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A possible role of histone-like DNA-binding protein of *Streptococcus intermedius* in the pathogenesis of bile duct damage in primary biliary cirrhosis

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Abstract Bacterial infection has become a focus of attention in the pathogenesis of primary biliary cirrhosis (PBC). It was reported that anti-histone autoantibody was detected in PBC, suggesting that bacterial histone-like DNA-binding protein (HLP) may be involved in the pathogenesis of PBC. To identify bacterial species in PBC to confirm this possibility, serum reactivity to bacterial cells was studied by ELISA. The IgM class *Streptococcus intermedius* titers were significantly higher in PBC than chronic hepatitis due to hepatitis C virus (CH-C) and healthy subjects. Among the streptococci, *S. intermedius* was selected for further study. The antigenic peptide of *S. intermedius* of HLP was synthesized to examine the serum reactivity to *Si*-HLP. IgM class anti-*Si*-HLP peptide titers were significantly higher in PBC. Immunoreactivity to anti-*Si*-HLP was detected in the cytoplasm of biliary epithelial cells and inflammatory cells in the portal area in PBC patients' livers. Streptococci, especially *S. intermedius*, might play a key role in the pathogenesis of PBC, possibly involving HLP.

Abbreviations: PBC, primary biliary cirrhosis; BEC, biliary epithelial cells; CNSDC, chronic non-suppurative destructive cholangitis; CH-C, chronic hepatitis due to hepatitis C virus; HLP, histone-like protein; LTA, lipoteichoic acid; AMA, anti-mitochondrial antibody; PDH-E2, E2 component of pyruvate dehydrogenase.

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Introduction

Primary biliary cirrhosis (PBC) is a chronic autoimmune disorder, characterized by chronic non-suppurative destructive cholangitis (CNSDC) of small intrahepatic bile ducts and epithelioid granuloma formation in the portal area, which leads to progressive ductopenia [1]. The pathogenesis of biliary epithelial cell (BEC) damages in PBC is still not clearly understood. Recently, the relationship between bacterial infection and the pathogenesis of PBC, via the mechanism of molecular mimicry, has become a focus of attention [2,3]. Fussey et al. reported that sera from patients with PBC reacted with both human and Gram-negative (G (−))-bacteria, *Escherichia coli* (*E. coli*) pyruvate dehydrogenase complex E2 (PDC-E2), and such reactivity of anti-mitochondrial antibodies (AMA) to both human and bacterial molecules has stimulated speculations that PBC may be induced by exposure to enterobacterial antigens, perhaps by sharing molecular mimicry with mitochondrial antigens in PBC [4].

Selmi et al. reported that human PBC sera had titers to aerobic bacteria, *Novosphingobium aromaticivorans* (*N. aromaticivorans*) proteins [5]. *N. aromaticivorans* is a G (−)-bacteria whose proteins are similar to those of human pyruvate dehydrogenase complex [5,6]. On the other hand, the 16S bacterial ribosomal RNA (rRNA) gene was detected from gallbladder bile and from epithelioid granuloma of PBC patients [7]. Sequencing revealed that *Staphylococcus aureus* or other Gram-positive (G (+)) bacteria were predominant [8]. We previously reported that the G (+)-bacterial cell wall component lipoteichoic acid (LTA) was detected at the portal tract in the livers of PBC patients with CNSDC [9]. LTA has been considered to be an antigen that mediates the attachment of certain bacteria to host tissues [10], and thought to be an immunostimulatory molecule as one of the pathogen-associated molecular patterns [11]. We also reported that defects in apoptosis inhibitor expressed by macrophages (AIM) affected portal inflammation as well as biliary epithelial cell damage in the livers of colitis-harboring female TCRα-deficient (TCR$\alpha^{-/-}$) mice [12]. In TCR$\alpha^{-/-}$xAIM$^{-/-}$ mice, LTA accumulation was observed in the liver, corresponding to portal inflammation and biliary epithelial cell damage followed by fibrosis, as seen in the human PBC liver [13,14].

Murakami et al. reported that the injection of killed *Streptococcus intermedius* into gingival mucosa caused inflammation of the gingiva as well as liver abscesses in mice [15]. However, its virulence factors involved in host immune responses were not determined. Recently, Liu et al. demonstrated that recombinant HLP of *S. intermedius* (r*Si*-HLP) strongly induces pro-inflammatory cytokines production, cooperating with bacterial cell wall components, such as LTA, in human monocytes [16]. Based on this observation, *Si*-HLP could be released from bacteria without cell lysis, since both intracellular and extracellular expression of *Si*-HLP occurs in the early stationary phase [16]. *S. intermedius* belongs to the *Streptococcus anginosus* group, forming part of the commensals of the mouth, gastrointestinal tract, and genitourinary tract, and often causes purulent infections [17–19]. HLPs are heat-stable proteins that bind to single- and double-stranded DNA without obvious sequence specificity [20]. Although the biological functions of HLPs are not fully understood, they are known to wrap DNA and restrain negative supercoiling [20–22].

Extracellular HLP formed soluble complexes with LTA *in vitro* and potentially resulted in the pathogenesis of streptococci-induced tissue inflammation [20]. In the oral cavity, streptococci constitute approximately 60% of the initial biofilm microflora on the tooth surface [23]. In addition, LTA is detected in the supernatant culture media of oral streptococci [24]. Collectively, some of the bacterial components could be involved in the crucial pathogenesis of PBC. In keeping with this possibility, Chou et al. reported that anti-histone antibodies were detected in 81% of PBC patients [25]. Stinson et al. reported that in glomerulonephritis, HLP of *Streptococcus pyogenes* may act as a fortuitous virulence factor and a nidus for *in situ* immune complex formation.

The aim of the present study was to investigate the serum reactivity to various bacteria, and to clarify the involvement of bacterial infection in PBC. Furthermore, the presence of antibodies and localization against bacterial HLP were studied in PBC patients to know their possible involvement in PBC.

Materials and methods

Patients and samples

The liver and serum samples examined in this series were obtained from 20 patients with PBC. Ten patients were diagnosed as stages 1–2 with CNSDC (all females; age 46–74 [median 55 years]). Ten patients were stages 3–4 (all females; age 35–72 [median 57 years]). All of the PBC patients were positive for AMA and/or anti-PDH autoantibodies. Among these 20 PBC patients, 3 patients suffered from Sjögren's syndrome and 2 patients suffered from chronic thyroiditis, simultaneously. For disease control, we examined liver and serum samples from 13 chronic hepatitis due to hepatitis C virus (CH-C) patients with lymphocytic cholangitis (female:male = 6:7; age 36–64 [median 51 years]), as lymphocytic cholangitis around the interlobular bile ducts without progressive bile duct destruction has been observed in CH-C [26]. The diagnosis of CH-C was based on clinical and laboratory data, and was confirmed histologically. In brief, in CH-C patients, serum alanine transaminase levels were elevated (range, 62–172 IU/L) for more than 6 months. All of the CH-C patients were positive for anti-hepatitis C virus (HCV) antibodies by enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Tokyo, Japan). The presence of HCV RNA was also confirmed by reverse transcriptase polymerase chain reaction (Rosche Diagnostics K.K., Tokyo, Japan). Liver biopsy specimens were evaluated according to the criteria of Desmet et al. [27]. Serum samples obtained from 11 healthy subjects (female:male = 10:1; age 39–63 [median 54 years]) were also examined by ELISA. A serum sample from each patient was taken and frozen at −20 °C until use. Serum samples were obtained with the informed consent of each patient [9]. All serum samples examined in the experiments were the same sera, taken from PBC, CH-C, and healthy subjects, as examined in our previous studies for the tests for serum reactivity to LTA [9].

Bacterial strains and culture conditions

The bacterial strains used for ELISA assays were *S. anginosus* ATCC33397^{T}, *Streptococcus constellatus* ATCC27823^{T}, *S.*