CASE REPORT

Changes in titers of antimitochondrial and antinuclear antibodies during the course of primary biliary cirrhosis

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Abstract A case of primary biliary cirrhosis (PBC) in whom a complete biochemical (serum bilirubin, transaminases and alkaline phosphatase) remission was noted after combination treatment with ursodeoxycholic acid (UDCA) and corticosteroid is reported. The antimitochondrial antibody (AMA) detected by indirect immunofluorescence was initially positive, and the antinuclear antibody (ANA) was negative, but these two antibodies subsequently fluctuated independently (AMA-positive/ANA-negative, AMA-negative/ANA-negative, AMA-negative/ANA-positive, AMA-positive/ANA-positive, and again AMA-negative/ANA-positive) in spite of a lack of histopathological improvement in the liver after treatment. The clinical presentation in our case suggests that in some cases the diagnosis of PBC or so-called autoimmune cholangitis (AIC) might depend on the ‘phase’ of the same disease. Our results also suggest that detailed immunoreactive profiles against 2-oxo-acid dehydrogenase complex (2-OADC) enzymes by using immunoblotting, together with a serial histological examination, should provide more precise information for a diagnosis of PBC.

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INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease associated with the damage of small and medium-sized bile ducts, which leads to cholestasis and progressive fibrosis. The cause of the disease is unknown, but is thought to be related to certain inherited disorders of immunoregulation.1 Antimitochondrial antibodies (AMA) are found in 95% of patients with PBC, and they have a specificity of more than 98% for this disease.2,3 It is well known, however, that AMA titers in PBC often fluctuate considerably even from a negative result to a positive one and vice versa during the course of the disease.4 Moreover, approximately 5% of patients who exhibit the clinical characteristics of PBC are AMA negative, and the majority of these patients have either antinuclear antibodies (ANA) or antismooth muscle antibodies (SMA).5,6

In the present report, we describe a patient with PBC in whom a complete biochemical remission was noted after combination treatment with ursodeoxycholic acid (UDCA) and corticosteroid. The AMA was initially positive and ANA was negative, but these two antibodies subsequently fluctuated considerably (AMA-positive/ANA-negative, AMA-negative/ANA-negative, AMA-negative/ANA-positive, AMA-positive/ANA-positive, and again AMA-negative/ANA-positive) in spite of a lack of histopathological improvement in the liver after treatment. We also examined serum immunoreactivity with enzymes of 2-oxo-acid dehydrogenase complex (2-OADC) by the use of immunoblotting.
CASE REPORT

Patient

A 43-year-old Japanese woman was referred to Nagasaki University Hospital in August 1993 for further management of pruritus and jaundice. A diagnosis of asymptomatic PBC had been made 3 months earlier at a local hospital based on biochemical and histopathological features of PBC with a positive AMA (1:160) by using indirect immunofluorescence. At that time, the serum level of immunoglobulin M (IgM) was 987 mg/dL (normal value, <359 mg/dL). The histopathological examination of a liver biopsy specimen obtained in May 1993 showed infiltration of mononuclear cells in the portal area with proliferation of small bile ducts (Fig. 1a), but no granuloma and copper deposition (by using Rubeanic acid stain) were observed. The diagnosis of PBC prompted treatment with UDCA at a dose of 600 mg daily, together with 7.5 g/day of Sho-saiko-to, an oriental herbal drug. The patient responded well to the therapy and showed improvement in serum levels of alkaline phosphatase (ALP), gamma-glutamyl transpeptidase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Three months later, however, she developed pruritus and jaundice. She was not a smoker, consumed alcohol only on rare occasions and had never received a blood transfusion. A physical examination on admission in our hospital showed jaundice and mild hepatomegaly. The serum level of total bilirubin was 3.4 mg/dL (normal value, <1.0 mg/dL), ALP was 318 IU/L (normal value, <270 IU/L), AST was 674 IU/L (normal value, <39 IU/L), ALT was 1074 IU/L (normal value, <33 IU/L), IgG was 1307 mg/dL (normal value, 2010 mg/dL) and IgM was 177 mg/dL. All tests for serum markers for hepatitis B virus infection, antibodies to hepatitis C virus (second-generation ELISA) and IgM antibody to hepatitis A virus gave negative results. Both ANA and AMA were negative, but AMA was positive at a titer of 1:40 (AMA-positive/ANA-negative). The human leukocyte antigen (HLA)-DR typing was DR 1 and DR 12. The lymphocyte stimulation test (LST) for Sho-saiko-to was negative. The computed tomographic scan of the abdomen showed no evidence of bile duct obstruction or liver tumor. Although there was no evidence of a drug-induced liver injury and ANA was negative, a provisional diagnosis of hepatitis caused by Sho-saiko-to or overlap syndrome with chronic active hepatitis was made. Accordingly, Sho-saiko-to was discontinued and the patient was treated with 40 mg/day of prednisolone (Shionogi & Co., Ltd, Osaka, Japan) together with UDCA. During the next 3 weeks, the serum levels of total bilirubin, ALP, AST and ALT returned to within normal limits, thus allowing a gradual tapering of prednisolone, with administration of prednisolone ceasing in July 1994. A second liver biopsy specimen obtained in October 1993 showed features similar to those observed in May 1993 (HE stain, ×150). A third liver biopsy specimen obtained in April 1997 showing significant mononuclear cells infiltration with chronic non-suppurative destructive cholangitis (CNSDC; arrow; HE stain, ×150). During the last 6 years of follow up, the patient has remained clinically and biochemically stable (serum levels of total bilirubin, ALP, AST, ALT and IgM were <1.0 mg/dL, 118–278 IU/L, 9–16 IU/L, 4–15 IU/L and 131–216 mg/dL, respectively), while being treated exclusively with UDCA.
However, the histopathological features of a third liver biopsy specimen obtained in April 1997 did not show any improvement and showed significant mononuclear cell infiltration with chronic non-suppurative destructive cholangitis (CNSDC) (Fig. 1c). The patient suffered from cancer of the left breast and underwent a mastectomy in August 1998 followed by adjuvant chemotherapy by using cyclophosphamide, epirubicin, and fluorouracil until February 1999.

From May 1997, the tests for ANA by using indirect immunofluorescence turned positive (1:40–1:160 titers) with a speckled pattern (AMA-negative/ANA-positive). The AMA also turned positive from March 1998 (1:40–1:160 titers; AMA-positive/ANA-positive) and this again reversed to negative in June 1999 (AMA-negative/ANA-positive) (Fig. 2).

Immunoreactivity against 2-oxo-acid dehydrogenase complex by using immunoblotting

Serially stocked sera were tested by the use of immunoblotting at a dilution of 1:5000 against bovine heart mitochondria (BHM) prepared by differential centrifugation from homogenized bovine heart,7 kindly provided by Dr Ian R Mackay (Monash University, Victoria, Australia), as previously described.8 Peroxidase-coupled affinity-purified goat antihuman IgG (H + L; Bio-Rad, Richmond, CA, USA) was used as the secondary antibody, and enhanced chemiluminescence (ECL) western blotting detection reagents (Amersham Inc., Buckinghamshire, UK) as the peroxidase substrate. The patient’s serum samples obtained during prednisolone treatment in November 1993 did not react with any of the BHM proteins (Fig 2, lane 1). However, after cessation of prednisolone, reactivity to proteins of 74 and 46 kDa appeared in August 1994 (Fig. 2, lane 2). Thereafter, the serum reacted weakly with a 74 kDa protein in February 1996 (Fig. 2, lane 3), December 1996 (Fig. 2, lane 4) and April 1997 (Fig. 2, lane 5), but did not react in September 1997 (Fig. 2, lane 6). The reactivity to this protein again weakly appeared in March 1998 (Fig. 2, lane 7) and July 1998 (Fig. 2, lane 8), but gradually decreased to low levels in September 1998 (Fig. 2, lane 9), and almost totally disappeared in March 1999 (Fig. 2, lane 10).

DISCUSSION

The possible cause of acute exacerbation of liver dysfunction observed in August 1993 in our patient included drug-induced hepatitis caused by Sho-saiko-to rather than overlap syndrome with chronic active hepatitis. According to the revised scoring system for diagnosis of autoimmune hepatitis (AIH) at the time of acute exacerbation,9 the aggregate score was zero, indicative of ‘non-AIH’ : female gender (+2); ALP : AST ratio (+2); AMA positive (–4); no hepatitis viral markers (+3); drug history (Sho-saiko-to; –4); average alcohol intake < 25 g/day (+2); biliary changes suggestive of PBC (–3); and a complete response to therapy (+2). Sho-saiko-to consists of a mixture of extracts from seven different herbs: Bupleurum root, Pinellia tuber, Scutellaria root, Jujube fruit, Ginseng root, Glycyrrhiza root and Ginger rhizome.10 Although herbal medicines such as Sho-saiko-to are believed to be safe, liver
damage induced by these agents has been reported.\textsuperscript{11} The major mechanism of such hepatotoxicity may be immunoallergic. Moreover, AIH triggered by herbal medicine has also been reported.\textsuperscript{12} Maeda \textit{et al.}\textsuperscript{13} reported a case of exacerbation of PBC during interferon therapy, which was thought to exert immunomodulatory effects. Therefore, the use of Sho-saiko-to in our patient may have induced acute exacerbation of liver dysfunction, although LST for Sho-saiko-to was negative.

Primary biliary cirrhosis is a slowly progressive chronic cholestatic liver disease, the pathogenesis of which is thought to be related to autoimmune mechanisms.\textsuperscript{1} However, a spontaneous or treatment-related remission of PBC is rarely encountered.\textsuperscript{14} The criteria for remission of PBC has been defined as follows: (i) a disappearance of pruritus and fatigue (symptomatic remission); (ii) normalization of serum bilirubin, AST and IgM, with ALP $< 1.5 \times$ normal (biochemical remission); and (iii) absence or restriction of inflammation to the portal tracts without bile duct destruction and absence of granulomas (histological remission).\textsuperscript{15} Based on the above criteria, Wolfhagen \textit{et al.}\textsuperscript{16} reported that treatment with UDCA resulted in the biochemical remission in 11\% of their patients with PBC, both biochemical and symptomatic remission in 6\%, and complete (symptomatic, biochemical and histological) remission in only 4\% of patients.\textsuperscript{15} Jorgensen \textit{et al.}\textsuperscript{17} also reported normalization of liver biochemical functions in 19\% of their patients with PBC 2 years after treatment with UDCA.\textsuperscript{16} Moreover, Kaplan \textit{et al.}\textsuperscript{18} reported that five of 19 patients with PBC responded completely to oral methotrexate.\textsuperscript{14} In our patient, a clear biochemical remission was evident after treatment with both UDCA and prednisolone. However, serial liver biopsy specimens did not show histological improvement, and most serum samples reacted against PDC-E2 when immunoblotting was conducted, even when the AMA by immunofluorescence was negative.

There is still no generally accepted treatment for PBC except UDCA therapy and liver transplantation. As osteoporosis is an important common complication of PBC, the use of a corticosteroid is not recommended.\textsuperscript{17} However, several recent studies have suggested that corticosteroid alone or in combination with UDCA may improve symptoms and certain biochemical parameters without worsening bone density.\textsuperscript{18–20} Thus, the new generation corticosteroids that have a lesser effect on bone density may hold hope for the future.\textsuperscript{17} In our patient, a complete biochemical remission occurred after treatment with both UDCA and prednisolone. Moreover, serum levels of ALP, AST, ALT and total bilirubin were persistently normal for 6 years following the cessation of prednisolone. Although the bone mineral content was not assessed before and after therapy, there were no clinical problems relevant to osteoporosis.

The interesting finding in our patient was the fluctuation of AMA and ANA reactivity during the course of the disease. Recently, considerable interest has been generated in a novel chronic liver disease recognized as autoimmune cholangitis/cholangiopathy (AIC), which is characterized by chronic cholangitis resembling PBC with a high titer of ANA and with seronegativity for AMA.\textsuperscript{21–23} However, the clinical entity of AIC is so far unestablished because 'sero-conversion' from AMA positivity to negativity by immunofluorescence, or vice versa, is a rare but recognized clinical situation in PBC,\textsuperscript{24} and an only consistently distinguishing feature between AIC and PBC is the autoantibody (AMA and ANA) profile, as both had otherwise virtually identical clinical and histopathological features.\textsuperscript{25,26} Goodman \textit{et al.}\textsuperscript{1} and Dhillon\textsuperscript{27} have proposed revised categories of a group of conditions in which chronic non-suppurative destructive cholangitis is seen histologically. They tentatively divided them into four groups according to the AMA and ANA results (i.e. AMA-positive/ANA-positive, AMA-positive/ANA-negative, AMA-negative/ANA-positive, and AMA-negative/ANA-negative). Our patient showed all of the above four serological patterns, and therefore our case report suggests that in some cases the diagnosis of PBC or AIC might depend on the 'phase' of the same disease.\textsuperscript{28,29}

Our results also suggest that detailed immunoreactive profiles against 2-OADC enzymes by using immunoblotting together with a serial histological examination should provide more precise information for the diagnosis of PBC. In our previous study, 31\% of sera from ‘AMA-negative’ PBC and 24\% of sera from AIC reacted by immunoblotting with the major autoantigenic component in PBC, PDC-E2.\textsuperscript{2,26} Unfortunately, stocked serum at the initial presentation in May 1993 was not available, but most of the stocked sera after the cessation of prednisolone treatment reacted to the 74 kDa protein (PDC-E2) even when the AMA was negative and serum levels of ALT and ALP were within normal limits in our patient. The serum reactivity to the 74 kDa protein was strong just after the cessation of prednisolone treatment, and it could be caused by an immunologic rebound phenomenon after the discontinuation of prednisolone. However, the reason why the AMA tested by immunofluorescence was negative at that time is still unclear.

In conclusion, the clinical presentation in our case suggests that in some cases the diagnosis of PBC or AIC might depend on the 'phase' of the same disease. Detailed immunoreactive profiles against 2-OADC enzymes by immunoblotting and serial histological examinations are useful for a diagnosis of PBC in such cases.

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REFERENCES

Antibodies in primary biliary cirrhosis
