Experimental Glomerulonephritis Induced by a Low Dose of Anti-Thy-1 Antibody

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Background: Thy-1.1 nephritis is a widely used model of proliferative glomerulonephritis. Lesions are characterized by diffuse mesangiolysis and prominent ballooning of capillary loops, followed by severe mesangial proliferation. However, such severe lesions are not common in human mesangial proliferative nephritis. We analyzed mild-to-moderate mesangial proliferative nephritis induced by a lower dose of anti-Thy-1.1 antibody.

Methods: We administered a low intravenous dose (0.02 mg/mL) of antibody to Wistar rats, and the histologic changes were observed by light and electron microscopy and by various immunopathologic methods. The serum complement level and distribution of polymorphonuclear leukocytes were also examined.

Results: Diffuse mesangial-cell lysis occurred at day 1, but mesangial-matrix lysis was mild, and ballooning of capillary loops was rare. Mesangial cell proliferation appeared at day 2 and reached its maximum at day 5, but it was milder than that seen in the usual dose (0.2 mg/mL) model. The glomerulus was repaired to almost normal structure by day 14. Residual mesangial matrices and endothelial cells seemed to prevent ballooning by making bridges with capillary basement membranes. In the healing stage, this low-dose model demonstrated mild vascular remodeling without active angiogenesis, while the usual-dose model of Thy-1 nephritis showed prominent angiogenic activity to reconstruct capillary tufts.

Conclusion: The low-dose model of Thy-1 nephritis, which does not require prominent angiogenesis, may be more useful in analyzing the mechanism of glomerulonephritis than the usually adopted Thy-1 nephritis model, which showed drastic glomerular destruction and prominent angiogenesis.


Key words: Thy-1 nephritis, mild mesangial proliferation, mesangiolysis, angiogenesis

There are many models of experimental glomerulonephritis, and recently, anti-Thy-1.1 antibody-induced glomerulonephritis (Thy1GN) has become one of the most important and widely used models of proliferative glomerulonephritis. Thy1GN was first reported in 1986 by Ishizaki et al.1 and Bagchus et al.2 and further basic studies were performed by Yamamoto and Wilson.3 The experimentally produced glomerulonephritis seemed to resemble the morphologic features of human proliferative glomerulonephritis, and has been used for many kinds of analyses in elucidating the mechanism of glomerulonephritis,4–6 although, pathologically, it is more severe than human glomerulonephritis. The lesions are characterized by severe mesangiolysis, cystic lesion (or ballooning lesion) of the capillary loop, and continuous mesangial hypercellularity with occasional crescents. After these changes, vascular remodeling and apoptosis of proliferated excess cells occur to reconstruct the glomerular structure, and the glomerulonephritis almost completely resolves within 4 to 6 weeks. Some studies induced chronic nephritis by using high-dose antibodies to mesangial cells.7–9 However, we have rarely observed such a severe mesangiolysis, with ballooning lesions as well as an active vascular remodeling, in usual human renal diseases. Therefore, the generally adopted Thy1GN model using the usual dosage of anti-Thy-1.1 antibody may not be suitable for studying and analyzing the mechanism of usual human glomerulonephritis.

In this study, we tried to establish and analyze a low-dose model of Thy1GN by administering a
reduced dose of anti-Thy-1.1 antibody to rats. There are a few studies on experimental nephritis using a reduced dose of monoclonal antibody to the mesangial cell, but there have been no previous reports with sufficient histologic analysis of Thy1GN, using a low dose of the antibody.

**MATERIALS AND METHODS**

Male Wistar rats (Saitama Experimental Animal Supply, Saitama, Japan) weighing 175 to 190 g were used, as in our previous studies. They were anesthetized by ether and given a single injection of 1 mL of the diluted monoclonal anti-Thy-1.1 antibody (Cedarlane, Hornby, Ontario, Canada) intravenously through the tail vein.

First, a preliminary histologic study was conducted by using variable dosages of the monoclonal anti-Thy-1.1 antibody. Antibody diluted 1/5, 1/10, 1/50, and 1/100 of the usual dosage used in our laboratory (0.2 mg/mL ascites) was administered to the rats. In the 1/5 group, the lesions were similar to those caused by the usual (high) dosage model and in the 1/100 group, the glomerular lesion was indiscernible at the microscopic level. In comparing lesions in groups with the 1/50 and 1/10 doses, the former group showed few glomerular injuries and very slight proliferative changes. We found the dosage of 10% diluted antibody was suitable for the low-dose model. Consequently, we decided to use a 10% diluted monoclonal anti-Thy-1.1 antibody (immunoglobulin G [IgG]) which contained 0.02 mg/mL ascites.

In the experimental animals (n = 20), 1 mL ascites of the antibody was carefully administered intravenously through the tail. (In this experiment, administration of the exact dosage of antibody is very important.) The open renal biopsies and/or harvest of kidneys were performed sequentially from very early times of each experimental group, but extended from 40 minutes to 14 days after administration (at 40 minutes, 1 and 3 hours, daily on days 1 through 5, then day 7, 10, and 14). Each experimental group consisted of 3 to 4 rats. As the control, we used previously collected specimens of the usual, high-dose model of Thy1GN, corresponding to the same time points of each experimental group, but extending farther, as normal recovery takes about 28 days.

**Histologic Examination**

After removal of the kidney, tissue blocks for light microscopic study were fixed in 20% formalin and embedded in paraffin for periodic acid-Schiff (PAS) and periodic acid-methenamine silver HE (PAM) stains. The naphthol AS-D chloroacetate esterase staining was performed by the modified Moloney method to detect neutrophils. Immunohistochemistry for proliferating cell nuclear antigen (PCNA) and α-smooth muscle actin (α-SMA) was performed. The 2.5-μm thick sections prepared from paraffin blocks were treated with 0.3% hydrogen peroxide in methanol for 30 minutes, and then incubated for 60 minutes with 1 of the primary antibodies, anti-PCNA monoclonal antibody (IgG3; Dakopatts, Glostrup, Denmark), anti-α-SMA monoclonal antibody (IgG; Dako, Kyoto, Japan) and type IV collagen (IgG; Southern Biotechnology, Birmingham, AL, USA). Subsequently, the tissue sections were incubated with biotinylated rat anti-mouse IgG antibody, at a dilution of 1:200 in phosphate-buffered saline (PBS) for 60 minutes, and were treated with avidin-biotin-peroxidase complex solution for 60 minutes. The reaction products were visualized by using hydrogen peroxides, containing 3,3′-diaminobenzidine in 0.05 mol/L Tris buffer, then examined under a light microscope.

In histologic sections, fragmented nuclear DNA associated with apoptosis was labeled with biotinylated deoxyuridine, introduced by terminal deoxynucleotidyl transferase (TdT), using the Gavrieli method and PAS counter stain was performed on serial sections to elucidate the correlation. After the exposure of nuclear DNA on histologic sections by 20 μg/mL proteinase K (Promega, Madison, WI, USA), TdT (Life Technologies, Rockville, MD, USA) was used to incorporate biotinylated deoxyuridine (Boehringer Mannheim GmbH, Mannheim, Germany) at the site of DNA breaks. The biotinylated signal was amplified with avidin-peroxidase (Nichirei, Tokyo, Japan), allowing it to be identified by light microscopy.

For the immunofluorescent study, polyclonal antithymocyte serum (ATS) was used as a marker of the mesangial cell; this has been widely applied for detection of mesangial cells. The tissue blocks were snap frozen in liquid n-hexan, cooled in dry-ice-acetone, and stored at −70°C. Cryostat sections of 4 μm in thickness were fixed in acetone for 5 minutes. Sections were stained for 40 minutes with ATS and washed in PBS at room temperature for 15 minutes. Sections were stained for 40 minutes with fluorescein isothiocyanate (FITC)-labeled anti-rabbit IgG as described. The stained sections were examined with an Olympus BHF (Olympus, Tokyo, Japan) fluorescence microscope.

For electron microscopy, the kidneys were quickly removed and cut into 1 mm³ cubes. Blocks were immersed in cold 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer, and post-fixed in 1% osmium tetroxide (OsO₄) in 0.1 mol/L phosphate buffer at pH 7.4 for 2 hours. After dehydration with ethanol, the tissues were embedded in Epok 812 (Okenshoji, Tokyo, Japan). Ultrathin sections were stained with uranyl acetate and lead citrate, then examined with a Hitachi H 7100 electron microscope (Hitachi, Tokyo, Japan).