

Anti-actin antibodies revealed by counter-immunoelectrophoresis

Relation to smooth muscle antibodies and bile canalicular antibodies

HANS DIEDERICHSEN AND KIRSTEN RIISOM

From the Blood Bank, the Department of Clinical Chemistry, and the Medical Department M, Odense University Hospital, Denmark

SUMMARY In investigations by counter-immunoelectrophoresis, anti-actin antibodies were found in 59% of patients with chronic hepatitis and in 8% of patients with non-hepatic diseases and normal blood donors. Anti-actin antibodies were found more frequently in patients with hepatitis and IgG smooth muscle antibodies than in other groups of diseases and normal subjects with IgG smooth muscle antibodies. Anti-actin antibodies showed no correlation with bile canalicular antibodies.

Antibodies against actin (AAA) have been found in some patients with smooth muscle antibodies (SMA) by immunodiffusion¹ and enzyme-linked immunosorbent assay.² Further, by absorption studies with thrombastenin and actin SMA-containing sera have been shown to contain AAA.^{1,3-5} Most sera with SMA and AAA have been obtained from patients with chronic hepatitis.¹⁻⁴ In this study we used counter-immunoelectrophoresis, a rather sensitive method, for the investigation of AAA. As material for the investigation we chose sera from patients with diseases with a known frequency of SMA (chronic and acute hepatitis and malignant disease)⁶⁻⁸ and other diseases and from normal subjects with an expected low frequency of SMA. Of further interest was the relation of AAA to the bile canalicular antibody described earlier in patients with chronic hepatitis.⁹

Patients

The following patients were studied:

- 36 patients with clinical and biochemical signs of chronic hepatitis of more than six months' duration; 33 had chronic aggressive hepatitis and/or cirrhosis and three had chronic persistent hepatitis at liver biopsy. All were HBsAg negative. None was alcoholic;
- 9 patients with biopsy-verified acute hepatitis of less than three months' duration. All cases were presumably of viral origin, and none was drug-

- induced. Three were HBsAg positive. One had anti HBs;
- 43 patients with various malignant diseases;
- 27 patients with discoid lupus erythematosus, verified by biopsy;
- 6 patients with systemic lupus erythematosus, fulfilling the American Rheumatism Association's criteria;¹⁰
- 41 patients with various diseases sent for blood type determination; and
- 30 healthy blood donors.

Methods

PREPARATION OF ACTIN

An acetone-dried powder of actin was prepared from rabbit striated muscle according to the method of Straub.¹¹ After further extraction with chloroform, actin was extracted with distilled water at 0-2°C, and, after addition of ATP, the extract was chromatographed on a Sephadex G 200 column (Pharmacia, Uppsala, Sweden)¹² at 6°C. The height of the gel was 93 cm and the flow 0.14 ml/min. Of the three peaks appearing at chromatography, the tubes from the second peak containing G-actin¹² were collected and concentrated. The protein concentration was determined at 280 nm (1% G-actin: E^{1 cm} = 10.97). The G-actin was dialysed against phosphate buffered saline, pH 7.2, with 0.02% sodium azide. The G-actin was stored at 4°C.

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed as described by Weber and Osborn.¹³ A 5% acrylamide gel was

made. The protein concentration was 2.1 mg/ml. A constant current of 40 mA/cm² for each sample was used. The voltage was variable. Electrophoresis was performed on the muscle extract before the gel filtration on Sephadex G 200 (Fig. 1a) and on the three peaks that emerged at gel filtration. The second top of the gel filtration showed only one band, corresponding to 46 000 daltons, the molecular weight of actin¹⁴ (Fig. 1b).

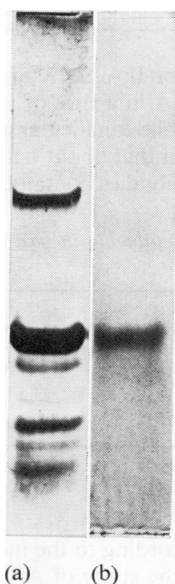


Fig. 1 (a) SDS PAGE on actin extract produced according to Straub. (b) SDS PAGE on gel filtrated actin extract.

COUNTER-IMMUNOELECTROPHORESIS

A modification of this method¹⁵ was used; 11.7 ml 0.85% agarose (l'Industrie Biologique Française) was layered on 10 × 10 cm glass plates. Three rows of paired wells were punched out, 5 mm in diameter and 3 mm from edge to edge; 22.5 μl serum (inactivated at 56°C after 30 minutes) were applied to the anodic well and 22.5 μl actin (0.3 mg/ml) to the cathodic well. Electrophoresis was performed for 1 hour at room temperature. The current was 4 V/cm, corresponding to 42-45 mA. The reading was performed immediately after the electrophoresis. Each electrophoresis run included a positive and a negative control.

IgG was produced by a DEAE Sephadex method.¹⁶ The IgG gave only one band corresponding to IgG by immunoelectrophoresis.

SPECIFICITY STUDIES

IgG from a serum reacting with actin, and containing

SMA, precipitated actin by counter-immunoelectrophoresis. IgG from pooled donor serum at the same protein concentration did not precipitate actin. Phosphate buffered saline did not precipitate actin either.

Immunofluorescence investigations for bile canalicular antibody and SMA were performed with 4 μ cryostat sections of bovine liver and stomach.^{9 17} The serum was inactivated at 56°C for 30 minutes and diluted 1/80. At this dilution sera from patients with chronic hepatitis should be positive for SMA.^{18 19} Fluorescein-isothiocyanate conjugated anti IgG, anti IgA, and anti IgM from the rabbit (Behring Werke, Marburg, Germany) was used. The fluorescein-isothiocyanate anti IgG had a molar fluorescein: protein (F:P) ratio of 2.5. The plateau end point titre was 1/256 and the working dilution was 1/64. FITC anti IgM had a F:P ratio of 2.2, the plateau end point titre was 1/160, and the working dilution was 1/40. FITC anti IgA had a F:P ratio of 2.0, the plateau end point titre was 1/80, and the working dilution was 1/20. The reaction for SMA was considered positive when more than half of the cells in the muscularis mucosae were stained. All sera were investigated for IgG and IgM SMA. Sera positive for AAA and negative for IgG/IgM SMA were investigated for IgA SMA. Positive reactions with bile canaliculi produced the characteristic staining described earlier.²⁰

Results

SDS-PAGE of the crude muscle extract showed

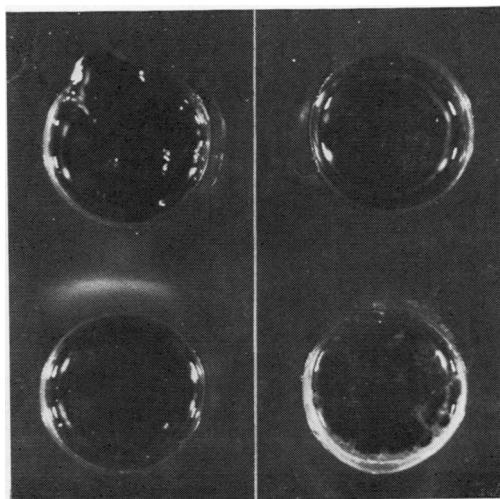


Fig. 2 (Left) A positive reaction indicated by a precipitin band. (Right) A negative reaction for comparison.

Table 1 Results of investigations for AAA and IgG/IgM SMA in various diseases

	Total No.	AAA positive SMA positive		AAA positive SMA negative		AAA negative SMA positive		AAA negative SMA negative	
		No.	(%)	No.	(%)	No.	(%)	No.	(%)
Chronic hepatitis	36	19	(53)	2	(6)	9	(25)	6	(17)
Acute hepatitis	9	3	(33)	2	(22)	2	(22)	2	(22)
Systemic lupus erythematosus	6	1	(17)	2	(33)	2	(33)	1	(17)
Discoid lupus erythematosus	27	4	(15)	0		10	(37)	13	(48)
Malignant disease	43	1	(2)	3	(7)	12	(28)	27	(63)
Miscellaneous inpatients	41	1	(2)	0		9	(22)	31	(76)
Blood donors	30	0		0		3	(10)	27	(90)

several bands (Fig. 1a). SDS-PAGE of the gel-filtrated muscle extract showed one band (Fig. 1b) of a molecular weight of 46 000 daltons corresponding to actin. The purified actin preparation was used for the test for AAA.

The precipitation between a serum from a patient with chronic active hepatitis and actin is shown in Figure 2. The results of tests for AAA are shown in Table 1. AAA was found in 21 of 36 patients (58%) with chronic hepatitis and in 12 of 147 patients (8%) with non-hepatic diseases and blood donors. The frequency of AAA in acute hepatitis was equal to that in chronic hepatitis (five positive out of nine). AAA was found more frequently in sera with SMA than in sera with SMA from patients with other diseases and normal donors (7 of 43 sera) ($P < 0.001$). AAA was found in sera with IgG SMA, IgM SMA, and combined IgG and IgM SMA (Table 2).

In nine patients with AAA, SMA was not found in a dilution of 1/80. Of these patients, two had SMA in a dilution of 1/40, three in 1/20, and one in

Table 2 Relation between IgG SMA and IgM SMA, and AAA in various diseases

	No. of positive reactions		
	IgG SMA/AAA	IgM SMA/AAA	IgG + IgM SMA/AAA
Chronic hepatitis	20/11	2/2	6/6
Acute hepatitis	0/0	2/1	3/2
Systemic lupus erythematosus	2/0	1/1	0/0
Discoid lupus erythematosus	8/2	2/1	4/1
Malignant disease	5/1	6/0	2/0
Miscellaneous inpatients	4/0	5/0	1/1
Blood donors	1/0	1/0	1/0

1/10. In the last three of the nine patients, SMA could not be demonstrated in a dilution of 1/10. When tested for IgG SMA these sera produced non-specific fluorescence of the tissue, not especially localised to the smooth muscle. Two of these sera were from patients with cancer and one was from a patient with systemic lupus erythematosus. All of the nine sera

were investigated for IgA SMA and all were found to contain IgA SMA in a titre of 1/80.

The findings for bile canalicular antibodies appear in Table 3. It is seen that about half of the sera with bile canalicular antibodies contained AAA.

Table 3 Anti-actin antibodies in sera with bile canalicular antibodies

	BCA (No. positive)	AAA (No. positive)
Chronic hepatitis	13	6
Discoid lupus erythematosus	1	1
Blood donors	1	0

Discussion

Actin produced according to the method of Straub¹¹ has been used for the study of AAA.¹ By using this method we found more bands by SDS-PAGE than corresponded to actin. By gel filtration with Sephadex G 200¹² of this extract three peaks emerged. By SDS-PAGE we found that the second peak contained only one band of a molecular weight of 46 000 daltons corresponding to G-actin.¹⁴ Because of these findings we used chromatographically purified actin for our tests for AAA. The actin was completely in solution. Comparable studies seem not to have been performed previously. Earlier investigations seem to have been performed only on sera containing SMA, mostly from patients with chronic hepatitis.¹⁻³ Thirty sera with SMA, of which 29 came from patients with chronic hepatitis, were found to contain AAA by the double gel diffusion technique with actin rendered diffusible by treatment with KCl, mercaptoethanol, and trisaminomethan.¹ We investigated sera from patients with various diseases and from normal donors for SMA, bile canalicular antibodies, and AAA. AAA was found most frequently in sera from patients with chronic and acute hepatitis. Most sera with AAA also had IgG and/or IgM antibodies to smooth muscle by the immunofluorescence technique. Of those sera with

AAA, not having IgG or IgM SMA in a dilution of 1/80, six had IgG/IgM SMA in a lower titre, and three had no IgG/IgM SMA in a titre of 1/10. All these nine sera with AAA and a titre of less than 1/80 of IgG/IgM SMA were found to have IgA SMA. As expected, we more often found SMA in patients with chronic and acute hepatitis⁶ than in patients with other diseases. These patients with hepatitis and SMA more often had AAA in their serum than patients with other diseases and SMA. This is in accordance with the findings in absorption studies performed by Kurki *et al.*²¹ The AAA showed no correlation with the bile canaliculi antibody described earlier in chronic hepatitis.⁹

Reactions between IgG and actin not due to specific antibodies have been described.²² The precipitations that we found between serum and actin do not seem to be non-specific because of the statistically significant correlation between the occurrence of AAA and IgG/IgM SMA. Further, the positive reactions between actin and IgG prepared from a serum with AAA and SMA, and the negative reaction between actin and IgG prepared from pooled donor serum, might indicate the antibody specificity of the precipitation.

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Requests for reprints to: Dr Hans Diederichsen, The Blood Bank, Odense University Hospital, 5000 Odense C, Denmark.