ANTIGEN-SPECIFIC DETECTION OF HBsAg-CONTAINING IMMUNE-COMPLEXES IN SERA FROM CHRONIC ACTIVE HEPATITIS PATIENTS WITH HEPATITIS B VIRUS PERSISTENT INFECTION BY ENZYME-IMMUNOASSAY (EIA)

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Summary

Clq levels in immune complexes (IC) were determined by a new enzyme-immunoassay (EIA) method to estimate serum antigen-specific HBsAg-containing immune complexes (specific HBsAg-IC). Serum was used from 15 hepatitis patients with persistent hepatitis B virus infection, 2 patients with non-B type chronic active hepatitis (CAH) and 5 healthy persons. This EIA technique detected and assayed HBsAg-IC in patients with hepatitis. Clq levels in specific HBsAg-IC were higher than optical density (OD) 5.00 in 10 of 15 patients, and all 10 patients had CAH and were positive for Fc reactive antibody to liver cell membrane. In the other 5 patients Clq levels in specific HBsAg-IC were lower than OD 5.00, and 3 of them had asymptomatic carrier with non-specific reactive hepatitis and 2 had inactive liver cirrhosis.

Key Words: immune complexes, HBsAg-immune complexes, HBsAg, HBV, enzyme-immunoassay, EIA, Clq, clinical immunological test.

Introduction

Pernice et al.1) had described an enzyme-immunoassay (EIA) technique for detecting circulating antigen specific HBsAg-containing immune complex (serum specific HBsAg-IC) found in persistent hepatitis B virus (HBV) carriers. This technique allows qualitative determinations of IgG in specific HBsAg-IC, but not quantitative determination. The present paper describes an EIA method that detects quantitative specific HBsAg-IC.

Materials and Methods

The subjects of the study were: 15 patients with persistent HBV infection (3 asymptomatic and 12 chronic), 2 non-B type chronic active hepatitis (CAH) and 5 healthy controls. Serum HBsAg and specific HBsAg-IC were examined by the EIA method with a micro plate and micro colorimeter (Photo-Elisa I Model of Organon Teknika2), using antibodies to human Clq, IgG and C3 (Behring Institute).
Each well was filled with 100 \( \mu l \) of the subject's serum inactivated at 57°C for 30 minutes, and incubated at room temperature overnight or at 37°C for 2 hours. After 5 times washings with 0.005 M phosphate buffered saline pH 7.4 (PBS), 100 \( \mu l \) of rabbit anti-human Clq, IgG or C3 (diluting by 100-fold) was added; incubated at 37°C for 40 minutes; washed 5 times with PBS, and peroxidase (PO)-labelled porcine anti-rabbit IgG (diluting the product of Dakopatz Laboratory 200-fold) was added. The mixture was incubated at 37°C for 40 minutes, was colored with O-phenylene diamine and urea peroxide (Organon Co) at room temperature for 20-30 minutes. After stopping the reaction with 4 N \( \text{H}_2\text{SO}_4 \) solution the mixture was transferred to a Ht tube for colorimetry. When the value obtained was higher than 20.00 at optical density (OD), the mixture was diluted 10-fold with 1.33 N \( \text{H}_2\text{SO}_4 \) solution and then the OD obtained was corrected. The subjects were judged positive for specific HBsAg-IC when the OD ratio \[(\text{sample OD} - \text{blank OD})/(\text{mean negative control OD} - \text{blank OD})\] was \( \geq 2.1 \). The same subjects were also checked for antibody to liver cell membrane (LMAb) by our technique\(^9\) and particularly in the positive cases, the site of binding to the hepatic cell membrane was examined. For evaluation of the dose response, using our EIA method, Clq levels were determined with specific HBsAg-IC prepared from purified HBsAg\(^4\), rabbit anti-HBsAg, and human fresh serum (blood type AB).

Results

It was shown in Fig. 1 the standard curve of HBsAg determined by the EIA method. When anti-human Clq, IgG and C3 were used for detection of specific HBsAg-IC, the cut-off values were 3.85, 2.28 and 2.51, respectively. However, as the value using anti-IgG and C3 exceeded the above cut-off values in some cases of non-B type hepatitis, anti-Clq was used for the present experiment. By our specific HBsAg-IC determination method, 10 of 15 patients with B type hepatitis showed a OD higher than 5.00, and all 10 patients were diagnosed as CAH or active liver cirrhosis. Three of the remaining 5 patients with OD lower than 5.00 were asymptomatics and the two were inactive liver cirrhosis (Fig. 2). As the relationship between HBsAg titer and IC levels, it was found the cases having high HBsAg titer showed lower IC level and had Fc reactive LMAb. These results were obtained in all the above 5 cases.