Reaction against liver cells by human antiactin antibodies purified by affinity chromatography

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SUMMARY Human antiactin antibodies were isolated from human smooth muscle antibodies by affinity chromatography using actin extracted from rabbit striated muscle and coupled to activated CH-Sepharose 4B. The eluate contained IgG and IgM smooth muscle antibodies reacting against muscularis mucosae of the stomach and blood vessels. Further the eluate contained IgM antibodies acting against the surface of isolated hepatocytes and the periphery of hepatocytes of sections of liver cells. These results indicate that antiactin antibodies possibly react with the hepatocyte membrane as has been described with regard to other antibodies.

Various tissue antibodies have been found in acute and chronic hepatitis. Of these the bile canalicular antibody¹ and the liver membrane antibody² react with hepatocytes. It has been suggested that smooth muscle antibodies (SMA), commonly found in hepatitis,³ also bind to liver, as many sera from patients with hepatitis and SMA give a so-called polygonal staining—that is, at the periphery of liver cells, with sections of liver.⁴ Smooth-muscle antibodies from patients with chronic hepatitis,^{5,6} and acute hepatitis^{7,8} have been found to contain antibodies against actin (AAA). Antibodies against actin produced by immunisation of rabbits have been described as binding to sections of frozen liver in a polygonal manner.⁹ Polygonal staining of liver sections by animal AAA and human SMA might be caused by reaction against smooth muscle antigens in the hepatocyte membrane. A model for testing for antibodies to the liver cell membrane has been reported by Hopf *et al* who used hepatocytes isolated by a non-enzymatic method.¹¹ The possibility of a reaction by AAA against the liver cell membrane does not seem to have been verified on isolated hepatocytes. If human AAA reacted with the liver cell membrane, it might indicate a possible pathogenetic role in chronic hepatitis. We studied purified AAA of human origin for reactivity against isolated hepatocytes and sections of fresh frozen liver. Specific AAA was produced by affinity chromatography with actin. Other antibodies against liver tissue in the human serum would thus be eliminated.

Material and methods

PREPARATION OF ACTIN

Actin was prepared by gel filtration of acetone-dried powder of striated muscle as described earlier.⁸ The purity of the actin was tested by SDS polyacrylamide gel electrophoresis (PAGE) (Fig. 1).

AFFINITY CHROMATOGRAPHY

Actin (30 mg) was coupled to 1 g activated CH-

Fig. 1 (a) SDS PAGE of extract of acetone-dried muscle (b) pure actin after gel filtration.

Sephacrose 4B (Pharmacia, Uppsala, Sweden) for one hour at room temperature. The coupling procedure recommended by the manufacturer was followed. Actin-Sepharose gel (3 ml) was poured into a column K9/15 (Pharmacia, Uppsala, Sweden). The column was washed with phosphate-buffered saline pH 7.2 (PBS) containing 0.02% Na-azide and stored at 4°C. Serum (3.3 ml) was applied to the column. After incubation at room temperature for half an hour, the column was washed with 40 ml PBS. The last one millilitre fraction of PBS was collected and investigated for negative immunofluorescence reaction against sections of liver and stomach to ensure that the washing had been effective. Antibodies bound to the actin-Sepharose column were eluted with 2.4 ml glycine-HCl solution, 0.2 M, pH 2.8 in 0.15 M NaCl. Fractions of 0.3 ml were collected. The pH of the eluted fractions was adjusted to pH 7 by the addition of NaOH.

Immunofluorescence studies were performed on 4 µm thick cryostat sections of fresh frozen bovine liver and stomach.¹⁰ Fluorescein-isothiocyanate (FITC) conjugated anti-human IgG and IgM from the rabbit (Behringwerke, Marburg, Germany) were used. The working dilutions were 1/64. The FITC conjugates were absorbed with lyophilised liver powder. The reactions were read using transmitted light in a Zeiss fluorescence microscope with a HBO 200 W mercury lamp, an exciter filter BG 12/3 and a Zeiss barrier filter 50.

Immunofluorescence investigations for antibodies against hepatocytes were performed according to the method of Hopf, *et al*¹¹ with rabbit hepatocytes. Investigations for antibodies in serum were performed with 200 µl of serum diluted 1/2. Investigations for antibodies in eluate were performed with 400 µl eluate and 0.1 ml hepatocyte suspension. The readings were carried out in a Leitz fluorescence microscope with incident light from a HBO 200 W mercury lamp and an I 2 filter block.

F(ab)₂ fragments from sera were prepared as described by Poulsen and Hjort¹² by pepsin diges-

tion. The pepsin hydrolysate was dialysed. The dialysate was tested for the effectiveness of the pepsin digestion by gel precipitation using anti-Fd (Behringwerke, Germany), anti-Fc (Behringwerke, Germany) and anti-CH2 (kindly provided by Dr Tage Hjort, Århus). The hydrolysate reacted with anti-Fd, but not with anti-CH2, and gave a spur as compared to normal serum with anti-Fc.

Selected sera sent for investigation for smooth muscle antibody (SMA) were used. The titre for SMA was 1/80 or more except U 5192 that had an IgM SMA titre of 1/40. Three sera were from patients with chronic hepatitis (U 5192, Y 1785 and R 6100), one from a patient with acute hepatitis (R 6103). As negative controls a serum without SMA (X 1785) and pooled serum without SMA were used. None of these sera was from patients with liver disease. The sera were stored at -30°C until used. They were inactivated at 56°C for 30 min.

Results

The sera were first studied with regard to IgG and IgM antibodies to smooth muscle and liver. Then an affinity chromatography was performed on each serum. The chromatographic eluates were investigated by immunofluorescence on sections of liver and stomach and on isolated hepatocytes. Three sera had IgM SMA and gave IgM peripheral hepatocyte staining in sections of liver cells (Table 1). The actin affinity chromatographic eluates of these three sera gave immunofluorescence staining with smooth muscle (Fig. 2) and sections of liver (Fig. 3) identical to the original sera. These three sera reacted with isolated hepatocytes before and after affinity chromatography. Positively stained hepatocytes are shown in Fig. 4. The results of the investigation of IgG antibodies are shown in Table 2. Three sera had IgG SMA antibodies. One of the sera reacted with isolated hepatocytes. After affinity chromatography with actin, these three sera reacted with smooth muscle. None reacted with liver.

Table 1 Results of investigations for IgM antibodies to smooth muscle cells and liver

Serum No	"Whole" serum				Chromatographed serum			
	ANA	Staining of smooth muscle	Peripheral staining of hepatocytes		ANA	Staining of smooth muscle	Peripheral staining of hepatocytes	
			Sections of liver	Isolated hepatocytes			Sections of liver	Isolated hepatocytes
R 6103	-	+	+	+	-	+	+	+
U 5192	+	+	+	+	+	+	+	+
Y 1785	+	+	+	+	-	+	+	+
X 1785	-	-	-	-	-	-	-	-
pooled negative sera	-	-	-	-	-	-	-	-

Fig. 2 Staining of smooth muscle by AAA eluate.

Fig. 3 Staining of sections of liver by AAA eluate.

Antinuclear antibodies (ANA) were found in the eluate of one of three sera containing ANA.

F(ab)₂ was produced from all sera giving IgG and IgM hepatocyte membrane staining. The F(ab)₂ gave the same immunofluorescence membrane

Fig. 4 Staining of isolated hepatocytes by AAA eluate.

staining as the original serum of isolated hepatocytes and the periphery of liver cells in sections of liver. A negative control gave no hepatocyte staining.

Discussion

Antibodies against actin from human serum has been isolated in the present investigation by affinity chromatography with actin purified by gel filtration. Even though the actin showed only one band at SDS PAGE, minor impurities with other cellular antigens may have been present as the eluate from one of two sera with SMA and ANA reacted both with smooth muscle and cell nuclei. The presence of AAA in the eluate was verified by immunofluorescence with smooth muscle cells.

The AAA extracted from other antibodies to smooth muscle in human sera by affinity chromatography was tested for reactions against the cell membrane of isolated hepatocytes. Our results showed that the IgM antibody eluted from the actin-Sepharose column reacted with the surface of isolated hepatocytes. The IgM antibody eluates also reacted with the periphery of hepatocytes in liver sections.

The staining pattern of liver sections was identical to that found by Lidman *et al*⁶ who used eluted AAA from antibody precipitates with actin and also to that of Trenchev *et al*⁹ who employed rabbit AAA. However, it differed from the pattern

Table 2 Results of investigations for IgG antibodies to smooth muscle cells and liver

Serum No	"Whole" serum				Chromatographed serum			
	ANA	Staining of smooth muscle	Peripheral staining of hepatocytes		ANA	Staining of smooth muscle	Peripheral staining of hepatocytes	
			Sections of liver	Isolated hepatocytes			Sections of liver	Isolated hepatocytes
R 6103	-	+	-	-	-	+	-	-
U 5192	+	+	-	-	+	+	-	-
R 6100	-	+	-	+	-	+	-	-
X 1785	+	-	-	-	-	-	-	-
pooled negative sera	-	-	-	-	-	-	-	-

obtained when using AAA produced by immunisation with actin of animals in which a staining of bile canaliculi-like structures were found.¹³

The difference between IgM and IgG AAA is in accordance with our previous investigation in which we found that the occurrence of peripheral IgM hepatocyte fluorescence was related to IgM SMA, whereas IgG peripheral hepatocyte fluorescence was not.¹⁰ This difference could possibly be due to different antiactin specificities of IgM and IgG AAA. Other investigators have made no distinction between IgG and IgM AAA.^{6,8,13} The possibility cannot be excluded that IgM antibody of the eluate might be directed against non-detectable impurities of the actin preparation used for the affinity chromatography column. The reaction of the IgM antibody against hepatocytes seems to be a true antibody reaction since the F(ab)₂ fraction also reacted with the hepatocytes.

Antibodies reacting with isolated hepatocytes have been described by Hopf *et al*¹¹ and others¹⁴ in sera from patients with hepatitis. These antibodies were considered to be directed against liver-specific membrane antigen (LMA). Our investigation appears to demonstrate that the non-organ specific AAA also react with hepatocyte membrane. This is in accordance with the finding of actin in liver cell membrane.¹⁵ The failing reaction against liver cells of the AAA eluate of R 6100 investigated for IgG antibodies might be due to antibodies reacting with antigens other than actin of the liver cell membrane—that is, LMA.

This work was supported by the Laegevidenskabeligt Forskningsråd. We thank Professor K-H Meyer zum Büschenfelde, who taught us to isolate hepatocytes and Dr Tage Hjort, Århus, who kindly provided the anti-CH2.

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