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Serum inflammatory cytokines, complement components, and soluble interleukin 2 receptor in primary biliary cirrhosis

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A B S T R A C T

Primary biliary cirrhosis (PBC) is a chronic cholestatic autoimmune liver disease characterized by selective destruction of the intrahepatic bile ducts and highly specific serum anti-mitochondrial auto-antibodies (AMA). Several studies have attempted to determine the cytokine pattern characterizing PBC, yet no definitive data have been gathered. The present study was designed to evaluate pro-inflammatory cytokines (IL-1β, IL-6, TNFα), soluble IL-2 receptor (sIL-2R, e.g. soluble CD25), and complement components (C1q, C3, factor B, properdin) levels in sera from 84 patients with PBC and 41 controls. PBC was characterized by significantly higher levels of all pro-inflammatory cytokines when compared to controls; these included IL-1β (433.3 ± 13.2 vs. 316.6 ± 14.7 pg/ml, $P < 0.001$), IL-6 (701 ± 17.4 vs. 158 ± 22.5 pg/ml, $P < 0.001$), TNFα (3.38 ± 0.6 pg/ml vs. undetectable, $P = 0.001$), and sIL-2R (1527.1 ± 106 vs. 566.4 ± 28.7 U/ml, $P < 0.001$). Similarly, all complement components were also significantly higher in PBC compared to control sera. In conclusion, PBC sera manifest higher levels of sIL-2R and complement components and this may reflect a perpetuated immune activation. As expected, we also report that all major pro-inflammatory cytokine levels are enhanced in PBC. Further longitudinal analyses could demonstrate a correlation between these markers and disease stage or inflammatory activity, to predict histological staging, disease activity, and response to treatment.

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1. Introduction

Primary biliary cirrhosis (PBC) is a chronic autoimmune liver disease affecting predominantly middle aged women and characterized by immuno-regulatory disturbances, destruction of the intrahepatic bile ducts, and liver fibrosis [1–3]. PBC is also characterized by hyposecretion of the lacrimal, salivary, biliary and pancreatic glands, therefore referred to as the "dry gland" syndrome [4]. Since many systems are affected in PBC, it was strongly suggested that PBC is a complex autoimmune syndrome [5], possibly representative of the autoimmunity spectrum [6] as most PBC cases have at least one other autoimmune comorbidity [7]. Serum anti-mitochondrial antibodies (AMA) are specific to PBC and often occur at high titers [8,9], being the major disease hallmark [10]. Along with CD8 T-cells surrounding the biliary ducts [11,12] serum AMA are associated with increased complement components in PBC [13], thus pointing to an activation of the complement system in the disease pathogenesis.

Quite surprisingly, few studies have addressed the issue of cytokine and complement levels in PBC. Elevated levels of inflammatory cytokines- i.e. interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor α (TNFα), have been reported in sera of patients with chronic liver diseases in a stage-dependent fashion. Of note, levels were only marginally affected by the disease etiology [14]. Whether elevated IL-6 levels represent a consequence of liver dysfunction and not of chronic inflammation in PBC remains to be determined.

Based on the abnormal immune derangement observed in PBC [1,11,14,17,18], we herein evaluated comprehensive panel of inflammatory cytokines (namely IL-1β, IL-6 and TNFα), complement (C)

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components (the early C1q and C3 and the alternative pathway Factor B and Properdin), and soluble interleukin-2 receptor (sIL-2R) associated with general immune activation [15] in sera from a large series of PBC cases and controls.

2. Materials and methods

2.1. Subjects

Eighty four patients with PBC were randomly selected at the University of Milan and evaluated in this study. The diagnosis of PBC was based on internationally-accepted criteria [1] including the presence of 2 out of 3 criteria: i.e. positive serum AMA, serum alkaline phosphatase $>2\times$ normal for $\geq$6 months and/or a compatible liver histology. In subjects without detectable AMA, patients had to fulfill both other criteria to be enrolled as AMA- negative PBC cases [16]. Serum AMA were determined using indirect immunofluorescence and titers $\geq$1:40 were considered as positive. Patients positive for serum hepatitis B surface antigen, HCV antibody, a history of alcohol abuse within one year of diagnosis, or bile duct obstruction (based on ultrasound, computer tomography and/or endoscopic evaluation) were excluded from the study. Healthy controls ($n = 41$) were age- and sex-matched and selected from blood donors. Serum samples from peripheral blood was obtained from all patients and controls and stored at -20′C until used for the study.

2.2. Cytokine levels

Serum levels of pro-inflammatory cytokines (IL-1β, IL-6, TNFα) and sIL-2R were analyzed by solid phase ELISA using a quantitative "sandwich" enzyme immunoassay technique. A polystyrene microtiter plate was pre-coated with a monoclonal antibody specific for each interleukin molecule. Standards and samples were introduced into the wells and the cytokine of interest was bound to the immobilized antibody. After washing away any unbound proteins, the second enzyme-linked polyclonal or monoclonal antibody specific for the interleukin was added to the wells to "sandwich" the interleukin immobilized during the first incubation. Following thorough washing to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and the color developed in proportion to the amount of interleukin bound in the initial step; the color development was then stopped and intensity measured. A curve was prepared by plotting optical density versus concentration of a given interleukin in the standard wells. By comparing the optical density of the samples to the standard curve, the concentration of the interleukin in unknown samples was then determined, as previously shown [17,18].

In addition, we also used high sensitive immunoassay kits for the determination of low levels of human cytokines (IL-1β, IL-6 and TNFα). These kits used an amplification system in which the alkaline phosphatase reaction provided a cofactor that activated a redox cycle leading to the formation of a colored product. The secondary enzyme system consisted of alcohol dehydrogenase and diaphorase (Amplifier). These assay kits were purchased from Quantikine, R&D Systems, Minneapolis, USA.

2.3. Complement levels

Normal serum levels of complement obtained from 27 healthy donors were used as standard control. Blood was aspirated and left to clot at room temperature for 1 h, and the serum was then separated after centrifugation at 4 °C. Data from patient sera were expressed as a percent of the results observed in pooled normal sera. Radial immunodiffusion was performed in 1% agarose gels containing monospecific goat antibodies to human C components.

2.4. Statistical analysis

Pairwise comparisons were performed using the Wilcoxon rank-sum test between groups. Continuous variables were expressed as mean ± standard error of measurement (SEM). Comparisons between groups were performed using the Student's *t*-test and the χ^2 test.

All analyses were two-tailed and P_c values < 0.05 were considered as statistically significant. Statistical analyses were performed using Intercooled Stata 8.0 (Stata Corporation, College Station, TX).

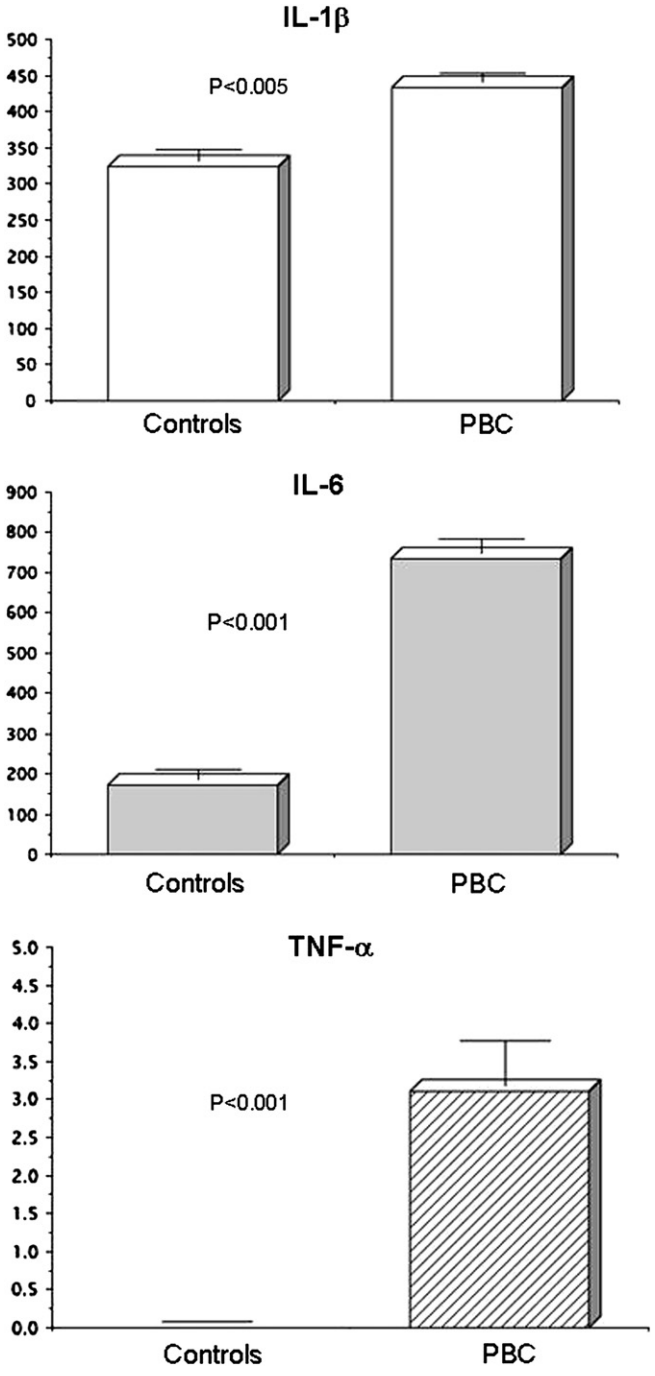

Fig. 1. Levels of pro-inflammatory cytokines in sera from patients with PBC and controls. Data are expressed in pg/ml and mean ± SEM.

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