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Identification of mitochondria in liver biopsies

A study by immunohistochemistry, immunogold and Western blot analysis

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Abstract Hepatocytes are rich in mitochondria, which play an important role in hepatic metabolism. In certain pathologic conditions (most often alcoholic liver disease) mitochondria became enlarged; nevertheless, even in these conditions they are hardly detectable on light microscopy. Recently an antimitochondrial antibody (mAM), which recognizes a 60-kDa protein, has been characterized. The purpose of the present study was to study immunoreactivity of this antibody in a series of liver biopsies. We studied 146 liver biopsies using an mAM. In 8 cases an ultrastructural study was also done, and in 2 cases Western blot analysis was performed. Cases were divided as follows: alcoholic liver disease (ALD, 31); steatosis (8); nonalcoholic steatohepatitis (NASH, 1); hepatitis C virus (HCV)-related hepatitis (83); hepatitis B virus (HBV)-related hepatitis (6); primary biliary cirrhosis (1); sclerosing cholangitis (1); haemosiderosis (1); sarcoidosis (1); alpha-1-antitrypsin deficiency (1); nonspecific findings (12). All the patients were investigated for alcohol or drug abuse, pharmacological treatment, hyperlipidaemia, hypercholesterolaemia and diabetes. Immunoreactivity was diffuse in cases of ALD, NASH and steatosis, and in patients with drug abuse. Electron microscopic immunogold and Western blot analysis confirmed that in the conditions examined the protein recognized by the mAM showed greater expression. Immunohistochemical staining was helpful in demonstrating a toxic or a metabolic insult even in cases in which the histological picture was blurred by viral infection.

Key words Alcohol · Steatosis · Hepatitis · Mitochondria · Megamitochondria

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Introduction

Hepatocytes are normally rich in mitochondria; mitochondria occupy about 18% of the entire liver cell volume [22] and each hepatocyte contains about 800 mitochondria. Mitochondria play an important part in hepatocyte metabolism, being principally involved in oxidative phosphorylation and oxidation of fatty acids [10, 22]. In normal conditions mitochondria are not visible at light microscopic level, but in certain pathological conditions, most frequently in alcoholic liver disease (ALD) and steatohepatitis, mitochondria become enlarged and swollen (so-called megamitochondria or giant mitochondria) and can be seen by light microscopy. Nevertheless, it is still difficult to detect them by light microscopy, and most morphological studies on mitochondria depend on ultrastructure [2, 8, 20].

Recently, Papotti et al. [11], using immunogold and immunohistochemical techniques, characterized an antimitochondrial antibody (mAM) that specifically recognizes human mitochondria. This mAM recognizes a 60-kDa nonglycosilated protein, the exact structure and function of which have not yet been published.

The purpose of the present paper is to confirm the specificity of the antimitochondrial antibody further to study the pattern of its immunoreactivity at both light and electron microscopic levels, in a series of liver biopsies, and to see whether it might be useful for diagnostic purposes.

Materials and methods

For the study, 146 liver biopsies were retrieved from the files of the Departments of Histopathology of the University of Bologna at the Ospedale Bellaria and of the Ospedale Generale of Treviso (Italy).

The cases were classified as follows: alcoholic liver disease (ALD) 31 cases; steatosis 8 cases; nonalcoholic steatohepatitis (NASH) 1 case; hepatitis C virus (HCV)-related hepatitis 83 cases; hepatitis B virus (HBV)-related hepatitis 6 cases; primary biliary cirrhosis (PBC) 1 case; primary sclerosing cholangitis (PSC) 1 case; haemosiderosis 1 case; sarcoidosis 1 case; alpha-1-antitrypsin deficiency 1 case; nonspecific findings 12 cases.

All the patients were investigated for the presence of HBV and HCV viral markers, hyperlipidaemia, hypercholesterolaemia and diabetes. Accurate histories were taken to define daily consumption of alcohol, therapy and drug abuse. Alcohol abuse was considered when a daily consumption of alcohol greater than 80 g was demonstrated clinically [12].

Tissues from all but 3 cases were from needle biopsies; in 1 case of ALD and 1 case with non-specific findings tissues had been obtained by wedge biopsies performed during cholecystectomy; in 1 other case with nonspecific findings the tissue available was liver tissue adjacent to a cavernous haemangioma. Tissues were formalin fixed and routinely processed to paraffin. From each block serial sections were cut and stained with haematoxylin and eosin (H&E), Sirius red, and PAS after diastase digestion. Immunohistochemistry was performed following a previously described method [7].

The antiserum used was a monoclonal antimitochondrial antibody (Biogenex 113/1; diluted 1:500). In the case of alpha-1-antitripsyn deficiency, polyclonal anti-alpha-1-antitrypsin antibody (Dako A012; diluted 1:300) was also applied. Positive and negative controls were added in each batch of slides.

For electron microscopy, tissues from 8 biopsies were fixed in glutaraldehyde and conventionally embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips 400T transmission electron microscope.

For immunoelectron microscopic investigation, Karnovsky (2% glutaraldehyde, 1% formaldehyde)-fixed samples were dehydrated in graded ethanols and infiltrated with pure hydrophilic resin Unicryl (BioCell, Cardiff, Wales) in Eppendorf tubes. Polymerization was achieved by UV irradiation for 72 h at 4°C as previously described [15]. Ultrathin sections were cut with glass knives and placed on nickel grids. The immunogold labelling was performed according to the protein A-gold technique [1]. Briefly, the sections were treated with PBS containing 1% ovoalbumin and incubated with the antimitochondrial monoclonal antibody diluted 1:20. Following incubation with a secondary antibody (diluted 1:20), sections were treated with a protein A-15 nm colloidal gold complexes diluted 1:20. After counterstaining, the ultrathin sections were observed in a Philips 400T transmission electron microscope. Sections with the primary antibody omitted served as negative controls.

For Western blot analysis, proteins were extracted according to the protocol previously described [14]. The same amount (30 µg) of protein from each sample was separated on 10% SDS-PAGE and transferred to 0.45-mm pore nitrocellulose paper with a Mini Trans-Blot Transfer Cell (Bio-Rad, Milan, Italy) as described by Towbin et al. [19].

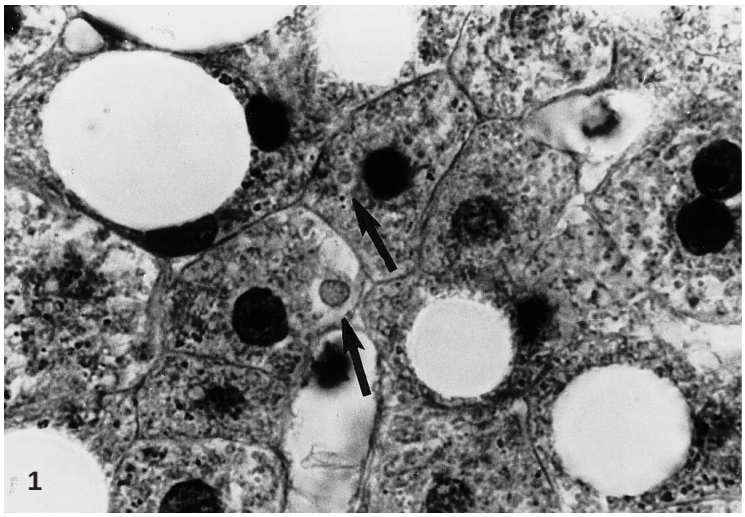
The nitrocellulose paper was washed for 2 h at room temperature in TBST (Tris-HCl 20 mM, NaCl 150 mM, 0.05% Tween 20 ad 0.1% BSA) containing 5% non-fat dry milk (Bio-Rad) to block nonspecific binding. Immunodetection was carried out using a mouse monoclonal anti-human mitochondrial antibody (BioGenex 113/1) at a dilution of 1:500, overnight, at 4°C. After four 10-min washes in TBST (Tris-HCl 20 mM, NaCl 150 mM, 0.05% Tween 20 and 0.1% BSA), the second of which also contained 0.1% Triton X-100, the nitrocellulose paper was incubated for 2 h at room temperature with an anti-mouse biotinylated antibody at 1:500 dilution. After four 10-min washes in TBST, the nitrocellulose paper was incubated for 1 h at room temperature with an avidin–alkaline phosphatase conjugate (Biomeda) diluted 1:1000. After four 10-min washes in TBST, the colour reaction was developed using the BCIP/NBT substrate system. For Western blot analysis 1 tissue specimen from a case of steatosis, a fragment of liver parenchyma adjacent to a cavernous haemangioma and a fragment of cardiac muscle obtained at autopsy (positive control) were utilized.

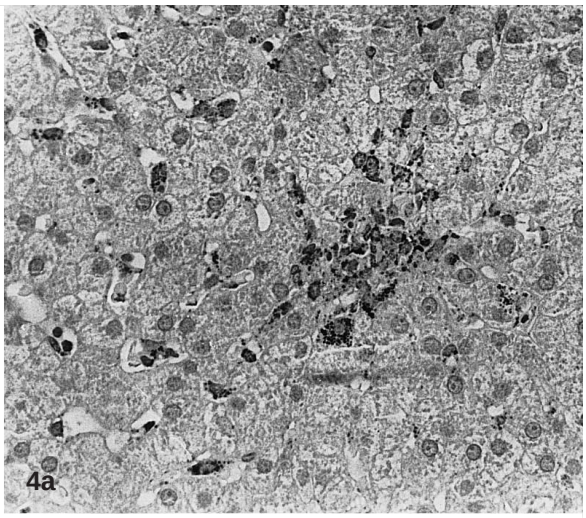
Results

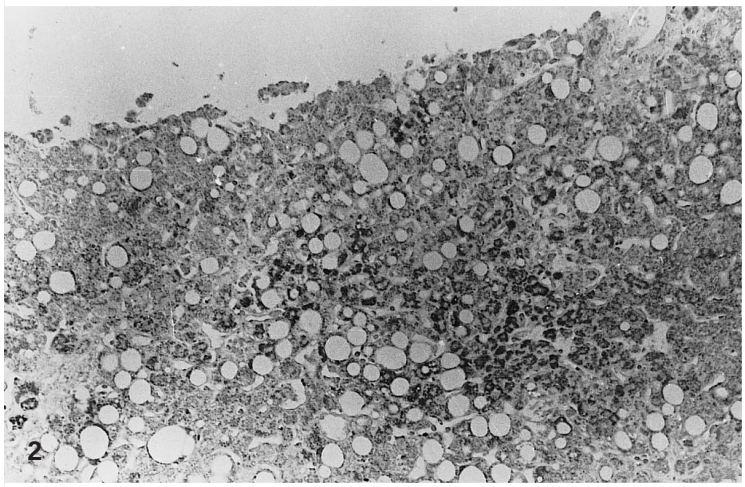
The clinical data of the patients are summarized in Table 1. In all cases (31) of ALD, H&E examination showed the typical features of alcoholic hepatitis, namely centrolobular steatosis, mild lobular inflammatory infiltration mainly composed of neutrophils and lipid-laden macrophages (so-called lipogranulomas), and pericellular fibrosis. Mallory bodies were observed. Oxyphilic changes in hepatocytes were focally observed in 19 cases. Cirrhosis was present in 7 of these cases. Following H&E staining megamitochondria appeared as intracytoplasmic, round to oval, brightly eosinophilic structures (Fig. 1); these were randomly distributed throught the parenchyma. Megamitochondria were observed in all cases of ALD under study. Immunohistochemistry revealed between 20% and 90% (average 60%) of hepatocytes with intense granular staining (Fig. 2) in all cases. Positive granules varied in size. The larger ones were round to oval in shape and were identical in mor-

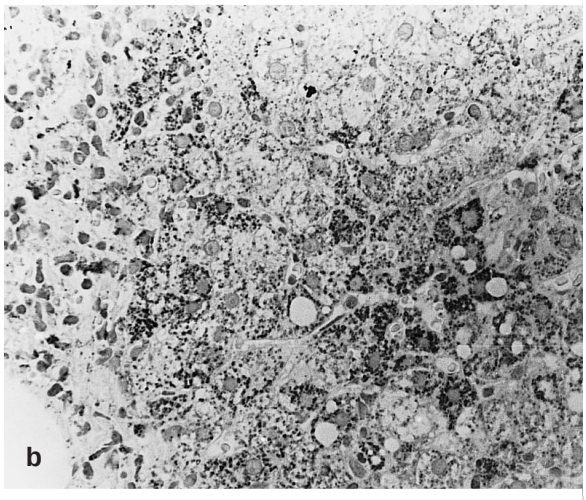
Table 1 Clinical data (*ALD* = alcoholic liver disease, *NASH* = nonalcoholic steatohepatitis, *A1AT* = Alpha 1-antitrypsin deficiency, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *PBC* primary biliary cirrhosis, *PSC* primary sclerosing cholangitis; figures in round brackets show no. of cases)

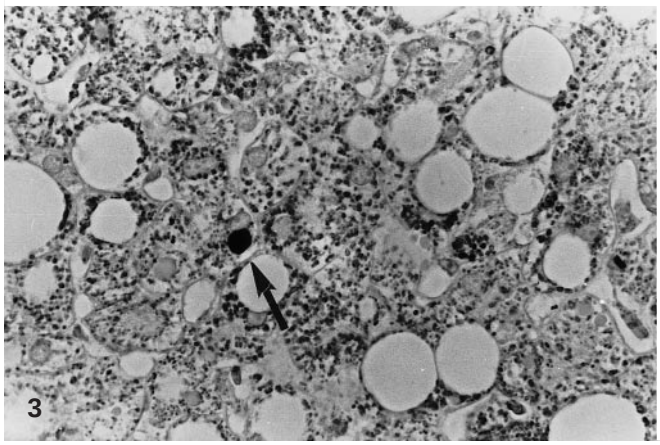
	No. of cases	Male/female	Age (mean)	Associated pathology
ALD	31	21/10	22–79 (46)	
Steatosis	8	5/3	21–62 (42)	Hyperlipidaemia hypercholesterolaemia (4), corticosteroids (1)
NASH	1	0/1	70	Diabetes
HBV-elated hepatitis	6	4/2	26–73 (44)	
HCV chronic hepatitis	83	37/46	26/67 (48)	ALD (8), hyperlipidemia (4), drug abuse (5)
PBC	1	0/1	50	
PSC	1	0/1	30	
Sarcoidosis	1	0/1	62	
Haemosiderosis	1	1/0	72	Corticosteroid
Non-specific	12	5/6	28–74 (42)	ALD (1)
A1AT	1	0/1	62	–











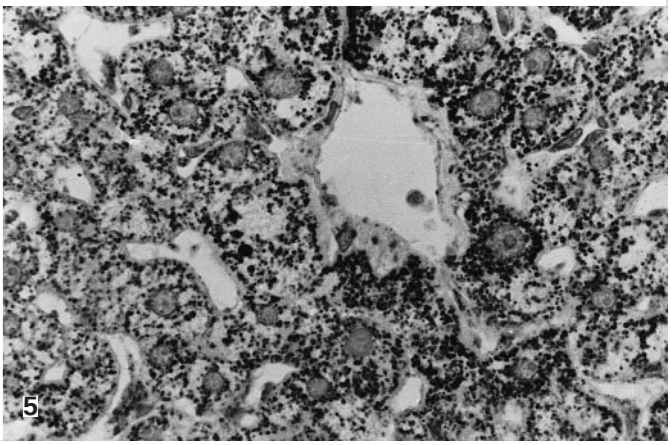


Fig. 1 Alcoholic liver disease (ALD): megamitochondria (*arrow*) present as roundish intracytoplasmic structures. Haematoxylin-eosin, ×400

Fig. 2 ALD: immunohistochemical positivity with antimitochondrial antibody (mAM) is widely distributed throughout the hepatic parenchyma. ×40

Fig. 3 ALD: same case as Fig. 1. Positive hepatocytes show intense granular staining. Granules vary in size, the larger ones were identified as megamitochondria (*arrow*). ×250

Fig. 4 **a** Hepatitis C virus (HCV)-related hepatitis, mAM stains rare hepatocytes around the necroinflammatory foci and Kupffer cells. **b** HCV-related hepatitis associated with alcohol abuse: immunoreactivity with mAM is similar to that of ALD. ×125

Fig. 5 Nonspecific findings: mAM stains numerous intracytoplasmic granules. The same patient admitted a heavy daily alcohol consumption. ×250

phology and distribution to the megamitochondria identified on H&E (Fig. 3). Positive hepatocytes were randomly distributed throughout the liver parenchyma; nevertheless, they were more numerous around the centrolobular veins.

In the 8 cases of steatosis panacinar macrovesicular steatosis was observed on H&E. In addition, lipogranulomas were present in 5 cases. Mild pericellular fibrosis was also seen in 3 cases, but pericentral fibrosis was absent. Four patients presented with hyperlipidaemia and/or hypercholesterolaemia. One patient was being treated with corticosteroids, and 1 patient was obese. The remaining 2 patients had normal serological values for cholesterol and lipids. No alcohol or drug abuse was demonstrated in these patients. Immunohistochemistry showed that in 5 cases 20–50% of the hepatocytes were immunostained with the mAM. Positive hepatocytes were randomly distributed through the parenchyma, although more numerous in the centrolobular area. Megamitochondria were demonstrated in 4 cases. In the remaining 3 cases only scattered hepatocytes were immunostained. These 3 cases corresponded to the patients with normal serological values of cholesterol and triglycerides and the obese patient.

In the case of NASH H&E examination showed features superimposable on those seen in alcoholic hepatitis. Megamitochodria were detected, but were extremely rare. The patient was affected by diabetes type II, and no alcohol or drug abuse was demonstrated. Immunohistochemistry with mAM revealed granular positivity in 30% of the hepatocytes. The type and distribution of immunoreactivity was similar to that observed in alcoholic hepatitis. Megamitochondria were also demonstrated.

All cases (83) of HCV-related chronic hepatitis showed the features of chronic hepatitis [5, 16]. Steatosis was present in 45 cases. In none of these cases were megamitochondria observed on H&E. Seven of these cases presented with cirrhosis, and 8 patients had a daily alcohol consumption of over 80 g; 4 patients had hyperlipidaemia or hypercholesterolaemia; 5 patients were intravenous drug abusers. Histories were negative in the remaining patients. Immunohistochemistry with mAM stained 1–10% of the hepatocytes in 62 cases. The staining was finely granular. Positive hepatocytes were mainly located around the necro-inflammatory foci (Fig. 4a). No megamitochondria were observed. In 21 cases immunostaining was obtained in 40–90% of hepatocytes. The pattern of immunoreactivity was similar to that observed in ALD (Fig. 4b). This group of 21 cases included the 17 patients presenting high alcohol consumption, hyperlipidaemia, hypercholesterolaemia and intravenous drug abuse. Histories were negative in 4 cases with diffuse imunostaining with mAM. No relationship was observed between steatosis and immunostaining with mAM.

All 6 cases of HBV-related hepatitis presented the typical features of chronic hepatitis, as described elsewhere [18]. Oxyphilic changes of the hepatocytes were observed focally in 2 cases. None of the patients had hypertriglyceridaemia or hypercholesterolaemia; nor did any have a history of alcohol or drug abuse. On immunohistochemistry 1–10% of the hepatocytes were immunostained in 5 cases. Positivity was mainly located around the necroinflammatory foci. In 1 case only the positivity reached 60% of hepatocytes.

The case of PBC showed typical findings of PBC stage 2/3. The patient was found to have antimitochondrial antibodies; she was not receiving pharmacological treatment, and neither metabolic alterations nor alcohol or drug abuse were demonstrated. About 20% of the hepatocytes were immunoreactive. Positive hepatocytes were mainly located in the periportal zone.

There was 1 case of primary sclerosing cholangitis. On H&E, typical findings of PSC were observed. The patient presented without hypertriglyceridaemia or hypercholesterolaemia; nor was alcohol or drug abuse suspected.

About 15% of hepatocytes were stained immunohistochemically; the distribution was similar to that seen in cases of PBC immunoreactivity and was mainly confined to periportal hepatocytes.

Mild portal inflammatory infiltration was present in the case of alpha-1-antitrypsin deficiency, together with a slight lobular inflammation. Intracytoplasmic eosinophilic globules were observed, which stained with PAS after diastase digestion. The intracytoplasmic globules immunostained with anti alpha-1-antitrypsin antiserum, but were negative with the mAM.

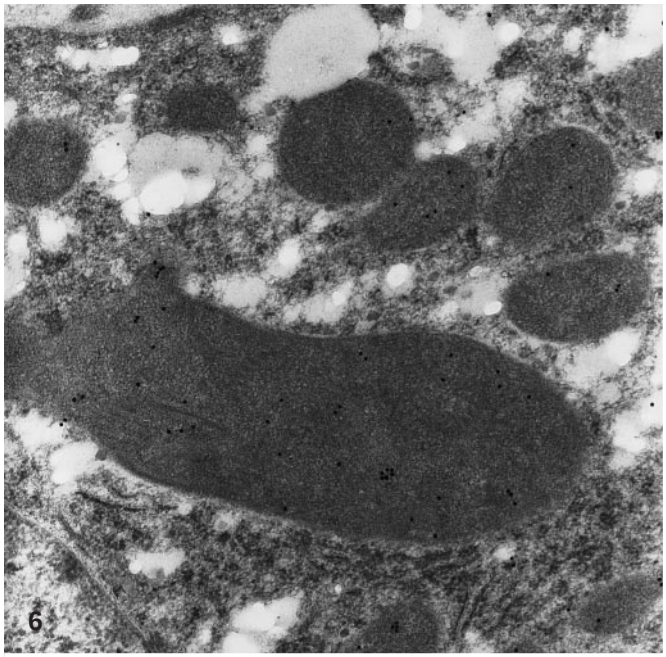
In haemosiderosis numerous hepatocytes contained brownish granules, which were stained with Perls method, and immunoreactivity was observed in 10% of the hepatocytes; the same cells as showed iron overload were decorated.

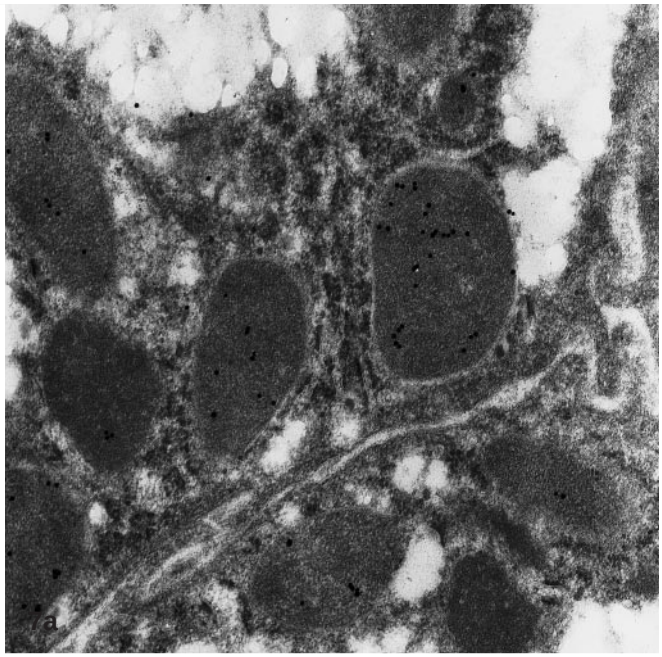
In the case of sarcoidosis typical intraparenchymal giant cell granulomas were present. The patient was affected by previously characterized pulmonary sarcoidosis, which had been treated with corticosteroids. Positivity was observed in 60% of the hepatocytes. In addition, rare megamitochondria were found.

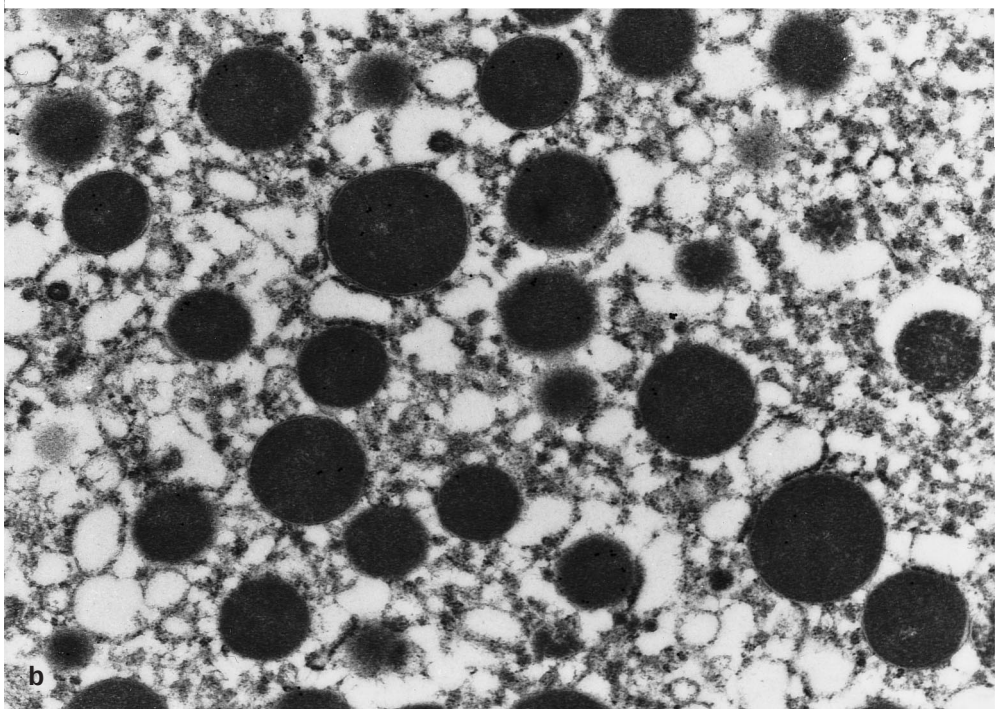
In the 12 cases with nonspecific findings minute necroinflammatory foci were present, scattered throughout the liver parenchyma. In addition, 2 cases showed focal sinusoidal dilatation. These patients underwent liver biopsy because of mild alterations in ALT and AST. Serological studies failed to reveal HBV- or HCV-related markers, hyperlipidaemia, or hypercholesterolaemia. On immunohistochemistry, all cases showed only scattered hepatocytes with finely granular positivity. In 1 case positivity was observed in 80% of the hepatocytes and megamitochondria were seen (Fig. 5). This same patient admitted a daily alcohol consumption of over 80 g.

In addition, antimitochondrial antibody gave a focal immunoreactivity in duct and in Kupffer cells of numerous cases investigated in the present study. Positive Kupffer cells were seen adjacent to necroinflammatory foci. Positivity was observed in proliferating ductules.

On electron microscopy, the monoclonal antimitochondrial antibody specifically labelled mitochondria, the gold particles being mostly localized over the mito-







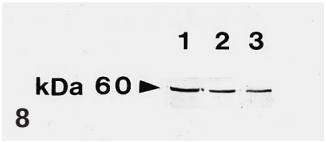


Fig. 6 ALD: immunoelectron microscopic labelling for mAM. Intense gold particle label is present over a megamitochondrion. Background staining is virtually absent. TEM, ×23,000

Fig. 7a, b Immunoelectron microscopic labelling for mAM. **a** ALD. TEM ×44,000 **b** liver adjacent to cavernous haemangioma. Compared with liver adjacent to a cavernous haemangioma, the ALD mitochondria show an increased gold labelling. TEM ×24,00

Fig. 8 Western blot analysis. The 60-kDa protein from liver with steatosis (*lane 1*) appeared more intensely labelled than liver parenchyma adjacent to a cavernous haemangioma (*lane 2*) and cardiac muscle (*lane 3*)

chondrial matrix. Nonspecific labelling was found on poorly infiltrated clear cytoplasmic areas, which corresponded to the extracted glycogen deposits in the conventionally processed samples. The monoclonal antimitochondrial antibody cross-reacted with residual bodies of Kupffer cell and mast cell granules.

Among the samples investigated the degree of labelling varied widely; although a few mitochondria were virtually unreactive, most of them (up to 24 per mitochondrion per section) were coated with numerous gold particles. As expected, megamitochondria displayed a heavy gold particle decoration (Fig. 6); however, taking into account their size the positivity did not exceed that of the neighbouring mitochondria of normal size. Interestingly, mitochondria of liver biopsies from patients with a clinical history of alcohol abuse or toxic/metabolic disease showed increased gold particle labelling (Fig. 7a). On these occasions an average of 9 gold particles/mitochondrion per section was counted. In contrast, mitochondria from HBsAg and HCV-related hepatitis displayed a lower positivity, that is to say 4 gold particles/mitochondrion per section, which was similar to that found in mitochondria from hepatocytes with nonspecific findings (Fig. 7b).

In Western blot analysis, mouse monoclonal anti-human mitochondrial antibody recognizes a band of 60 kDa in extracts from livers and from cardiac muscle. The 60-kDa protein from liver with steatosis (lane 1) appeared more intensely labelled than that of liver parenchyma adjacent to a cavernous haemangioma (lane 2) and of cardiac muscle (lane 3) (Fig. 8). An experiment in which the nitrocellulose paper was incubated without the anti-human mitochondrial antibody showed that the faint bands heavier than 60 kDa were due exclusively to the reaction of anti-mouse biotinylated antibody or to avidin–alkaline phosphatase conjugate.

Discussion

Specificity of the antimitochondrial antibody used in the present study has been tested previously with the immunogold technique [11] and Western blot analysis [13]. The same mAM has been used to detect mitochondrion-rich tumours [4, 11, 13, 18]. Specificity was also confirmed by the results obtained with electron microscopy and immunogold in the cases studied here. The antibody specifically labelled the internal matrix of the mitochondria. On average, four gold particles were normally localized in each mitochondrion. More numerous gold particles (nine on average) were localized in mitochondria in cases of ALD, steatosis and drug abuse.

These findings probably explain why, on light microscopy, little or no immunostaining is normally obtained in hepatocytes. Most probably the reaction is not detectable with light microscopy, but is visible on electron microscopy only. In contrast, when the protein is expressed more vigorously mitochondria became clearly visible with immunohistochemistry, presenting as intracytoplasmic granules of variable size.

In the present series the antimitochondrial antibody showed diffuse positivity in all cases of ALD steatosis and NASH and in biopsies from drug abusers. The data obtained indicate that the 60-kDa protein recognized by the monoclonal antibody is probably overexpressed in these conditions. In particular toxic (e.g. from alcohol or drugs) or metabolic (e.g. from lipids and cholesterol) damage seems to stimulate the appearance of the protein.

Mitochondria contain numerous enzymes involved in cellular metobolism. By screening the molecular weight of these enzymes we found four with molecular weights of aproximately 60 kDa, namely ATP synthetase alpha chain, glutamate dehydrogenase, sterol-26-hydroxylase, and amine oxidase (flavin containing) A. The present mAM may recognize one of these proteins.

In ALD and NASH, immunohistochemical staining with antimitochondrial antibody reveal megamitochondria and granular positivity in numerous hepatocytes. Specifically, it was helpful in identifying megamitochondria in cases of ALD, in which they were extremely rare on H&E, and in 4 cases of steatosis, in which they were not seen on H&E-stained sections. The distribution and possible significance of megamitochondria in ALD has been extensively studied on H&E and by ultrastructural investigation [2, 3, 6, 17, 20]. According to Chedid et al. [3] the presence of megamitochondria in ALD is indicative of a good prognosis, as such patients experience a low incidence of cirrhosis. Immunohistochemistry demonstrating megamitochondria could thus be helpful in evaluating patients with ALD.

A totally different type of immunoreactivity was observed in PBC, PSC, haemosiderosis, nonspecific illness, and HCV- and HBV-related chronic hepatitis. In these cases only occasional hepatocytes revealed finely granular immunostaining. Positive hepatocytes were mainly located around necroinflammatory foci. Most probably the positive hepatocytes correspond to the hepatocytes rich in enlarged mitochondria, with the “oxyphilic” features described in chronic active hepatitis by Lefkowitch et al. [9]. In contrast in the cases of HCV-related chronic hepatitis associated with ALD, metabolic disorders and drug abuse, the type and distribution of immunostaining were similar to those observed in cases of ALD, NASH and steatosis uncomplicated by viral infection. Steatosis is a common finding in HCV-related hepatitis [5, 16]. The cause and significance of steatosis in HCV-related hepatitis are not known. However, in alcoholics with chronic HCV infection the histopathological features of chronic viral hepatitis can mask those of alcoholic injury [21]. In 21 cases in which the biopsy was performed to grade and stage HCV-related hepatitis, diffuse immunoreactivity for antimitochondrial antibody prompted further clinical investigations which disclosed hyperlipidaemia, and alcohol or drug abuse in 17 of these cases. Only in 4 cases was no explanation for the diffuse immunostaining apparent. The same antibody failed to stain hepatocytes with steatosis in cases without toxic or metabolic damage. Immunohistochemical staining with antimitochondrial antibody may be helpful in the diagnosis of an associated toxic injury or metabolic disorder.

Interestingly, no immunostaining was observed in globules of alpha-1-antitripsin deficiency. Globules of alpha-1-antitrypsin deficiency are easily distinguished from megamitochondria, as they are PAS after diastase positive. Nevertheless, immunostaining with antimitochondrial antibody could be an additional feature useful for differential diagnosis.

It seems that immunostaining with antimitochondrial antibody can be helpful in demonstrating megamitochondria; furthermore it discloses diffuse positivity in cases of ALD, metabolic disorders or drug abuse. This may be apparent even in cases in which a complete histological picture of ALD is not evident or is blurred by viral infection.

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References

1. Bendayan M (1984) Protein A-gold electron microscopic immunohistochemistry: methods, applications and limitations. J Electron Microsc Tech 1:243–270
2. Bruguera M, Bertran A, Bombi MB, Rodes J (1977) Giant mitochondria in hepatocytes. A diagnostic hint for alcoholic liver disease. Gastroenterology 73:1383–1387
3. Chedid A, Mendenhall CL, Tosch T, Chen T, Rabin. Garcia-Pont P, Goldberg SJ, Kiernen T, Seeff LB, Sorrell M, Tamburro C, Weessner RE, Zetterman R, and the Veterans Administration Cooperative Study of Alcoholic Hepatitis (1986) Significance of megamitochondria in alcoholic liver disease. Gastroenterology 90:1858–1864
4. Damiani S, Eusebi V, Losi L, D'Adda T, Rosai J (1998) Oncocytic carcinoma (malignant oncocytoma) of the breast. Am J Surg Pathol 22:221–230
5. Fischer HP, Willisch E, Bierhoff E, Pfeifer U (1996) Histopathologic findings in chronic hepatitis C. J Hepatol 24 [Suppl 2]:35–42
6. French SW, Nash J, Shibata P, Kachi K, Hara C, Chedid A, Mendenhall CL, and the VA Cooperative Study Group (1993) Pathology of alcoholic liver disease. Semin Liver Dis 13:154–169
7. Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedure. J Histochem Cytochem 29:577–581
8. Inagaki T, Kobayashi S, Ozekei N, Suzuki M, Fukuzawa Y, Shimizu K, Kato K, Kato K (1992) Ultrastructural identification of light microscopic giant mitochondria in alcoholic liver disease. Hepatology 15:46–53
9. Lefkowitch JH, Arborgh BAM, Scheuer PJ (1980) Oxyphilic granular hepatocytes. Mitochondrion rich liver cells in hepatic diseases. Am J Clin Pathol 74:432–441
10. Lindros KO (1995) Alcoholic liver disease. Pathobiological aspects. J Hepatol 23 [Suppl 1]:7–15
11. Papotti M, Gugliotta P, Forte G, Bussolati G (1994) Immunocytochemical identification of oxyphilic mitochondrion-rich cells. Appl Immunohistochem 2:261–267
12. Perquignot G, Cyrulnik F (1970) Chronic disease due to overconsumption of alcoholics drunks (excepting neuropsychiatric pathology). In: International encyclopaedia of pharmacology and therapeutics, vol II, chap 14. Pergamon Press, Oxford, pp 375–412
13. Roncaroli F, Riccioni L, Cerati M, Capella C, Calbucci F, Trevisan C, Eusebi V (1997) Oncocytic meningioma. Am J Surg Pathol 21:375–382
14. Sambrook J, Fritsch EF, Maniatis F (eds) (1989) Molecular cloning, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
15. Scala C, Cenacchi G, Ferrari C, Pasquinelli G, Preda G, Manara GC (1992) A new acrylic resin formulation. A useful tool for histological, ultrastructural and immunohistochemical investigations. J Histochem Cytochem 40:1799–1804
16. Scheuer PJ, Ashrafzadeh P, Sherlock S, Brown D, Dusheiko GM (1992) The pathology of hepatitis C. Hepatology 15:567–571
17. Stewart RV, Dincsoy HP (1982) The significance of giant mitochondria in liver biopsies as observed by light microscopy. Am J Clin Pathol 78:293–298
18. Tickoo SK, Amin MB, Linden MD, Lee MW, Zarbo RJ (1997) Antimitochondrial antibody (113-1) in the differential diagnosis of granular cell tumors. Am J Surg Pathol 21:922–930
19. Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfers of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354
20. Uchida T, Kronborg I, Peters RL (1984) Giant mitochondria in the alcoholic liver disease – their identification, frequency and pathologic significance. Liver 4:29–38
21. Uchimura Y, Sata M, Kage M, Abe H, Tanikawa K (1995) A histopathological study of alcoholics with chronic HCV infection: a comparison with chronic hepatitis C and alcoholic liver disease. Liver 15:300–306
22. Wight DG (ed) (1994) Liver, biliary tract and exocrine pancreas. In: St Symmers W (ed) Systemic pathology, 3rd edn. Churchill Livingstone, Edinburgh