Supplementation of Heterologous Complement Induces Anti-Thy-1.1 Nephritis in the Mongolian Gerbil (Meriones unguiculatus)

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(Received 5 September 2001/Accepted 18 February 2002)

ABSTRACT. Anti-Thy-1.1 nephritis in the rat is a popular experimental model for mesangial proliferative glomerulonephritis (GN). This model is characterized by direct binding of anti-Thy-1.1 antibody with Thy-1.1 antigen expressed on mesangial cells (MCs) of glomeruli in the rat. A single injection of anti-rat thymocyte serum (ARTS) results in GN with proteinuria and extensive mesangiolysis. Development of mesangiolysis and proteinuria are complement-dependent. We previously demonstrated Thy-1.1 antigen, similar to the rat, in thymocytes, brain cells and MCs of the kidney in the Mongolian gerbil (MG). In this study, we attempted to develop a MG nephritis model, but an injection of ARTS did not induce GN. An additional injection of guinea pig serum as a complement after ARTS injection resulted in anti-Thy-1.1 nephritis in MG. Degeneration of MCs and neutrophil infiltration were observed 1 hr after GP serum injection. Mesangiolysis and fibrin exudation occurred 12 hr after the injection and MC proliferation was apparent 7 days after the injection. In the complement-dependent hemolytic test, MG serum could not hemolyze sheep erythrocytes. These results suggested low activity, or depletion of some factors, in complements of MG serum.

KEY WORDS: complement, Mongolian gerbil, nephritis, Thy-1.1 antigen.

Anti-Thy-1.1 antibody induced glomerulonephritis (GN) is a well-characterized and frequently used model of mesangial proliferative GN [24]. A single intravenous (iv) injection of Thy-1.1 antibody results in proteinuria and GN with lysis of mesangial cells (MCs), called mesangiolysis, followed by MC proliferation and accumulation of extracellular matrix (ECM) [3, 10].

The Thy-1 antigen, a cell surface glycoprotein expressed on thymocytes and thymus-derived lymphocytes but not on B-lymphocytes in mice, was first reported in mice and has been used as a specific cell marker for T-lymphocytes in mice [16]. The Thy-1 antigen also is present in the brain, nervous tissues [7], fibroblasts [22] and epidermal cells [18]. The subtypes of Thy-1 antigen are defined in several strains of mice such as Thy-1.1 in AKR and RF, and Thy-1.2 in other strains including Balb/c and C3H. In rats, Thy-1.1 antigen exists in the thymocytes, brain [5], fibroblasts [22] and MCs of the kidney [9]. Humans [4], dogs [4, 14], chickens [17] and other animals also have Thy-1 antigen but its distribution pattern in tissues and its biological role may be different among species.

Our previous work demonstrated Thy-1.1 antigen in Mongolian gerbils (MGs) in which it was located on thymocytes, brain cells and MCs of the kidney, but it did not represent a cell marker for T-lymphocytes as in the rat [11]. In the present study, we attempted to induce nephritis in MG by injection of anti-Thy-1.1 antibody, but anti-Thy-1.1 antibody alone did not cause pathological changes in the kidney. An additional injection of guinea pig serum led to the development of nephritis. Our results demonstrated that the complement activity of MG was very low and supplementation of heterologous complement was necessary to develop anti-Thy-1.1 nephritis in MG.

MATERIALS AND METHODS

Animals: Two-week (w)-old male Wistar-Imamichi (WI) rats weighing 30–35 g (Imamichi Institute for Animal Reproduction, Tsukuba, Japan) and 12-w-old male Japanese white rabbits weighing 2.0 kg (Saitama Experimental Animal Supply Co., Ltd., Saitama, Japan) were used for preparation of anti-rat thymocyte serum (ARTS). Sera were obtained from 4-w-old male Hartly guinea pigs (GPs) weighing 200–250 g as a source for the heterologous complement in GN induction and the hemolytic test, and sera from 8-w-old male Wistar rats weighing 250–300 g was used for the hemolytic test (Saitama Experimental Animal Supply Co., Ltd.). These sera were stored at –20°C until use. MGs were originally supplied from the Department of Parasitology, Institute of Medical Science, Tokyo University in 1973. They have been bred with sister-brother mating in our laboratory and called Mon/Nms strain after reaching the F20 generation. Twelve-w-old male MGs weighing 60–65 g were used in GN induction and the hemolytic test. The animals were reared with commercial food (MF for rat and MG, GC4 for rabbit and GP, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water under clean conventional conditions (24 ± 2°C of room temperature and 60 ± 5% of relative humidity).

Preparation of anti-rat thymocyte serum (ARTS): ARTS was prepared in rabbits as previously described for polyclonal anti-Thy-1.1 antibody [9]. Three rabbits were immunized twice with 1 × 10⁹ WI rat thymocytes by iv injection at 2-w intervals. The sera were obtained 1 w after the last
injection, then inactivated at 56°C for 30 min and absorbed with packed erythrocytes and hepatic homogenates of WI rats. They were pooled and stored at −20°C until use. Immunohistochecmical study revealed that ARTS reacted with the mesangium area alone, but did not react with the other structures of the renal glomeruli. Its binding capacity to the mesangium of rat glomeruli was completely blocked by monoclonal anti-Thy-1.1 antibody (Sera-Lab., Sussex, UK). The data confirmed that ARTS reacted with Thy-1.1 antigen alone.

**Induction of GN:** Thirty male MGs were injected iv with 0.5 ml of ARTS (4.0 mg of rabbit IgG) and allocated into 2 groups to examine the effect of heterologous complement. Fifteen MGs each received an additional iv injection of 0.3 ml saline (group 1 as a control) or 0.3 ml of fresh GP serum as complement (group 2) 1 hr after ARTS administration. Three MGs from each of the 2 groups were sacrificed under ether anesthesia at 1, 6, 12 hr, 2 and 7 days (d) after ARTS injection and their kidneys were immediately removed.

**Microscopic examinations:** The kidney tissue was fixed with 10% buffered formalin and embedded in paraffin, sectioned, and stained with periodic acid-Schiff (PAS) for light microscopy examination. For immunofluorescence microscopy, renal tissue of MG was snap-frozen in OCT compound (Tissue-Tek, Sakura Finetechical Co., Ltd., Tokyo, Japan), cooled in dry ice-acetone, and stored at −70°C. Cryostat sections of 4 µm were cut and fixed in acetone for 10 min. The sections were washed in phosphate buffered saline (PBS) at room temperature for 5 min, then examined for the presence of rabbit IgG and GP C3 using fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG and FITC-conjugated goat anti-GP C3 (ICN Pharmaceuticals Inc., CA, U.S.A.), respectively.

For electron microscopy, the kidney tissue was immersed in cold 2.5% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4) for 3 hr at 4°C, washed with 0.1 M PB and post-fixed in 1% OsO4 in 0.1 M PB for 2 hr at room temperature. After dehydration with ethanol, the tissues were embedded in Epon 812.

**Complement-dependent hemolytic test:** Rabbits and MGs were immunized iv with sheep red blood cells (SRBC) (Nippon Bio-supply, Center, Tokyo, Japan) twice at 2-w intervals and were bled 1 w after the last injection to prepare anti-SRBC rabbit and MG serum, respectively. SRBC was suspended in gelatin veronal-buffered saline (GVB++) at a concentration of 0.5% after three washes with veronal buffered saline (VBS) and sensitized with two types of inactivated anti-sera, respectively, using the standard procedure. Fifty µl of normal GP, Wistar rat or MG serum was mixed with an equal volume of sensitized SRBC in a microtest plate. Complement activity of MG serum was compared to that of GP or Wistar rat sera based on hemolytic titer after incubation at 37°C for 60 min.

All experiments in this study were performed with the approval of the Animal Experimental Committee of Nippon Medical School.

**RESULTS**

**Detection of ARTS and GP C3 in the kidney:** Deposition of rabbit IgG was noted in the renal glomeruli in a widespread diffuse mesangial pattern 1 hr after the injection of ARTS (Fig. 1a). Deposition of GP C3 was also observed in the mesangial region of MG injected with ARTS 1 hr after iv injection of GP serum (Fig. 1b).

**Effect of administration of ARTS (group 1):** No significant changes except migration of inflammatory cells were found in the mesangial region 12 hr after the administration of ARTS (Fig. 2a-12 hr). The mesangial region appeared normal after 7 d (Fig. 2a-7d). These results indicate that ARTS alone did not induce GN in MG.

**Effect of GP serum supplementation after ARTS injection (group 2):** The cytoplasmic structures of MCs were degenerated and neutrophils had infiltrated into the mesangial region 1 hr after the administration of GP serum (Fig. 3-1 hr). Decrease of glomerular cells and urinary casts were observed 12 hr after administration (Fig. 3-12 hr). The MCs were completely destroyed and fibrin exudation was also found in the lumen of some capillary vessels indicating mesangiolysis (Fig. 3-12 hr). Many neutrophils and mono-
cytes migrated into the mesangial region 2 d after the injection (Fig. 3-2 d) and MC proliferation was apparent 7 d after the injection (Figs 2b-7 d, 3-7 d).

**Complement activity of MG**: Complement-dependent hemolytic pattern induced by normal sera of GP, rat, and MG is shown in Fig. 4. Complement-dependent hemolysis was observed in GP serum diluted less than 2^7 and in rat serum diluted less than 2^5 in both tests using anti-SRBC rabbit and MG sera. MG serum did not induce hemolysis at any dilution in both tests.

**DISCUSSION**

Anti-Thy-1.1 nephritis in the rat is a popular experimental model for mesangial proliferative GN caused by type II allergy. MCs are acutely damaged directly by the immune reaction between ARTS and Thy-1.1 antigen on the surface of the MC, followed by activation of the complement system [23]. Mesangiolysis is mediated by the membrane attack complex, C5b-9 [1]. Platelets respond to C3b-derived chemotactic factors and MC proliferation is initiated by platelets [12]. Neutrophils and macrophages are recruited by C3b and C5a [8]. Complement depletion by cobra venom factor (CVF) did not affect the amount of ARTS bound in glomeruli but prevented the degeneration of MCs, accumulation of inflammatory cells in the glomeruli and GN [23].

In this study, we attempted to develop a GN model of MG induced by injection of ARTS similar to that in the rat. Single ARTS injection caused ARTS binding on the mesangial region, but did not induce chemotaxis of inflammatory cells, proteinuria in the kidney and GN in MG. These findings were similar to those shown by rats given complement depletion by CVF administration. An additional injection of GP serum as a complement after ARTS injection resulted in mesangiolysis and accumulation of leukocytes in the glomeruli suggesting that supplementation of heterologous complement is necessary to induce anti-Thy-1.1 nephritis in MG. In the complement dependent hemolytic test, 2-fold dilution of MG serum did not induce hemolysis of sheep erythrocytes. These results suggest low activity or the depletion of some factors in the complements of MG.

There are no descriptions of the complement system in previous studies on MG. MG accepts various parasitic infections including filaria [20], strongyloides [15] and toxocara [2] of human or the other animals even though parasitic worms usually have host specificity and make symbiotic association only with their definitive hosts. In non-
definitive hosts, the complement system activated by a worm infection directly attacks worm bodies by the lytic effect or by inhibiting worm growth with immunological reactions to prevent host damage [19, 21]. But in specific hosts, a worm structure does not activate the complement system [6], or worms are resistant to the lytic effect of complement [13]. Low activity of complement may be one of the factors behind high and wide susceptibility to various parasites in MG.

The present study clearly demonstrated that anti-Thy-1.1 nephritis in MG needed additional administration of heterologous complement because of its low complement activity. In addition, MG could be a novel model for investigating the role of the complement system in host-parasite relationships.

REFERENCES


