Protective Activity of Rabbit Polyclonal Anti-idiotypic Antibody against Leptospira interrogans Infection in Hamsters

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We prepared an anti-idiotypic (Id) antibody against leptospirosis. Serum from rabbit immunized with monoclonal antibody (MAB) LW2, which reacted to the main protective antigen prepared from Leptospira interrogans serovar lai, inhibited agglutination of the organism by MAB LW2. The immune rabbit serum was applied to a column coupled with normal mouse IgG as a ligand (first column), and the unbound fraction eluted was applied to a column coupled with MAB LW2 as a ligand (second column). The bound fraction (anti-Id antibody) eluted from the second column inhibited the binding of MAB LW2 to sonicated leptospiral cells in ELISA. Mice produced antibodies against Leptospira by intraperitoneal immunization with the anti-Id antibody at doses of 2 μg/mouse or more. Hamsters were protected by immunization with the anti-Id antibody at doses of 2 and 20 μg/hamster from the lethal infection of Leptospira. This is the first report concerning the use of an anti-Id antibody against leptospirosis.

Key words Leptospira; leptospirosis; anti-idiotypic; vaccine

Leptospirosis in humans and domestic animals is still widespread around the world. At present, vaccination is the best way to control leptospirosis.1) In such a vaccine, the outer envelope fraction of Leptospira induced better protection than lyophilized whole cells.2,3) We showed that glycolipid protective antigens (PAGs) of the serovars lai4) and canicola5) protected hamsters from homologous infection. Tetra-valent PAGs prepared from serovars copenhageni, autumnalis, hebdomadis, and australis induced the production of agglutinating antibody and opossonising antibody, and the level in serum was almost the same as those of commercial vaccines now in use in Japan.6) Furthermore, endotoxic activities, such as a lethal effect, of PAGs were not observed, and the biological safety of PAGs was proven.7) These findings suggested that PAG is potent enough to be used as a leptospiral vaccine. PAG of serovar lai strain 017 was composed of rhannose, arabinose, xylose, mannose, galactose, glucose and several unknown sugars and various sizes of fatty acids.8) Since PAG has a complicated chemical makeup, there are difficulties in using PAG as a component vaccine, in addition to the fact that gene manipulation cannot be applied in the production of this glycolipid antigen. Anti-idiotypic (Id) vaccines against antigens of viruses, parasites, and bacteria have been studied.9–15) A vaccine based on an anti-Id antibody is one method of raising immunity against non-protein antigens.

In this study, we attempted to prepare an anti-Id antibody from immune rabbit serum to a monoclonal antibody (MAB) LW216) against PAG of L. interrogans serovar lai, which is widely found in East Asia, for example, China and Korea,17–21) but not in Japan.

MATERIALS AND METHODS

Monoclonal Antibody and Normal Mouse IgG Previsously established hybridoma cells producing MAB LW216) against PAG of L. interrogans serovar lai were intra-peritoneally (i.p.) inoculated into BALB/c mice (Japan SLC, Hamamatsu, Japan) and the MAB was collected from the mice ascites fluid. Normal mouse sera were collected from 8-week-old ddY mice (SLC) by cardiac puncture. Normal mouse IgG and MAb LW2 were purified from the serum and ascites fluid, respectively, by protein A-affinity column chromatography (Pharmacia LKB, Uppsala, Sweden) according to the manufacturer’s instructions.

Immunization of MAb Purified MAB LW2 (1.0 mg) or a normal mouse IgG fraction (0.5 mg) which had been emulsified in an equal volume of complete Freund’s adjuvant was injected subcutaneously (s.c.) into New Zealand white rabbits (SLC) 4 times at 3-week intervals. Six days after the last injection, sera were collected from the rabbits.

Purification of Anti-Id Antibody (Ab-2) Collected immune rabbit sera were precipitated with 33% saturated ammonium sulfate solution. Normal mouse IgG was coupled with CNBr-activated Sepharose 4B (Pharmacia LKB) according to the manufacturer’s instructions (first column). An affinity column coupled with purified MAb LW2 (second column) was prepared using an Affi-gel Hz immunoaffinity kit (Bio-Rad, Richmond, CA, U.S.A.). The crude Ig fraction precipitated from the antiserum to MAB LW2 was applied to the immunoaffinity column, coupled with IgG fraction prepared from normal mouse IgG (first column). The unbound fraction was eluted from the column with phosphate-buffered saline (PBS, pH 7.2) and was applied to the affinity column, coupled with MAb LW2 (second column). The bound fraction to the second column, designated as LW2-2, was eluted with 0.1 M citric acid and was immediately neutralized with 1.5 M tris-(hydroxymethyl)aminomethane. These fractions were concentrated by ultrafiltration using Omegacell (Filtron Technology Co., Clinton, MA, U.S.A.), and dialyzed intensively against PBS. The protein concentration of each fraction was determined by the Bradford method.22)

Detection of Anti-Id Antibody (Ab-2) The anti-Id antibody was detected by the microscopic agglutination inhibition test and the enzyme-linked immunosorbent assay (ELISA) inhibition test. The activity of anti-Id serum © 1996 Pharmaceutical Society of Japan
was determined on the basis of the agglutinating inhibition of L. interrogans serovar lai by MAb LW2.16. Leptospira culture (50 μl), MAb LW2 (25 μl) and two-fold serial dilutions of anti-Id serum (25 μl) were mixed and incubated at 30°C for 2 h. The agglutination titer was expressed as the reciprocal number of the highest dilution in which 50% of the leptospires were agglutinated. The ELISA inhibition test was performed as follows. The sonicated antigens were prepared from whole cells of L. interrogans serovar lai by the method of Adler et al.23) and were attached to immunoassay plates (Falcon No. 3915; Becton Dickinson Labware, Lincoln Park, NJ, U.S.A.) overnight at 4°C. Purified MAb LW2 (10 μg/ml) and serial dilutions of anti-Id serum as an inhibitor were added and incubated at 37°C for 1 h. After 3 washes, peroxidase-conjugated anti-mouse IgG-IgM-IgA (Organan Teknika Co., West Chester, PA, U.S.A.) was added to each well. After incubation at 37°C for 1 h, the plate was washed and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid was added as a substrate. The optical density was measured at 415 nm and inhibition activity was calculated.

Induction and Detection of Antibody (Ab-3) against Leptospira by Immunization with Anti-Id Antibody (Ab-2) LW2-2, affinity-fractionated anti-Id antibody (Ab-2) and Normal-2, unbound fraction to the first column from rabbit serum immunized with normal mice IgG were injected i.p. to 4-week-old male ddY mice (SLC) 5 times at 7-d intervals. Serum was collected from each mouse before immunization and at 7-d after the final immunization. Antibody (Ab-3) against Leptospira in each mouse serum (diluted 1/50) was detected by ELISA using the sonicated whole cells of serovar lai as an antigen.

Protective Activity Test The protective activity of anti-Id antibody, LW2-2, was examined in 4-week-old male golden Syrian hamsters (SLC).4,7,19,24) The antibody was given i.p. to hamsters on days 0, 7, 14, and 21. Hamsters were challenged i.p. with a virulent strain of serovar lai (1.0 x 10⁶ cells/animal) on day 38. After observation for 21 d, survivors were killed and their kidneys were incubated in Korthof medium25) at 30°C for 14 d to check the exclusion of leptospires.

RESULTS AND DISCUSSION

Anti-Id Antibody (Ab-2) Induction in Rabbits In this study, we prepared anti-Id antibody from immune rabbit sera to MAB LW2,16 which recognized the PAg of L. interrogans serovar lai as an antigen. Immunization with MAB LW2 induced inhibitory activity against leptospiral agglutination of the MAB (Table 1). In contrast, serum from rabbit immunized with normal mouse IgG fraction did not show any inhibitory activity. Immune rabbit serum was applied to an immunoaffinity column coupled with normal mouse IgG fraction (first column). In this column, IgG proteins as ligands were randomly bound to CNBr-activated Sepharose 4B at its free amino groups. This binding form was favorable and effective in eliminating antibodies against the constant region of mouse IgG. The second column coupled with MAb LW2 was prepared using an Aff-gel HZ immunoaffinity kit. Since sugar moieties at the Fc region in IgG were specifically linked to the carriers in this column, antigen-binding sites were effectively expressed on the surface of the carrier. Competitive inhibition activity of anti-Id antibody against whole cells with MAB LW2 were tested by the ELISA inhibition test (Table 2). Serum from rabbit immunized with MAB LW2 showed inhibitory activity depending on the dilution of the sera in ELISA. However, inhibitory activity of the fraction bound to the first column was weaker than that of the original immune serum applied to the column. The fraction bound to the second column (LW2-2) showed higher inhibitory activity in comparison with that of the fractions unbound to the first column and the second column. In contrast, unbound fraction to the first column from rabbit serum immunized with normal mice IgG (Normal-2) exhibited no inhibitory activity in ELISA. These findings suggested that the anti-Id antibody, LW2-2, inhibited the binding of MAB LW2 to leptospiral antigen in ELISA.

Induction of Antibody and Protective Activity against Leptospira by Immunization with LW2-2 An antibody against serovar lai was produced in mice immunized i.p. with anti-Id antibody, LW2-2, at more than 2 μg/mouse doses (Table 3), whereas antibodies reactive to leptospiral antigens were not induced by immunization with the Normal-2 fraction. The anti-Id antibody, LW2-2, protected hamsters from lethal infection of L. interrogans serovar lai upon administration of 2 and 20 μg/hamster doses (Table 4). Leptospires were not found in the culture of kidneys from 21-d survivors. On the other hand,
Table 3. Detection of Antibody against *Leptospira* in Mice Immunized with Anti-Ig Antibody

<table>
<thead>
<tr>
<th>Immunized with</th>
<th>Antibody titer (Optical density at 415 nm × 1000 ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 µg</td>
</tr>
<tr>
<td>LW2-2</td>
<td>658 + 88</td>
</tr>
<tr>
<td>Normal-2</td>
<td>NT a</td>
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Five mice in each group were immunized i.p. with LW2-2 or Normal-2 five times at 7-d intervals. Sera were collected from each mouse before the initial immunization (0-d) and at 7-d after the final immunization (35-d). Antibody titers were calculated using the following formula: (optical density (OD) of sera at 35-d/OD of sera at 0-d) × 1000. a NT, not tested.

Table 4. Protective Activity of Polyclonal Anti-Ig Antibody against *L. interrogans* Serovar *lai* Infection in Hamsters

<table>
<thead>
<tr>
<th>Immunized with</th>
<th>Dose (µg/hematom)</th>
<th>21-d survivors/No. of hamsters tested (% survivors)</th>
<th>Kidney culture positive/survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LW2-2</td>
<td>20</td>
<td>4/5 (80)</td>
<td>0/4</td>
</tr>
<tr>
<td>Normal-2</td>
<td>20</td>
<td>0/5 (0)</td>
<td>NT a</td>
</tr>
</tbody>
</table>

Hamsters were injected i.p. with LW2-2 or Normal-2 on days 0, 7, 14, and 21, and were then challenged i.p. with *L. interrogans* serovar *lai* (10 × 10 ^6 cells/hematom) on day 38. Kidneys were removed from hamsters and cultured on day 59. a NT, not tested.

Normal-2 did not show any protective activity. The anti-Ig antibody was equivalent in protective activity to PAg (FrII) extracted from serovar *lai*, as previously reported. 41 These findings indicate that the anti-Ig antibody, LW2-2, induces protective immunity against *L. interrogans* and an anti-Ig antibody is beneficial when the protective antigen is not a protein.

In conclusion, we confirmed the production of the anti-Ig antibody (Ab-2) against MAb LW2 which was reactive to leptospiral glycolipid PAg, and the anti-Ig antibody protected hamsters against lethal leptospiral infection.

REFERENCES