Detection of Antibodies to 65 KD Heat Shock Protein and to Human Superoxide Dismutase in Autoimmune Hepatitis-Molecular Mimicry between 65 KD Heat Shock Protein and Superoxide Dismutase

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Summary  The antibody to 65 KD mycobacterial heat shock protein (HSP65) and antibody to human superoxide dismutase (H-SOD) were measured by ELISA in patients with autoimmune hepatitis (AIH), and results were compared with those of patients with chronic active hepatitis C (CAH-C) or systemic lupus erythematosus (SLE) and normal subjects (NS). Patients with AIH had significantly higher OD values of anti-HSP65 antibody and anti-H-SOD antibody compared with those of patients with CAH-C or SLE and NS. OD values of anti-HSP65 antibody were correlated with those of anti-SOD antibody. Affinity-purified anti-SOD antibody reacted with HSP65. Analysis of the amino acid sequence of human SOD showed that 7 segments, corresponding to r to 25 amino acid residues, exhibited 50 to 71% homology with that of mycobacterial HSP65.

Key words  Autoimmune Hepatitis, Mycobacterial 65 KD Heat Shock Protein, Superoxide Dismutase, Human 60 KD Heat Shock Protein, Molecular Mimicry

INTRODUCTION

Patients with autoimmune hepatitis (AIH) express various autoantibodies, including antibodies to nuclear antigens, smooth muscle antigens, liver/kidney microsomes(1) and superoxide dismutase (SOD)(2). Among them, antibody to SOD was considered as a possible cause of false positive results in Ortho HCV ELISA(2).

Heat shock protein (HSP), a protein conserved among several species(3) and mycobacterial 65 KD HSP (HSP65), for example, has been implicated in autoimmune disorders. Although self antigens and widely distributed antigens are expected to be poor immunogens, HSP families are so immunogenic, despite the high degree of homology among various species, that they are believed to have the potential to destroy immunity(4). SOD is also a conserved protein (5).

In this context, we investigated the presence of antibody to HSP65 and antibody to SOD in patients with AIH and compared the results of patients with chronic active hepatitis C (CAH-C) or systemic lupus erythematosus (SLE) and normal subjects (NS).

MATERIALS AND METHODS

Patients

Serum samples were obtained from patients with AIH, CAH-C or SLE. Subjects were in- or outpatients at our department. The diagnosis for AIH was based on the criteria proposed by Johnson and McFalane(6). The diagnosis of CAH-C was based on histopathological examination of liver biopsy specimens, antibody titers determined by first- or second-generation enzyme-linked immunosorbent assay (ELISA) and examination for hepatitis C virus RNA by multicyclical RT-PCR(7). The diagnosis for SLE was based on the 1982 revised SLE criteria of ARA(8). Serum samples obtained from NS in our department were used as control samples.
Recombinant HSP 65 and recombinant H-SOD

Recombinant mycobacterial HSP65(9) which originated from Mycobacterium leprae was kindly supplied by Dr. H. Nomaguchi (National Institute for Leprosy Research, Tokyo). Briefly, the recombinant HSP65 was produced in an E. coli strain carrying a plasmid harboring the re-cloned gene coding for the protein. The protein was purified by affinity chromatography with the IgG fraction of a monoclonal antibody that was produced against HSP65(9). Recombinant human SOD (H-SOD), copper zinc dismutase, was kindly provided by Fujisawa Central Research Institute.

ELISA for antibody to HSP65

The ELISA was performed as described previously(10). Briefly, the ELISA plate (Dynatech Laboratories Inc., Chantilly, VA, USA) was coated with 0.1 μg/ml of recombinant HSP65 in carbonate-bicarbonate buffer (0.05 M, pH 9.6) and incubated for 3 hours at 37°C and overnight at 4°C. The plate was washed three times in phosphate-buffered saline (0.1 M, pH 7.4) containing 0.05% Tween 20 (PBS-T) followed by blocking by addition of PBS containing 3% bovine serum albumin (PBS-BSA). Serum samples diluted at 1:600 in PBS-BSA were placed in wells and incubated for 1 hour at 37°C. The wells were then washed three times with PBS-T. Alkaline phosphatase-conjugated F(ab')2 goat anti-human IgG antibody (Tago Inc., Burlingame, CA, USA) diluted at 1:1000 in PBS-BSA was placed in wells and incubated for 1 hour. After wells were washed three times with PBS-T, 0.5 mg/ml of nitrophenyl phosphate (Sigma Diagnostics, St. Louis, MO, USA) in carbonate-bicarbonate buffer (0.05 M, pH 9.8) was added to the wells. The optical density (OD), measured at 405 nm, was used to estimate the level of the antibodies. The OD values were corrected based on OD values obtained for a positive control in each plate to permit interassay comparison.

ELISA for antibody to SOD

The ELISA plate was incubated with 3 μg/ml of either recombinant H-SOD or bovine-SOD (B-SOD) (Sigma Chemical Company, St. Louis, MO, USA) in carbonate-bicarbonate buffer (0.05 M, pH 9.6) for 3 hours at 37°C and overnight at 4°C. The subsequent procedure was carried out as described above. OD values were corrected as described above to permit interassay comparison.

Affinity-purified anti-SOD antibody

Twelve grams of CN-activated Sepharose 4B (Pharmacia, AB Laboratory Separation Division, Uppsala, Sweden) was swollen in 1 mM HCl, and then washed in distilled water and sodium bicarbonate buffer (coupling buffer). H-SOD (60 mg) in coupling buffer was applied to the swollen gel and shaken for 2 hours at room temperature. Serum samples obtained from two AIH patients, H.K. and Y.S., diluted at 1:600 in sterilized saline were applied to H-SOD affinity columns, and incubated for 1 hour at room temperature. The antibodies adsorbed to the SOD column were eluted by the addition of Tris-HCl buffer (pH 3.2). The eluates were neutralized and concentrated five times by Minicon (Amicon Division, W.R. Grace & Co., Beverly, MA, USA) for detection of IgG anti-SOD antibody and IgG anti-HSP65 antibody by ELISA.

Homology search

Homology search for amino acid sequences of B-SOD, H-SOD, HSP65, and human 60 KD heat shock protein (HSP60) was performed using Microgenie (Beckman Co., CA, USA).

Statistical analysis

Statistical analysis was carried out using the Stat View 2 software package for an Apple Macintosh computer. Differences between two groups were analyzed by Student's t-test. Correlations were determined by F test.

RESULTS

The OD values of IgG antibody to H-SOD and those to B-SOD were significantly correlated in NS and in patients with AIH (Fig. 1) (r=0.806, p<0.0001). The following results for SOD all refer to H-SOD.

The OD values of anti-HSP65 antibody and anti-SOD antibody in sera of 22 AIH, 26 CAH-C, 26 SLE and 36 NS are summarized in Table I. The mean OD values ± standard deviation (SD) of IgG antibody to HSP65 and IgG antibody to SOD in NS were 670±204 and 350±190, respectively. The OD value of IgG anti-den was swollen in 1 mM HCl, and then washed in distilled water and sodium bicarbonate buffer (coupling buffer). H-SOD (60 mg) in coupling buffer was applied to the swollen gel and shaken for 2 hours at room temperature. Serum samples obtained from two AIH patients, H.K. and Y.S., diluted at 1:600 in sterilized saline were applied to H-SOD affinity columns, and incubated for 1 hour at room temperature. The antibodies adsorbed to the SOD column were eluted by the addition of Tris-HCl buffer (pH 3.2). The eluates were neutralized and concentrated five times by Minicon (Amicon Division, W.R. Grace & Co., Beverly, MA, USA) for detection of IgG anti-SOD antibody and IgG anti-HSP65 antibody by ELISA.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>AIH (n=22)</th>
<th>CAH-C (n=26)</th>
<th>SLE (n=26)</th>
<th>NS (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody to HSP65</td>
<td>814±222*</td>
<td>715±200</td>
<td>679±222</td>
<td>670±204</td>
</tr>
<tr>
<td>Antibody to SOD</td>
<td>818±293***</td>
<td>336±205</td>
<td>360±119</td>
<td>350±190</td>
</tr>
</tbody>
</table>

*: Number of patients; **: p<0.05, ***: p<.001, significant difference from patients with CAH-C or SLE and NS.