The presence of the Thy-1.1 antigen in Mongolian gerbils

MASAMICHI ISHIZAKI¹, MASUMI SHIMIZU², KAZUHIRO SHICHINOE², YUKINARI MASUDA¹, YUICHI SUGISAKI¹, NOBUAKI YAMANAKA¹ and YOZO MASUGI¹

¹Department of Pathology, and ²Department of Laboratory Animal Science, Nippon Medical School, Bunkyo-ku, Tokyo 113, Japan

ABSTRACT

Rabbit anti-rat thymocyte antiserum (ARTS) and monoclonal anti-Thy-1.1 antibody (Thy-1.1 mAb) were used in the absorption assay and complement-dependent cytotoxic test to define the antigenic relationship between rats and Mongolian gerbils. ARTS was absorbed by the gerbil brain and thymocytes and lost its cytotoxicity on rat thymocytes. Thy-1.1 mAb was also absorbed by gerbil thymocytes, but not by spleen cells. Immunofluorescent studies showed that cortical thymocytes were strongly positive to both antibodies, whereas medullary thymocytes exhibited a weakly positive reaction. In the spleen and lymph nodes, only a small number of Thy-1.1 antigen-positive cells were observed. In the kidney, a positive reaction to both antibodies was observed in the glomerular mesangial region. The results indicate that the Thy-1.1 antigen exists in Mongolian gerbils, and its distribution in tissues is similar to that in rats.

The existence of the Thy-1 antigen, a cell surface glycoprotein with a molecular weight of 18,000, was first reported by Reif and Allen (8), and this has been known as a cell marker for T-lymphocyte in mice. In mice the Thy-1 antigen consists of two types, *i.e.* Thy-1.1 and Thy-1.2 (8). Later, occurrence of the Thy-1.1 antigen was shown also in rats (2). More recently, the presense of Thy-1 antigens has been reported in humans (1), chickens (10), dogs (6), and in some other animals. The present investigation demonstrates immunologically that Thy-1.1 antigen exists also in the Mongolian gerbil (*Meriones unguiculatus*).

Gerbils used (JMS strain) were originally supplied in 1973 from the Department of Parasitology, Institute of Medical Science, Tokyo University. Since then, this strain has been bred and maintained in our animal colony at the Department of Laboratory Animal Science.

ARTS was raised in rabbits as previously

described (5). ARTS was absorbed with an equal volume of thymocytes or of the cerebral homogenate of the Wistar rat or the gerbil. Each of these mixtures was allowed to stand overnight at 4°C for absorption. Each lot of ARTS thus obtained was examined for its cytotoxicity to rat thymocytes. ARTS was strongly absorbed by rat thymocytes and brain. Further, the absorption effect on gerbil thymocytes and brain was also observed (Fig. 1). These results suggest that the Thy-1.1 antigen exists in the gerbils. Next, the Thy-1.1 mAb (Sera-Lab, Sussex, England) was absorbed with gerbil thymocytes in various numbers and with non-adherent spleen cells, and a cytotoxic test was subsequently performed with rat thymocytes as the target. Simultaneously, cytotoxic tests were also conducted with Thy-1.1 mAb absorbed with the rat and AKR mouse thymocytes and with spleen cells. Cytotoxicity of Thy-1.1 mAb disappeared by the absorption of thymocytes

Fig. 1 The complement-dependent cytotoxic effects on rat thymocytes exerted by ARTS (●), ARTS absorbed with rat thymocytes (■), with rat cerebral homogenate (▲), with gerbil thymocytes (□), or with gerbil cerebral homogenate (△). For the method of absorption and complement-dependent cytotoxic test, see reference 5. Cytotoxic index = (% killed with the antibody - % killed with the complement alone) / (100% - % killed with the complement alone)

Fig. 2 The Thy-1.1 mAb at a 1:16 dilution was absorbed with gerbil spleen cells (○), with gerbil thymocytes (●), with rat spleen cells (□), with rat thymocytes (■), with AKR mouse spleen cells (△) or with AKR mouse thymocytes (▲) at 5×10^7 – 5×10^4 . The residual antibody was assayed for the complement-dependent cytotoxicity on rat thymocytes.

(1×10^7 cells) from gerbils, rats, and AKR mice. In contrast, no absorption effect was noted with spleen cells from gerbils and rats even with 5×10^7 cells, whereas an absorption effect was noted with spleen cells from AKR

mice (Fig. 2). The results indicate that the Thy-1.1 antigen exists in Mongolian gerbils.

The gerbil thymus was examined immunohistologically using Thy-1.1 mAb and ARTS. Both antibodies showed a similar immunofluo-

Fig. 3 Immunofluorescent staining with Thy-1.1 mAb. Fresh frozen sections of the gerbil thymus (a), spleen (b) and kidney (c) were allowed to react with Thy-1.1 mAb (1:128 dilution) at room temperature for 45 min. Then the sections were reacted with FITC-labeled rabbit F(ab')₂ fragment of anti-mouse IgG (Cooper Biomedical, Malvern, PA, U.S.A.) at room temperature for 45 min. The FITC-labeled antibody was diluted to 1:50 with phosphate-buffered saline containing 10% normal gerbil serum. a: Frozen sections of a normal gerbil thymus. Cortical thymocytes (on the right) are strongly positive to the Thy-1.1 mAb, whereas the medullary thymocytes (on the left) are weakly positive. $\times 180$. b: Frozen section of a normal gerbil spleen. Thy-1.1 mAb-positive cells are observed in the periphery of the periarterial lymphoid sheath. $\times 180$. c: Frozen section of a normal gerbil kidney. Thy-1.1 mAb reacted with the mesangial area. $\times 360$

rescent pattern in the gerbil thymus. Cortical thymocytes were strongly positive, whereas medullary thymocytes were weakly positive (Fig. 3a). Further, the response to Thy-1.2 mAb (Cedarlane Laboratories, Harnby, Ontario, Canada) was completely negative. In the spleen, a small number of Thy-1.1 mAb-positive cells were observed in the periphery of the periarterial lymphoid sheath (Fig. 3b) and in the red pulp. The other cells in the T-cell region were negative. The results suggest that the Thy-1.1 antigen does not exist in peripheral T-cells of gerbils like in rats (3). The Thy-1.1 mAb-positive cells exist in a small quantity in the spleen and lymph nodes. This finding coincides with that in rats, where these cells were considered as early B-cells (9). This may hold also in gerbils, but further research is required to conclude this.

Thy-1.1 mAb and ARTS showed a similar immunofluorescence in the gerbil kidney; the reaction was observed in the glomerular mesangial region (Fig. 3c). No reaction in the basement membrane was noted. The reaction to ARTS and Thy-1.1 mAb, absorbed with rat and gerbil thymocytes, was negative in the mesangium. Occurrence of the Thy-1.1 antigen in the kidney has been reported in rats and dogs, though its distribution entirely differs; the antigen occurs in the glomerular mesangium in rats (5, 7), whereas in the tubular basement membranes of the medulla in dogs (6). We have previously reported in rats with experimental nephritis that the mesangium is the site of the Thy-1.1 reaction (4). Similarly, it is possible that experimental nephritis may be induced in gerbils with the anti-thymocyte serum.

It is interesting to note that the distribution of Thy-1.1 antigen in tissues differs between mice and rats, despite that these two species belong to the same family, whereas the distribution is almost the same between gerbils and rats, though these species belong to different families.

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