with chronic active hepatitis (CAH) and 15 patients with primary biliary cirrhosis (PBC). SMA of IgG class were found in 92% of sera from patients with CAH but in only 20% of sera from PBC patients, whereas the incidence of IgM-SMA was higher in PBC (67%) than in CAH (38%). All six patients with the atypical cholestatic form of CAH had SMA of IgM class, whereas other CAH patients had SMA of mainly IgG class. SMA reacting with rabbit liver (bile canaliculus antibodies, BCA) and with rat glomeruli (glomerulus antibodies) were of anti-actin specificity and were more common in CAH than in PBC. Organ specific BCA or glomerulus antibodies were not found. Anti-actin antibodies were detected in the majority of the investigated sera by an immunoenzymatic anti-actin assay. The results suggest that the determination of SMA titres with heavy chain specific antisera may help in the assessment of diagnosis and prognosis of chronic hepatitis.

Chronic active hepatitis (CAH) and primary biliary cirrhosis (PBC) are characterised by the presence of high titres of non-organ specific autoantibodies; mitochondrial antibodies (AMA) in PBC and anti-nuclear antibodies (ANA) and smooth muscle antibodies (SMA) in CAH. These autoantibodies have differential diagnostic importance¹ but this is disturbed by the considerable overlap between the serological findings in CAH and PBC. AMA occur in 83-100% of cases with PBC and in 11-35% of cases with CAH. SMA are found in 61-86% of patients with CAH and in 32-49% of patients with PBC.²⁻⁸ Antinuclear antibodies are frequently found in both diseases. Some authors have described glomerulus antibodies⁹ and bile canaliculus antibodies^{10,11} in CAH and PBC, and a relationship to SMA has been proposed.¹²⁻¹⁴

The determination of different types of AMA may have diagnostic significance.¹⁵ There are also several types of smooth muscle antibodies.^{16,17} SMA in

chronic active hepatitis are claimed to be of anti-actin specificity^{18,19} but the specificity of SMA in primary biliary cirrhosis is not known.

The aim of this study was to investigate the specificity and diagnostic significance of the different types of SMA in primary biliary cirrhosis and chronic active hepatitis. For that purpose we determined the immunoglobulin class and the immunofluorescence staining patterns of SMA. Anti-actin antibodies were assayed by a solid phase immunoenzymatic technique, anti-actin-ELISA.²⁰

Methods

PATIENTS

Stored sera (-20°C) from 24 patients with chronic active hepatitis (CAH) and 15 patients with primary biliary cirrhosis (PBC) were examined. All sera were HBsAG-negative by complement fixation and radioimmunoassay (Austria II, Abbot Laboratories, Chicago, Illinois). Patients with CAH had mean age of 36 years (range 21-69 years) and female/male ratio of 16/8. The diagnosis was based on accepted histological criteria.²¹ Six patients had an atypical form of CAH characterised by bio-

*Address for correspondence: Pekka Kurki, MD, Clinical Immunology Section, Department of Bacteriology and Immunology, University of Helsinki, Haartmaninkatu 3, SF-00290 Helsinki 29, Finland.

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chemical signs of cholestasis and a poor response to customary immunosuppressive treatment.^{22,23} All patients with PBC were females and had a mean age of 53 years (range 32–69 years). The diagnosis was based on typical clinical, biochemical, immunological, and histological findings as published in detail before²² and the diagnostic criteria were similar to those reported by Sherlock and Scheuer.²⁴ The presence of smooth muscle antibodies was not included in the diagnostic criteria of either disease. At the time when serum samples were drawn, seven patients with CAH were treated with prednisone and azathioprine, four patients with prednisone, and four patients with azathioprine. One patient with PBC was treated with azathioprine and two with a combination of azathioprine and prednisone.

TISSUE ANTIBODIES

Tissue antibodies were detected by the indirect immunofluorescence (IFL) technique. Smooth muscle antibodies (SMA), antinuclear antibodies (ANA), mitochondrial antibodies (AMA), and antibodies reacting with glomeruli were assayed by indirect

immunofluorescence using unfixed cryostat sections of rat kidney and stomach as substrate and bile canaliculus antibodies using rabbit liver as substrate.²⁵ The diagnostic criteria for SMA were: definite fluorescence in the muscularis mucosae and interglandular smooth muscle fibres of rat stomach and in rat renal arteriolar walls. FITC labelled sheep anti-human immunoglobulin (Lot SH 074510, National Bacteriological Laboratory, Stockholm, Sweden) had a molar F/P ratio 3.5 and FITC labelled heavy chain specific antisera to human IgG (Lot 89361), IgA (Lot 89473), and IgM (Lot 89374, Meloy Laboratories, Springfield, Virginia) had molar F/P ratios of 2.9, 2.3, and 3.0, respectively. Bile canaliculi were visualised by the histochemical ATP:ase staining.²⁶

ABSORPTION AND ELUTION EXPERIMENTS

Rheumatoid factors were determined by slide agglutination (Latex-RF reagent, Behringwerke, Marburg, GFR). Absorption of rheumatoid factors was performed with 20 mg aggregated (30 minutes at 63°C) human IgG (Kabi, Stockholm, Sweden)/

Fig. 1 Reaction of smooth muscle antibodies with cryostat sections of rat kidney (a) and rabbit liver (b) by indirect immunofluorescence. Note the immunofluorescence staining of arteriolar walls and glomerulus (a) and the periphery of hepatocytes (b). $\times 500$.

ml serum. SMA were immunologically purified by eluting the antibodies from cryostat sections of human uterus by low pH. A serum pool was made from 10 SMA positive sera. It contained also antibodies reacting with glomeruli and nuclei in rat kidney and bile canaliculi in rabbit liver and with mouse parietal cells.

The serum pool was allowed to react with cryostat sections of human uterus for 30 minutes. The sections were then washed twice with phosphate buffered saline, pH 7.2, for 10 minutes and the bound antibodies were eluted by 0.1 M citrate buffer, pH 2.8, for five to 30 minutes. The eluate was then neutralised by 1M NaOH and tested for tissue antibodies.

ANTI-ACTIN ANTIBODIES

Anti-actin antibodies were determined by enzyme-linked immunosorbent assay (ELISA) as described earlier.²⁰ Disposable polystyrene tubes were coated with purified actin (0.02 mg/ml buffer). Patient sera were diluted 1:100 and the anti-actin antibodies were detected using immunologically purified alkaline phosphatase-coupled goat anti-human IgG. Actin was prepared from bovine skeletal muscle as described earlier.¹⁷

Results

INCIDENCE, TITRE, AND IMMUNOGLOBULIN CLASS OF TISSUE ANTIBODIES

The incidence and titre of smooth muscle, antinuclear and mitochondrial antibodies and antibodies reacting with rat glomeruli (Fig. 1a) and rabbit bile canaliculi (Fig. 1b) are shown in Table 1. Smooth muscle antibodies were found in 88% of patients with CAH at serum dilution 1:10 and in 54% at serum dilution 1:200 using a polyvalent FITC

labelled anti-human immunoglobulin. Seventy-five per cent of patients with PBC had SMA in their serum at dilution 1:10 and only one patient (7%) at serum dilution 1:200 ($P < 0.01$, when compared with CAH). All sera containing glomerulus and bile canaliculus antibodies were also SMA positive. The immunoglobulin class of SMA, ANA and AMA is seen in Table 2, which shows that SMA in chronic active hepatitis were mainly of the IgG class, whereas patients with primary biliary cirrhosis had more IgM-SMA (Fig. 2). SMA of IgA class were found in three patients with CAH. Twelve out of the 24 patients with CAH had rheumatoid factors at the titre 1:4; two had titres exceeding 1:32. Thirteen out of the 15 patients with PBC had rheumatoid factors at the titre 1:4 and three patients had titres exceeding 1:32. Neutralisation of the rheumatoid factors with aggregated IgG did not change the titres of tissue antibodies of IgM class.

ANTI-ACTIN ANTIBODIES

In the anti-actin ELISA test 21 out of the 24 patients with chronic active hepatitis (88%) and 10 out of the 15 patients with primary biliary cirrhosis (67%) had values exceeding the mean + 2SD (71 Units, Ref. 20) of the 157 control sera from students and blood donors. The mean anti-actin-ELISA value in CAH was 113 units (SD 42) and in PBC 120 units (SD 91).

SMOOTH MUSCLE ANTIBODIES AND THE CLINICAL COURSE OF CAH

Six patients with CAH of atypical cholestatic form and poor response to treatment²² were compared with the other 18 patients (Fig. 3). SMA of IgM class were found in all six patients in the former group but in only three patients in the latter group. In this group all patients had SMA of IgG class.

Table 1 Incidence of smooth muscle antibodies (SMA), antibodies to rat glomeruli (GA) and rabbit bile canaliculi (BCA), antimitochondrial antibodies (AMA) and antinuclear antibodies (ANA) in sera from patients with chronic active hepatitis and primary biliary cirrhosis*

Diagnosis	Number of sera	Serum dilution	SMA (%)	Incidence %			
				GA	BCA	AMA	ANA
Chronic active hepatitis	24	1:10	88	63	50	21	71
		1:50	67	46	42	17	71
		1:200	54	29	29	13	21
		1:800	29	13	8	13	13
Primary biliary cirrhosis	15	1:10	73	27	27	100†	47
		1:50	33	20	20	100	40
		1:200	7	—	7	100	27
		1:800	—	—	—	93	13

*Antibodies were assayed by indirect immunofluorescence technique using rat stomach and kidney and rabbit liver as substrate. A sheep anti-human immunoglobulin conjugate was used.

† $P < 0.001$.

Table 2 Smooth muscle (SMA), antinuclear (ANA), and antimitochondrial (AMA) antibodies determined by heavy chain specific antisera to human IgG, IgA and IgM*

Diagnosis	Number of sera	Percentage of positive sera								
		SMA			ANA			AMA		
		IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM
Chronic active hepatitis	24	92†	13	38	54	13	33	21	—	8
Primary biliary cirrhosis	15	20	—	67	40	—	47	100†	40‡	60‡

*SMA, ANA and AMA were assayed by indirect immunofluorescence at patient serum dilution 1:10 using unfixed cryostat sections of rat stomach and kidney as substrate.
†P<0.001. ‡P<0.01.

Three out of the six patients with atypical CAH and one of the other patients had mitochondrial antibodies. The anti-actin-ELISA revealed a mean value of 79 Units (range 47–103) in the group of patient with atypical CAH and 119 Units (range 56–209) in the other group of CAH.

SPECIFICITY OF GLOMERULUS AND BILE CANALICULUS ANTIBODIES

The specificity of BCA and glomerulus antibodies (Fig. 1a) was studied by absorption with purified skeletal muscle actin in 16 sera containing both

smooth muscle antibodies and antibodies reacting with bile canaliculi and glomeruli. A typical bile canalicular staining pattern is seen in Fig. 1b. The staining pattern of BCA positive sera corresponded to the histochemical ATPase staining of bile canaliculi. In all 16 sera both SMA and antibodies to glomeruli and bile canaliculi could be neutralised by 100 mg skeletal muscle actin/ml undiluted serum. The organ specificity of BCA and glomerulus antibodies was studied also in elution experiments.

The serum pool consisting of 10 SMA positive sera was allowed to react with unfixed cryostat

Fig. 2 The titres of smooth muscle antibodies of IgG and IgM class in sera from patients with chronic active hepatitis (squares) and primary biliary cirrhosis (circles).

Fig. 3 The titres of smooth muscle antibodies of IgG and IgM class in sera from patients with chronic active hepatitis (CAH). Patients with the atypical cholestatic form of CAH (filled squares) are compared with other CAH patients (open squares).

sections of human uterus and the antibodies were eluted with citrate buffer for five minutes. The neutralised eluate reacted with smooth muscle and with glomeruli in rat kidney and with bile canaliculi in rabbit liver. When the elution time was prolonged to 30 minutes also antinuclear activity was seen. This finding was not due to non-specific binding of immunoglobulins, as human antibodies to mouse parietal cells could not be eluted in the same way.

Discussion

The present study confirms the high incidence of SMA in chronic active hepatitis (CAH) and primary biliary cirrhosis (PBC). There are several types of CAH. CAH patients in this material had either the 'autoimmune' or the 'cryptogenic' type of the disease. Patients with CAH had higher titres and a higher incidence of smooth muscle antibodies (SMA) of IgG class. In our material SMA titres exceeding 1:50 were rarely seen in PBC. This is in agreement with earlier reports.^{2 27 28} A new finding was that 67% of patients with PBC had SMA of IgM class compared with 38% in sera from patients with CAH. This marked difference was not seen in antinuclear antibodies, which occurred in about one half of the sera.

The incidence of glomerulus antibodies is known to be higher in patients with CAH than in patients with viral hepatitis, cryptogenic cirrhosis, and chronic persistent hepatitis.^{12 9 29} The present results and our previous investigations show that the incidence of glomerulus antibodies in CAH is higher than in PBC or in patients with malignant disease.²⁵ An increased incidence of bile canicular antibodies (BCA) has been described in patients with viral hepatitis (38%), PBC (35%),¹¹ and CAH (66%).¹⁰ Our results support the earlier suggestion³⁰ that the BCA assay can be useful in the diagnosis of chronic active hepatitis. Organ specificity of glomerulus antibodies and BCA has been proposed based on absorption studies and on the finding of these antibodies without other antibody activities.^{9 31} In our material the glomerular and bile canicular fluorescence was always associated with SMA. The presence of organ specific glomerulus antibodies and BCA was not ruled out but our findings indicate that these antibodies cannot be determined by the standard IFL test without actin absorption. However, the recording of glomerular and bile canicular staining helps to distinguish SMA of anti-actin specificity from 'non-actin' SMA.

SMA in CAH have previously been shown to be

of anti-actin specificity.^{18 19} Our results in the IFL test and in the ELISA assay indicate that most patients with CAH and PBC have anti-actin antibodies. The anti-actin ELISA seems to give more positive results than the IFL-assay. This may be due to the fact that the ELISA test measures also antibodies to denatured actin.²⁰ Anti-actin antibodies have been found in some patients with non-hepatic diseases,^{17 32-34} but the majority of SMA in these diseases seem to have other specificities.^{35 17}

Some patients with chronic active hepatitis are resistant to the customary immunosuppressive treatment.^{23 22 36} These cases are characterised by cholestatic features: high serum levels of alkaline phosphatase, gammaglutamyltranspeptidase, and of bile acids.²² Hepatic copper levels are increased and an intracellular copper binding protein can be demonstrated histologically by orcein staining.³⁷

With early administration of high doses of immunosuppressive drugs the progression of the disease may be prevented.²³ A distinct subclass of mitochondrial antibodies has been shown to occur in patients with the cholestatic form of CAH.¹⁵ The prognostic significance of other tissue antibodies is controversial.^{23 36} In this material all patients with the cholestatic form of CAH had SMA of IgM class which were rare in other CAH patients (Fig. 3). The determination of IgM-SMA may thus help to recognise cases which need higher doses of immunosuppressive drugs. Interestingly, the determination of SMA with heavy chain specific anti-immunoglobulin antisera was previously shown to be useful in the assessment of prognosis in HBsAG-positive viral hepatitis.^{38 39} Mitochondrial antibodies also seemed to be associated with atypical CAH, although the different subtypes of AMA were not determined.

It can be concluded that the titration and more defined classification of the tissue antibodies, notably smooth muscle antibodies and mitochondrial antibodies, may give important information for the assessment of the diagnosis and prognosis of chronic hepatitis. However, there is considerable overlap in the serological findings of CAH and PBC. This is in agreement with the knowledge that these diseases sometimes have common clinical, biochemical, and histological features.¹

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