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Autoantibody formation against a canalicular epitope found in a patient with acute intrahepatic cholestasis with PFIC-like presentation

To the Editor:

Cholestatic liver injury can be caused by progressive familial intrahepatic cholestasis (PFIC), which are hereditary diseases related to defects in hepatocellular proteins involved in canalicular membrane polarization, export of bile components, and tight junction (TJ) formation.[1] However, some reports suggest that PFIC-like disorders might develop as a result of *de novo* allo- or autoantibody formation against PFIC-related proteins in susceptible hosts.[2,3] Herein, we present a case of severe intrahepatic cholestasis with previously unknown autoantibodies directed against a canalicular epitope mimicking a PFIC-like phenotype in a young patient with ulcerative colitis.

A 26-year-old patient presented with severe jaundice and itching following a febrile respiratory infection the week before admission. The only pre-existing clinical condition was ulcerative colitis in clinical remission under mesalazine. Laboratory parameters upon admission are presented in Table S1. Serology testing excluded infections and the patient had no recent changes in medication or intake of xenobiotics. MRI with cholangiopancreatography showed no liver parenchyma or bile duct abnormalities, but a liver biopsy revealed canalicular cholestasis with moderate periportal and lobular inflammation. No interface hepatitis, siderosis or histological signs of primary sclerosing cholangitis could be detected. We suspected an autoimmune hepatitis (AIH)-like disorder, and therefore initiated prednisolone treatment. As alanine aminotransferase (ALT) levels increased during prednisolone treatment, we decided to start plasmapheresis therapy, under the hypothesis that in a susceptible host with autoimmune colitis the preceding respiratory infection might have triggered a liver-directed cytokine or antibody response that was non-responsive to the systemic steroid therapy. A remarkable biochemical response was seen after 5 cycles of plasmapheresis (Fig. 1A) and the severe itch subsided. The patient was discharged, and close follow-up visits were scheduled in our outpatient clinic. Prednisolone was tapered when liver function tests (LFTs) normalized. Six weeks after discharge, alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT), which were normal during the acute phase, increased. Maintenance therapy with azathioprine was initiated as it has been shown to be an effective therapeutic option for maintaining remission in IgG4-related cholangitis, a disease thought to be mediated by autoantibodies.[4–6] ALP and GGT serum levels stabilized but remained at slightly elevated levels. A follow-up MRI showed again no bile duct irregularities, and genetic testing was negative for PFIC-associated mutations (Table S1). The correlation between plasmapheresis, reconstitution of LFTs and normalization of initially reduced levels of complement factor 3 and 4 are suggestive of an underlying humoral immune response. Serum samples taken before and after plasmapheresis were analysed for the presence of autoantibodies directed against liver antigens by using immunofluorescence staining of human liver sections. Besides diffuse staining of parenchyma, we observed an anti-canalicular staining with a potential TJ-like staining pattern in the pre-plasmapheresis sample at a serum dilution down to 1:1,600 (Fig. 1B). This staining pattern was reproduced after direct staining of patient liver sections with an anti-human secondary antibody (Fig. S1). After plasmapheresis and normalization of LFTs the titre had reduced to 1:50 (Fig. S2). Follow-up liver biopsy showed fibrotic parenchyma. Incubation of patient liver sections with serum samples again showed a diffuse parenchymal and, interspersed, anti-canalicular TJ-like staining pattern. In our opinion, the ineffectiveness of the steroid treatment suggests that the pathomechanism of acute liver injury was different from a typical liver-directed autoimmunological reaction. In contrast, plasmapheresis was able to interrupt the damaging process, which could be caused by circulating factors, such as cytokines, damage-associated molecular patterns or antibodies.

To summarize, we observed serum autoantibodies directed against a canalicular epitope in a susceptible host with acute intrahepatic cholestasis with PFIC-like presentation. High titres coincided with the acute phase of liver injury while clinical remission was associated with a reduction of antibody titre. *De novo* formation of antibodies against PFIC-associated proteins, that potentially induce a PFIC-like disorder, can be triggered by preceding viral or bacterial infections.[2,3] Notably, the symptoms of our patient developed after a febrile respiratory infection. However, anti-canalicular antibodies might have occurred as an epiphenomenon of an unidentified acute autoimmune-like liver disease. Moreover, polyclonal antibody formation might arise in acute inflammatory states, which can result in a non-specific cross-reactivity with cholangiocytes. We emphasise that further research is needed to identify potential epitopes before clear conclusions can be drawn from our observation. Nevertheless, here we describe for the first time the presence of anti-canalicular antibodies in acute liver disease. The ineffectiveness of steroid treatment and the

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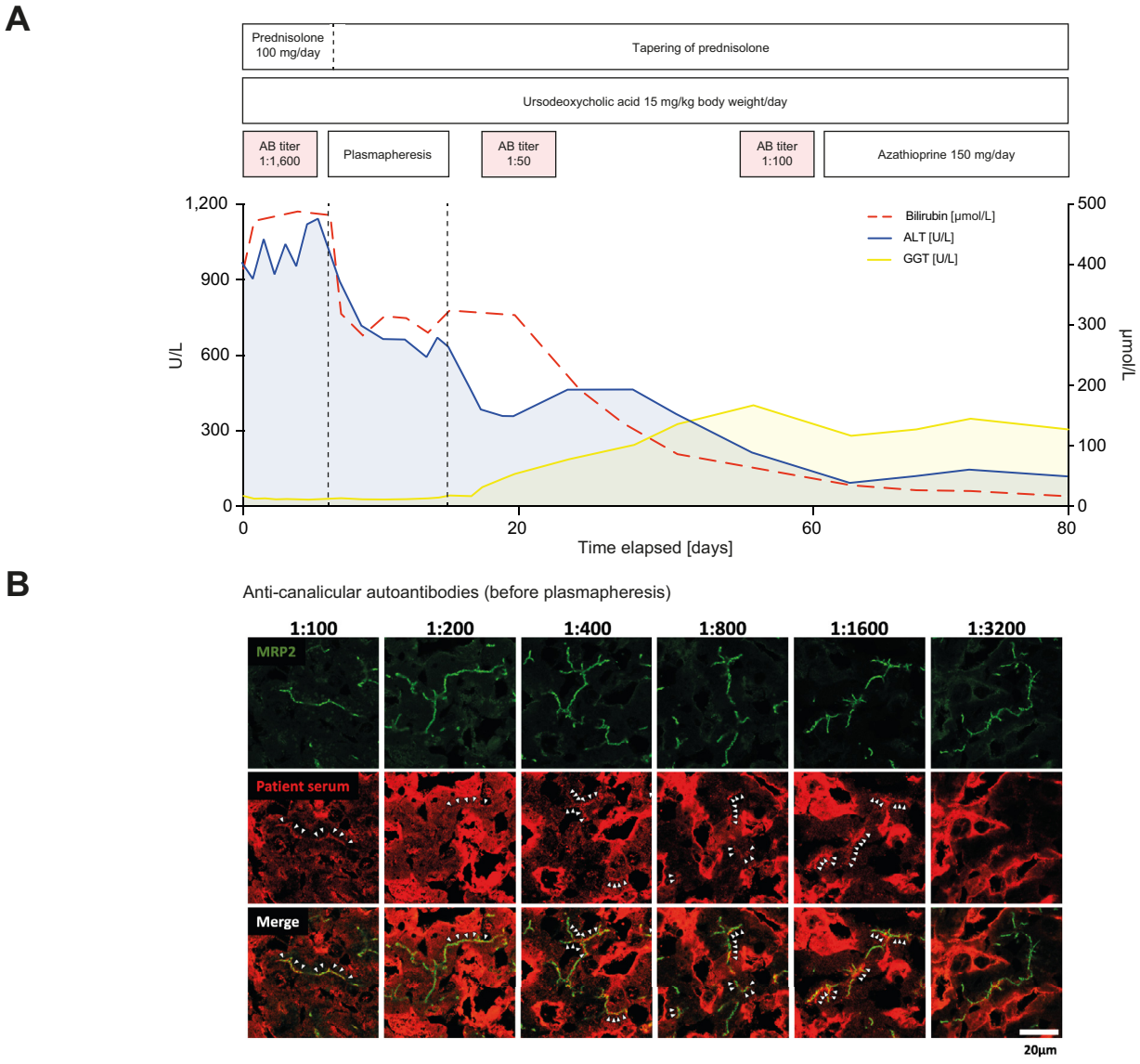

Fig. 1. Course of laboratory parameters and titration of serum autoantibodies with canalicular staining pattern before plasmapheresis. (A) Course of laboratory parameters and treatment. AB, antibody titre with canalicular staining pattern; bilirubin, bilirubin serum levels (red dashed line, [µmol/L] right coordinate); ALT, alanine-aminotransferase (blue curve, [U/L] left coordinate); GGT, gamma-glutamyl transferase (yellow curve, [U/L] left coordinate). (B) Cryosections of human liver tissue (obtained from a non-cholestatic patient resected for hepatic metastasis of colorectal cancer) were fixed for 3 minutes with methanol, blocked for 30 minutes with UltraVision Block (Thermo Scientific) and incubated for 1 hour with 1:25 anti-human MRP2 primary antibody (Enzo, catalogue #ALX-801-015-C250) and pre-plasmapheresis patient serum at different dilutions (1:100 to 1:3,200). Sections were then incubated with the following secondary antibodies: 1:500 anti-mouse Alexa Fluor 488 (Invitrogen, catalog #A-11001) and 1:500 anti-human Cyanine Cy3 (Jackson ImmunoResearch, catalogue #109-165-003). Microscope: ZEISS LSM 880. MRP2, multidrug resistance-associated protein 2 (green, canalicular marker); ► tight junction-like canalicular staining pattern.

remarkable response to plasmapheresis in our patient is suggestive of an underlying liver-directed cytokine or antibody response. Patients with acute liver injury with PFIC-like features should be screened for the presence of anti-canalicular antibodies to unravel a potential diagnostic or pathomechanistic role of this phenomenon.

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Conflict of interest
The authors declare no conflict of interest with regard to this work.

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Authors' contributions
JS and TB were involved in patient's care and sample collection. JSt and VK performed immunofluorescence. TH and JSt prepared the figure. TH, JS and TB wrote the manuscript. All authors revised and approved the final manuscript.

Informed consent
Written informed consent was obtained from the patient before submitting the manuscript.

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Supplementary data
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