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CASE REPORT

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A male patient who developed late-onset primary biliary cirrhosis presenting with antinuclear envelope antibodies

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Abstract An 81-year-old man who had previously shown high levels of alkaline phosphatase (ALP), γ-glutamyltransferase (GTP), and total bilirubin presented with acute liver damage. He was positive for serum anti-gp210 and anti-p62 antibodies, but negative for serum antimitochondrial antibody. A liver biopsy revealed massive interstitial fibrosis and pseudolobulus, which were compatible with a diagnosis of primary biliary cirrhosis (PBC) at Scheuer's stage 4. He was given ursodeoxycolic acid at 600 mg/day. However, his condition deteriorated, and he eventually died of hepatic insufficiency in a state of malnutrition. We hypothesize that the presence of anti-gp210 and anti-p62 complex protein antibodies, rather than that of antimitochondrial antibodies, was correlated with the progression of PBC in this particular case.

Key words Anti-gp210 antibody · Antinuclear envelope antibody (ANEA) · Anti-p62 complex antibody · Primary biliary cirrhosis (PBC) · Sjögren's syndrome (SS)

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Introduction

A variety of autoantibodies have been found in patients with primary biliary cirrhosis (PBC). They include antimitochondrial antibody (AMA), anticentromere antibody, and antimultiple nuclear dot antibody. Recently, antinuclear envelope antibody (ANEA) has received special attention.[1–3] The cognate antigen of ANEA is an integral glycoprotein, gp210, located in the nuclear pore membrane. Among PBC patients, 9.4%–21% have been reported to possess ANEA which react with gp210 upon immunoblotting.[4,5] In contrast, the presence of antilamin B receptor antibody has been reported in less than 5% of PBC patients.[4] In 1996, Gadek et al.[6] reported that PBC sera reacted with a 62-kDa protein known to be a major component of the nuclear pore complex.[7] In this study, we report on a rapidly progressive late-onset case of PBC having both anti-gp210 and anti-p62 antibodies.

Case report

The patient, who was born in 1909, had been followed for the past 15 years at Keigu Clinic. Initially he was being seen two to three times a month because of hypertension. Liver dysfunction was never observed (Fig. 1). Toward the end of 1991 he began to complain of itching and fatigue, and his abnormal liver functions included ALP 772 IU/l, γ GTP 473 IU/l, TB 2.0 mg/ml, GOT 111 IU/l, and GPT 134 IU/l, suggesting the presence of a combination of cholestatic liver damage and liver cell damage. Antinuclear antibodies (ANA) were positive at a 1:160 dilution with homogeneous staining, and AMA was negative at a 1:40 dilution. The patient was subsequently sent to a hospital for further evaluation, where various malignant diseases and drug-induced diseases were ruled out. He was retested in July 1994 and tested positive for ANA at a dilution of 1:1280, as well as for ANEA. He was referred to a hepatologist to undergo liver biopsy. His general details were height 167 cm

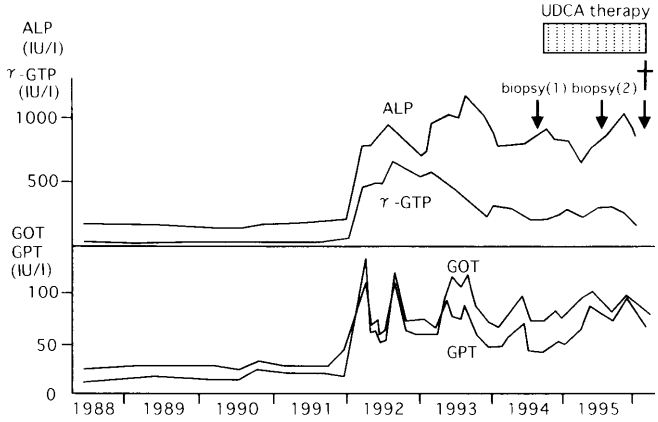

Fig. 1. Clinical course of the primary biliary cirrhosis (PBC) patient who presented with abnormal liver function in December 1991. He died of hepatic insufficiency and malnutrition in January 1996

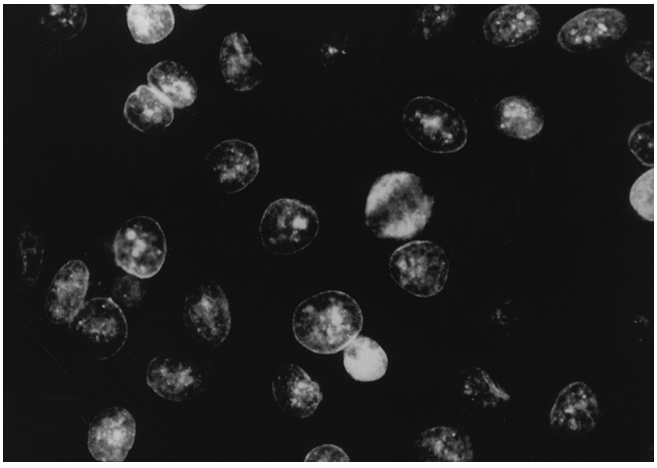

Fig. 2. Indirect immunofluorescence showing typical nuclear envelope staining of Hep-2 cells at high magnification. The patient's serum was diluted 1:160. Original magnification ×400

and weight 63 kg. He had jaundice, but no anemia. He had no noticeable skin rash on his body. His heart and breathing sounds were normal. Hepatomegaly and splenomegaly were not marked. Ascites and leg edema were not observed. His laboratory and immunological findings included: TB 3.1 mg/dl, DB 2.1 mg/dl, GOT 76 IU/l, GPT 42 IU/l, rGTP 215 IU/l, ALP 879 IU/l, TTT 12 U, and ZTT 23 U. Hepatitis B surface antigen (HBsAg) and hepatitis C virus (HCV) Ab were negative. Abnormal peripheral blood examination results included hemoglobin (Hb) 11.1 g/dl, and platelets $11 \times 10^4/mm^3$. Erythrocyte sedimentation rate was 39 mm/h. IgG and IgM levels were 2000 mg/dl and 585 mg/dl, respectively. AMA, as determined by indirect immunofluorescence and by enzyme-linked immunosorbent assay (ELISA) using E2 of M2, was negative. Antismooth muscle antibody was also negative at a 1:40 dilution. However, ANEA was positive at a 1:1280 dilution (Fig. 2).

Microscopic examination of the liver biopsy specimen showed the presence of massive interstitial fibrosis and pseudolobulus at low magnification (Fig. 3a). Mononuclear cells were moderately infiltrated in Glisson's area, and interlobular ducts were not observed. Furthermore, under high magnification, mononuclear cells were seen to have infiltrated the liver cell plate and parenchyma (Fig. 3b). Taken together, these findings led to this particular patient being diagnosed as having stage 4 (by Scheuer's criteria) PBC.

Subsequently, the patient's serum ANEA was characterized by immunoblot analysis. The reactivities of various PBC sera with extracts of sonicated Hela cells are shown in Fig. 4a. Serum from a patient with Sjögren's syndrome (SS) was put in lane 6. PBC sera with AMA were put in lanes 1–4, and the patient's serum without AMA was put in lane 5. Bands at 72 kDa and 52 kDa were seen in lanes 1–4, and a band at 200 kDa was seen in lanes 4 and 5. The 100-kDa band in lane 5 is thought to represent multiple nuclear dot antigen. Figure 4b shows immunoblot results using nuclear envelope proteins and the same groups of PBC sera. Figure 4c shows immunoblot results using a Triton/high-salt ex-

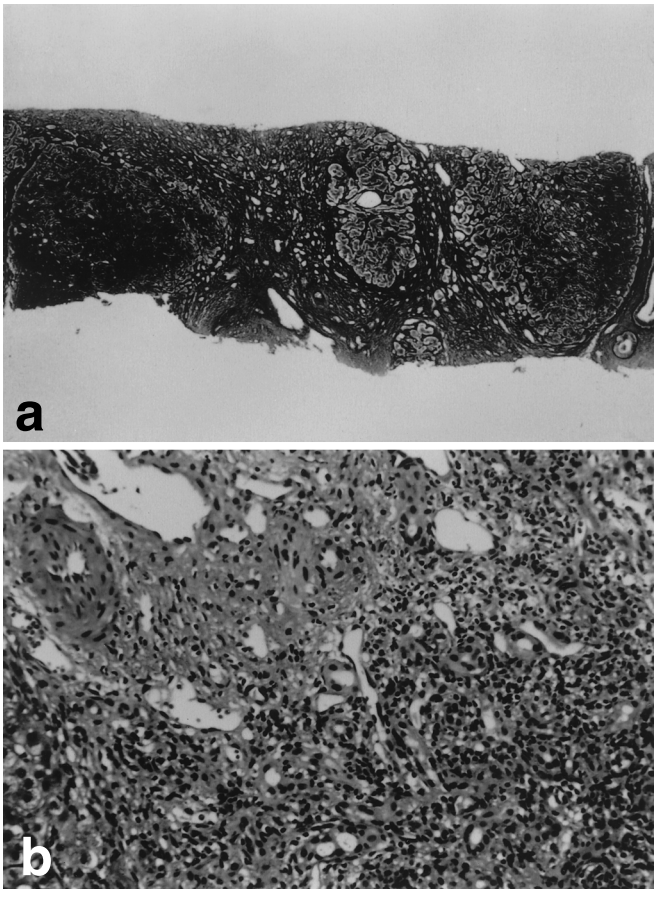

Fig. 3. The first liver biopsy was performed in August 1994. **a** Azan Mallory staining at low magnification, showing massive interstitial fibrosis and pseudolobulus, including large and small lobulus formation. Original magnification ×50. **b** Haematoxylin eosin staining at high magnification, showing small round cells infiltrating the liver cell plate and Glisson's area, where small bile ducts were destroyed. Original magnification ×200

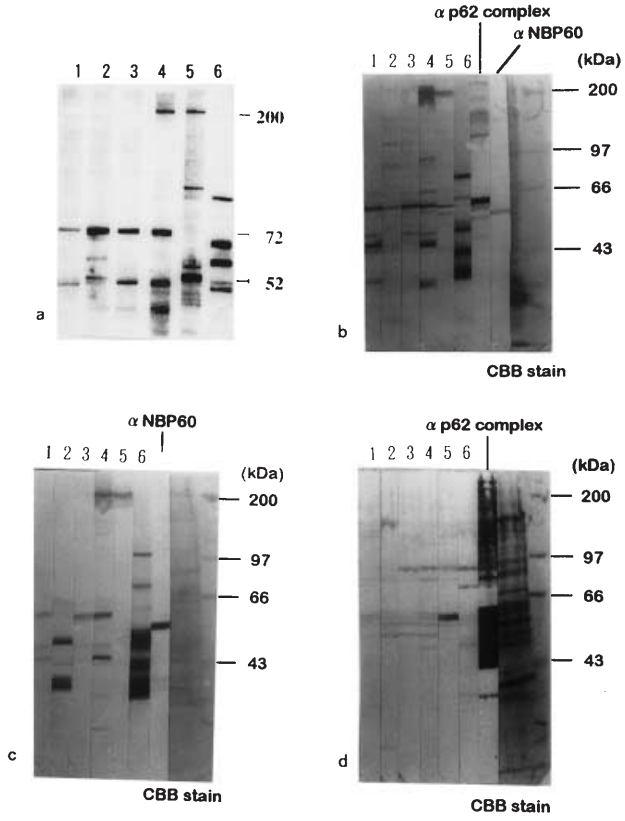

Fig. 4. **a** Reactivities of five PBC sera with sonicated HeLa cell extracts. The patient's serum, which was negative for antimitochondrial antibody (AMA) and positive for antinuclear envelope antibody (ANEA) was put in lane 5. **b** Reactivities of the same sera with nuclear envelope proteins. **c** Reactivities of the same sera with Triton-X/KCl extract of rat liver nuclear envelope. Antirat lamin-B receptor serum was put in lane "α NBP60." **d** Reactivities of the same sera with the wheat-germ agglutinin (WGA) bound fraction of rat liver nuclear envelope. Rabbit antirat p62 complex serum was put in lane "α p62 complex"

tract of rat liver nuclear envelope and the same group of PBC sera. These extracts contain nuclear membrane proteins such as gp210, lamina-associated polypeptide (LAP), lamin B receptor, and others. The gp210 band is seen in lanes 4 and 5. The bands of 53, 43, and 41 kDa observed in lanes 2 and 6 are thought to be derived from LAP 2.

Figure 4d represents an immunoblot assay using as antigen the wheat germ agglutinin (WGA) bound fraction, and the same group of PBC sera. A 62-kDa band corresponding to a component of the nuclear pore p62 complex is present in lane 5. A putative nucleoporin band of 86 kDa is seen in lanes 3, 4, and 5.

A second liver biopsy was performed in June 1995. The findings were similar to those from 1994. By December 1995, the patient had accumulated massive ascites and pleural effusion. He was admitted to a neighboring hospital, but died of hepatic insufficiency in a state of malnutrition in January 1996.

Discussion

We have described the case of an elderly man with PBC and with serum ANEA. This case was unusual in several respects.[8,9] First, male PBC cases are rare (M/F ratio 1:10). Second, the onset of symptoms usually occurs at 50–55 years of age, but in this case, it occurred very late at 84 years. Third, AMA is present in 90%–95% of patients with PBC, but serial AMA tests, including indirect immunofluorescence on rodent liver cryostated sections and ELISA using pyruvate dehydrogenase (PDH)-E2, consistently showed negative results. Fourth, ANEA are thought to be present in only 29%–53% of PBC cases,[1–5] but in this case the serum contained very high titers of ANEA. Finally, late-onset PBC cases are generally asymptomatic or mild,[10] but in this case the disease was rapidly progressive. The possibility of this case being a PBC–CAH overlap was ruled out when tests for serum anti-M4 and anti-M8 antibodies were found to be negative (data not shown).

We reported previously that a high percentage of PBC sera recognize gp210, which is localized in the nuclear pore membrane.[5,11] This patient's serum was found to recognize not only gp210 but also p62 complex proteins bearing *N*-acetylglucoseamine residues from rat liver nuclear envelope. Gadek et al.[6] reported that anti-p62 antibodies appear to be a novel marker for PBC. However, another study reported that although anti-p62 antibodies were not found in patients with RA, they were detected in 13% of patients with Sjogren's syndrome.[12] Thus, anti-p62 antibodies may not necessarily be specific for PBC.

We do not know why PBC patients show chronic cholestasis and parenchymal damage in the late stage of the disease. As far as we can gather from this case, the presence of anti-gp210 and/or anti-p62 may be more relevant to the progression of PBC than that of AMA.[13] Furthermore, Itoh et al.[14] reported the utility of anti-gp210 as an independent prognostic marker for a poor outcome in patients with PBC.

We cannot yet explain how these antibodies elicit cholestasis of biles and damage to parenchymal cells. Based on data concerning expression of the E2 component of pyruvate dehydrogenase (PDH),[15] it is suggested that the actual mechanism involves recognition by activated T lymphocytes (CD4) of selected molecules on the cell membrane surface of the small bile duct. However, the precise mechanism of cholestasis and cell damage remains to be clarified.

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