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Primary biliary cirrhosis in classmates: Coincidence or enigmatic environmental influence?

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ABSTRACT

Two former classmates aged 58 with a history of more than 20 years of elevated liver function tests presented to our clinic. As they were both tested positive for antimitochondrial antibodies and liver biopsy showed distinctive bile duct lesions, they were given the diagnosis of primary biliary cirrhosis. To confirm presence of AMAs and subtype of AMAs western blotting was performed showing two distinct bands at 62 kD (PDH-E2) and 48 kD (2ODH-E2) in patient 1 and only one band at 62 kD in patient 2. Searching for potential explanations for this unusual occurence of this rare disease in two close friends a comparison of restriction fragment lengths polymorphism was performed and excluded any familial relationship. Interestingly, histocompatibility leukocyte antigen typing revealed strong similarities as they both shared A2, B51, DR4, DR8 and DR53. These results may point to an unknown environmental factor what caused primary biliary cirrhosis in immunologically susceptible individuals.

Keywords: Primary biliary cirrhosis, etiology, antibody, frequency, autoimmunity

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic disease involving immune mediated destruction of the intrahepatic bile ducts. The course is slowly progressive leading to periportal fibrosis and ultimately cirrhosis requiring transplantation of the liver. Recently, using cryopreserved primary hepatocytes and transdifferentiation of stem cells to hepatic phenotypes future options for therapies of slowly progressive liver diseases have been demonstrated (Steinberg et al., 1999; Hengstler et al., 2000a,b; Beerheide et al., 2002). The most characteristic immune abnormality is the presence of antimitochondrial antibodies (AMA; Kaplan, 1987). Different types of mitochondrial autoantigens have been described, but only antibodies against the M2 autoantigen have been demonstrated to be specific for primary biliary cirrhosis. For the M2 autoantigen two subunit enzymes of a functionally related enzyme family have been identified, the E2 subunit of the pyruvate dehydrogenase complex (PDH-E2) and the E2 component of the 2-oxo-acid-dehydrogenase complex (2ODH-E2; Gershwin and Mackay, 1991). The question as to what triggers the initial bile duct lesion in primary biliary cirrhosis, - genetical, environmental, or defective immunoregulation alone – has yet not been answered.

PATIENTS

Case report 1

Patient 1 was the 58-year-old female first presented in 1995 with a 26 year history of intermittent elevations of serum aminotranferases. As demonstrated in table 1 serologic studies were positive for AMA and antinuclear autoantibodies (ANA).

Table 1: Comparison of serology and serum chemistry of the presented patients

Parameter	Patient 1	Patient 2
AMA (IFT)	1:20	1:80
anti-PDH-E2 (ELISA, %)	neg	56
anti-2ODH-E2 (ELISA, %)	51	Neg
ANA (IFT)	1:80	Neg
SMA (IFT)	neg	Neg
SLA (IFT)	neg	Neg
LKM (IFT)	neg	Neg
ALT (U/L)	42	54
AST (U/L)	36	28
AP (U/L)	125	282
γ-GT (U/L)	38	48
Total Bilirubin (µmol/L)	18.0	24.1
IgG (g/L)	16.3	10.2
IgM (g/L)	1.96	1.69
Total γ-globulin (relative %)	21.6	13.9

Smooth muscle antibodies (SMA), soluble liver antigen antibodies (SLA), and liver-kidney-microsomal antibodies (LKM) were not detectable. Serology for hepatitis A, B, C, and D was negative. Electrophoresis showed moderate hypergammaglobulinemia primarily due to elevated immunoglobulin G (IgG) levels (see table 1). Laparoscopy showed moderate liver fibrosis and histological findings on liver biopsy were chronic hepatitis with moderate inflammatory activity, occasional piecemeal necroses, distinct portal fibrosis, and accompaning cholangitis. On the basis of these findings the patient was considered to suffer from PBC, better termed chronic nonsuppurative destructive cholangitis, with secondary autoimmune hepatitis (AIH). The patient was started on treatment with ursodeoxycholic acid (UDCA, 750 mg/d) and immunosuppression with tapering doses of prednisolone and azathioprine (50mg/d) as is our standard practice (Meyer zum Büschenfelde and Lohse, 1995). During follow-up the patient showed a good response with histologically less inflammatory activity without necroses and complete normalization of liver function tests (alanine aminotransferase (ALT) 17 U/L, aspartate aminotransferase (AST) 32 U/L, alkaline phosphatase (AP) 128, γ-glutamyl tranpeptidase (γ-GT) 19 U/L, and total bilirubin 14.2 µmol/L).

Case report 2

In 1997 a former classmate of the first patient came to our clinic. They shared the same table at primary school from 6th till 10th year of age and had been friends since then. Though they were born in a relatively small town, they denied any familial relationship. The second patient was the 58-year-old female with a past medical history including 23 years of abnormal liver function tests and two episodes of jaundice: (i) At the age of 7 the patient experienced an acute hepatitis probably explained by a hepatitis A virus (HAV) infection, as family members and

close friends were also be affected. (ii) During her first pregnancy at the age of 22 she complained of recurrent colicky pain in the upper abdomen and developed scleral icterus. Cholangiography performed after this pregnancy was normal pointing to a pregnancy jaundice frequently being the first symptom of primary biliary cirrhosis.

As demonstrated in table 1 serum chemistry showed moderately elevated ALT, AP, γ-GT, and total bilirubin and a normal AST. She also had a combined hyperlipoproteinemia with triglycerides of 3.59 mmol/L and cholesterol of 5.34 mmol/L under treatment with a HMG-CoA reductase inhibitor. Gamma-globulins, IgG and IgM fractions were within the normal range. Serologic tests were positive for AMA and anti-HAV IgG. Anti-HAV IgM and Hepatitis B, C, and D testing was negative. Histology showed primary biliary cirrhosis grade 2 with lymphocytic inflammatory infiltration of bile ducts, necrosis of bile duct epithelia, and focal bile duct proliferation. Therefore, the patient was given the diagnosis of primary biliary cirrhosis with combined hyperlipoproteinemia and received treatment with UDCA (750 mg/d).

MATERIALS AND METHODS

ELISA. Mitochondrial autoantibodies against PDH-E2 and 2ODH-E2 were diagnosed by inhibition enzyme-linked immunosorbent assay (ELISA) based on the description of Manns et al. (1987). This assay is based on the capability of a test serum to inhibit the binding of a labelled indicator AMA-positive serum to PDH-E2 or 2ODH-E2 indicating the presence of anti-PDH-E2 or anti-2ODH-E2-antibodies in the test serum. Microtiter plates were coated with 50 µl of gamma globulins (20µg/ml) of an anti-PDH-E2-positive or anti-2ODH-E2-positive serum and postcoated with 1% BSA in PBS. After washing, 50 µl (200 µg/ml) of mitoblasts from rat prepared as 100,000 g supernatant by differential centrifugation as described previously (Manns et al., 1987) were added. After washing of unbound antigen, the plates were incubated with 50 µl of biotinylated gamma globulins of the indicator serum (10 µg/ml) in 1% BSA/PBS. Binding of the indicator serum was detected with 50 µl of avidin-peroxidase (1:1500 in 1% BSA/PBS) and ABTS solution (Sigma, Deisenhofen, Germany). Samples were measured at λ402nm in a Titertec Multiscan MC (Flow Laboratories, Meckenheim, Germany). Sera were considered anti-PDH-E2 or anti-2ODH-E2 positive, if binding of the indicator serum was inhibited by more than 40% (i.e. above two standard deviations of inhibition by normal sera).

Western Blot. SDS PAGE was performed with a 10% running gel and a 4.5% stacking gel based on the method of Laemmli (1970) as described previously (Herkel et al., 1997). A Mini Protean II Electrophoresis chamber (BioRad, Munich, Germany) was used and 200 µg of rat mitoblasts (prepared as described above) were applied to the gel. Proteins were transferred to a polyvinyl-difluoride (PVFD) membrane (Millipore, Eschborn, Germany). For immunoenzymatic detection patient sera were diluted 1:200 and peroxidase conjugated anti-human IgG, IgA, IgM, kappa, lambda (Dako, Kopenhagen, Denmark) were diluted 1:1000. Immunoblots were developed with metal-enhanced diaminobenzidine kit (Boehringer, Mannheim, Germany). The following immunoblots were carried out according to Towbin et al. (1979). Sera were diluted 1:200.

Additionally, histocompatibility leukocyte antigen (HLA) typing was performed and restriction fragment lengths polymorphism of both patients was analysed as previously described (Hohler et al., 1996; Kaluza et al., 2001).

RESULTS

At the day of presentation both patients were tested positive for either anti-PDH-E2 or anti-2ODH-E2 (see table 1). During follow-up results of ELISA in patient 1 were

repeatedly positive for anti-PDH-E2 and anti-2ODH-E2, and in patient 2 anti-PDH-E2 occasionally was not detectable. To confirm presence of AMAs and subtype of AMAs western blotting was performed showing two distinct bands at 62 kD (PDH-E2) and 48 kD (2ODH-E2) in patient 1 and only one band at 62 kD in patient 2 (**Fig. 1**). Histocompatibility leukocyte antigen typing revealed strong similarities as both patients shared A2, B51, DR4, DR8 and DR53 (**Table 2**). Genetic differences between both individuals could be evidenced by analysis of restriction fragment lengths polymorphism showing a wide chromosomal variability.

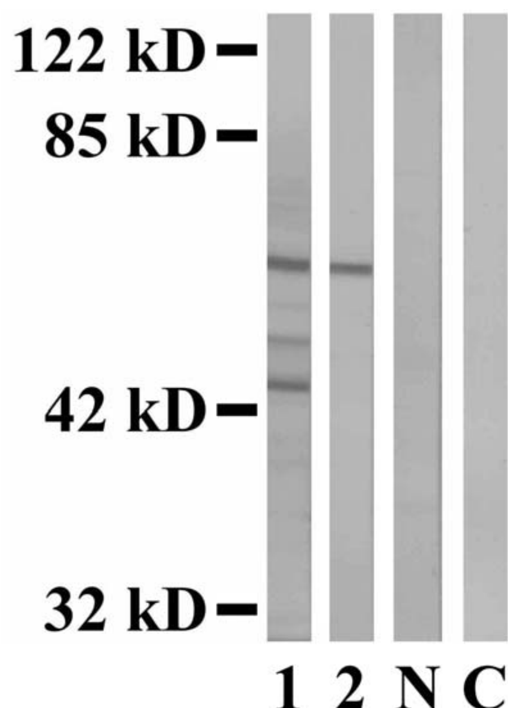

Fig. 1: Western Blot showing two distinct bands at 62 kD (the E2 subunit of the pyruvate dehydrogenase complex; PDH-E2) and 48 kD (the E2 component of the 2-oxo-acid-dehydrogenase complex; 2ODH-E2) in patient 1 (1) and only one band at 62 kD in patient 2 (2) as compared to a control with no human serum (N) and a pooled serum of healthy human subjects (C).

Table 2: Histocompatibility leukocyte antigen (HLA) typing of the presented patients

HLA	A	B	C	DR	DQ	
Case 1	2/3	51/50	w6	4/8	7/8	DR53
Case 2	2/24	51/60	w3	4/8	3/4	DR53

DISCUSSION

We described the occurence of primary biliary cirrhosis in adulthood in two patients who were close friends for most of their early schoolyears. Four explanations for this observation came to mind: (i) chance occurence; (ii) common environmental risks; (iii) common genetic risks; (iv) common immunological susceptibility.

(i) As the prevalance of primary biliary cirrhosis in West Germany was shown to be only 0.7 per 100,000 inhabitants (Triger et al., 1984) chances are almost zero that patient 1 and patient 2 who visited the same class at primary school developed primary biliary cirrhosis by coincidence.

(ii) In both patients intermittent elevations of liver enzymes were known for about 25 years. Considering the slow and insidious progression typical for primary biliary cirrhosis, the exposure to an unknown causative agent could possibly have taken place during their visit of primary school till 10th year of age. What particular agent could have been responsible for the development of

primary biliary cirrhosis in these patients? In 1980 Triger et al. demonstrated for the city of Sheffield, that there occured an apparent clustering of cases in districts supplied by the same water reservoir, but further investigations failed to show any striking difference in this water compared to other reservoirs. Burroughs et al. (1992) supported the hypothesis of infection as a trigger for primary biliary cirrhosis when Escherichia coli was found to share epitopes with PDH-E2 indicating that molecular mimicry may lead to primary biliary cirrhosis. The existence of a transmissible infectious agent was recently discussed by Delpre and Niv (1998) when they reported of primary biliary cirrhosis occuring in a married couple. Nevertheless, thus far, no environmental agent causing this chronic liver disease could be determined.

(iii) Primary biliary cirrhosis has been described in certain families (Bach and Schaffner, 1994; Caldwell et al., 1992), and Myszor and James (1990) who carried out a comprehensive epidemiological study in 347 patients with histologically confirmed primary biliary cirrhosis determined a familial incidence of 2.4%. Between the present two patients any familial relationship was denied and the genetic difference could be evidenced by analysis of restriction fragment lengths polymorphism showing a wide chromosomal variability among the two individuals.

(iv) As defective immune response seemed to play a significant role in the pathogenesis of primary biliary cirrhosis, characteristics like HLA were investigated in affected individuals. Associations of primary biliary cirrhosis with HLA-DR2, DR3, DR4, and DR8 have been shown, being the closest to HLA-DR8 accounting for about one third of Caucasian patients (Mackay and Gershwin, 1998; Neuberger and Thomson, 1999). The present two patients shared 5 of 11 HLA loci tested and were positive for HLA-DR8. HLA-DR4 could also be detected in both patients possibly contributing to the hepatitic picture in patient 1. This allel has recently been described as an important HLA type causing susceptibility to AIH and leading to overlapping features of autoimmune hepatitis in primary biliary cirrhosis patients (Lohse et al., 1999).

In accordance to the findings of the present investigation one may presume that the two patients could have been exposed to an environmental agent in childhood what made them develope primary biliary cirrhosis after decades depending on their particular HLA constellation. Whether this presumed agent was infectious, i.e. bacterial, viral, fungal, etc., or an unknown substance, and as to what degree certain HLA types may influenced the progress of disease remains an enigma.

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