The presence of Thy-1.1 antigen in rat glomerular mesangial cells

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ABSTRACT

Glomerular mesangium cells are known to contain actomyosin and fibronectin. We found in this study that Thy-1.1 antigen was also present in rat glomerular mesangial cells. Anti rat thymocyte serum (ATS) was raised in rabbits by immunization of Wistar Imamichi rat thymocytes. After *in vivo* administration of ATS to rats or *in vitro* application of ATS to frozen rat kidney sections, ATS was found by immunohistological methods to be strictly confined to glomerular mesangium. ATS absorbed by rat thymocytes and brain homogenate failed to show such activity. Recently, mouse Thy-1.1 antigen has been found in rat thymocytes, brain, and other cells. Therefore, mono-clonal Thy-1.1 antibody derived from hybrid myeloma cells was also tried in the same way. It was found that this mono-clonal antibody was also localized in the rat glomerular mesangium *in vitro* just like ATS. It is thus concluded that the antigen common to both glomerular mesangium and thymocyte is Thy-1.1 antigen. By immunoelectron microscopic study using peroxidase labeled antibody, the site of Thy-1.1 antigen localization in the mesangium was observed to be the surface of the mesangial cell.

KEY WORDS Thy-1.1 antigen / ATS / mesangium / mono-clonal antibody

It is a general practice to carry out cell typing immunologically by taking advantage of the cell surface differentiation antigens. One of these antigens is Thy-1 antigen, which was reported by Reif and Allen (8), and is known to be the major antigen serving as a cell marker for thymocytes and thymus-derived lymphocytes in mice. In mice the Thy-1 antigen consists of two types, Thy-1.1 and Thy-1.2. Every strain of mice has been confirmed to possess either one or other of the two types. Thy-1.1 antigen was reported to exist also in rats (2).

It was reported that Thy-1 antigen was present also in the nervous system (1, 3), epidermal cells (9), and fibroblasts (10) in mice and rats. In the present investigation, we have further demonstrated immunohistologically that Thy-1.1 antigen exists also in the mesangial cells of the renal glomeruli of rats.

In preparing anti-thymocyte serum, the method of Sutthiwan *et al.* (11), which had originally

been developed for mice, was applied to a strain of rats. To prepare cells for immunization, thymocytes were collected from two-week-old inbred Wistar-Imamichi (WI) rats, and 1×10^9 of the thymocytes were injected intravenously into rabbits twice at an interval of 2 weeks for immunization. These rabbits were bled totally 1 week after the second immunization. The serum was separated from the blood and inactivated at 56°C for 30 min. The inactivated serum was mixed with equal volumes of packed erythrocytes and hepatic homogenate and allowed to stand overnight for absorption before it was used as anti-thymocyte serum (ATS). ATS was mixed with an equal volume of lymph node cells, splenic cells, thymocytes, or cerebral homogenate. Each mixture was allowed to stand overnight for absorption. Each lot of ATS thus obtained was examined for cytotoxicity to thymocytes of WI rats and for the capacity of mesangium binding. When the cytotoxicity test

was applied to ATS samples already subjected to absorption with lymph node cells and splenic cells, no distinct reducing effect was seen on cytotoxicity to thymocytes. On the other hand, the ATS sample mixed with the cerebral homogenate revealed a remarkable reduction in cytotoxicity. The ATS sample subjected to absorption with thymocytes exhibited no cytotoxicity at all (Fig. 1).

Fig. 1 Complement dependent cytotoxic effects on WI rat thymocytes exerted by ATS (○), ATS absorbed with rat lymph node cells (■), ATS absorbed with rat splenic cells (□), ATS absorbed with rat cerebral homogenate (▲) and ATS absorbed with thymocytes (●). The complement dependent cytotoxicity test was carried out in the following manner. To 0.05 ml of a suspension of single thymocyte cells containing 5×10^6 cells/ml were added 0.05 ml of serially diluted ATS and 0.05 ml of 1:4 dilution of guinea pig serum, as a complement source. The mixture was incubated at 37°C for 45 min. After incubation 0.1 ml of 0.16% trypan blue in saline was added to it to count dead cells. The cytotoxic index (CI) was calculated from the following formula: $CI = (\% \text{ kill with antiserum} - \% \text{ kill with complement alone}) / (100 - \% \text{ kill with complement alone})$. The cytotoxicity of the guinea pig serum used for rat thymocytes was determined by the 0.16% trypan blue exclusion test. Serum samples exhibiting a cytotoxicity of less than 5% were pooled and used.

Next a series of twofold dilutions of these ATS samples was made and applied to frozen sections of the kidney of normal WI rats. These sections were washed with PBS and subjected to the indirect fluorescent antibody technique in which FITC-labeled anti-rabbit IgG was used as a secondary antibody in the reaction. Up to a 1:256 dilution, the ATS samples showed a positive reaction with the mesangium of the renal glomeruli (Fig. 2). When the ATS samples were mixed with lymph node cells and splenic cells for absorption, their mesangium binding capacity was hardly reduced, but when they were absorbed by thymocytes and the cerebral ho-

mogenate, their mesangium binding capacity disappeared completely.

Thus it is clear that an antigen possessed by mesangium is commonly present on the surface of the thymocytes. The antigen was scanty on the surface of lymph node cells and splenic cells, but was abundant in the brain. As reported by Williams (13), the antigen exhibiting the localization mentioned above was Thy-1.1 antigen in rats. It is highly possible that this antigen is present also in the rat mesangium. However, with such a poly-clonal antibody as the ATS used in this study, it was difficult to identify tissue antigen immunohistologically. Therefore, another experiment was carried out with mono-clonal antibody. In 1975, Köhler and Milstein (5) developed a method to prepare mono-clonal antibody from hybrid myeloma cells. The antibody used in our experiment was prepared by their method. In this experiment, the mono-clonal anti-Thy-1.1 mouse IgG was a product of the Sera-Lab. Company (Sussex, England) and the mono-clonal anti-Thy-1.2 mouse IgM was a product of the Olac Company (Oxon, England).

The experiment was performed in the same manner as that with ATS. A series of twofold dilutions of the mono-clonal antibody was made and tested on frozen sections of the rat kidney. As secondary antibodies, FITC-labeled anti-mouse IgG and anti-mouse IgM were used in the indirect fluorescent antibody technique. Up to about 1:16,000 dilution, mono-clonal anti-Thy-1.1 antibody reacted with the mesangium of the rat glomeruli in the same pattern as in the experiment with ATS (Fig. 3). Mono-clonal anti-Thy-1.2 mouse IgM, however, showed no reaction at all.

The blocking test was carried out between ATS and mono-clonal anti-Thy-1.1 antibody. Both antibodies were blocked by each other. ATS reacted with the mesangium was confirmed to be an antibody against Thy-1.1 antigen.

From these results, the antigen possessed commonly by the mesangium of the renal glomeruli and the thymocytes of the rat was confirmed to be Thy-1.1 antigen.

The detailed distribution of Thy-1.1 antigen in the mesangium was observed by immunoelectron microscopy. We used mono-clonal anti-Thy-1.1 mouse IgG and peroxidase-labeled anti-mouse IgG, and found that Thy-1.1 antigen was present on the surface of the mesangial cells (Fig. 4). Recently Morris *et al.* (6) demonstrated the presence of Thy-1 antigen in rat glomerulus by immunofluorescence. They

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Fig. 2 A series of twofold dilutions of ATS was made. Each serum dilution was allowed to react on fresh frozen sections of the adult WI rat kidney at room temperature for 45 min. After reaction the sections were washed with PBS. As a secondary antibody, FITC-labeled anti-rabbit IgG goat antibody was reacted at room temperature for 45 min. After being washed with PBS, each section was observed by fluorescent microscopy. As shown in the photograph, ATS reacted clearly with the mesangial areas. The photograph presents the reaction of 1:128 dilution of ATS in the mesangium of the glomerulus. The urinary tubuli and Bowman's capsule exhibited granular auto-flourescence.

Fig. 3 A series of twofold dilutions of mono-clonal anti-Thy-1.1 mouse IgG was made. Each dilution of antibody was allowed to react on fresh frozen sections of the adult WI rat kidney at room temperature for 45 min. After reaction, each section was washed with PBS. As a secondary antibody, FITC-labeled anti-mouse IgG was used at room temperature for 45 min. After being washed with PBS, the section was examined under a fluorescent microscope. Monoclonal anti-Thy-1.1 antibody reacted with the mesangial areas, as shown in the photograph. This photograph presents the fluorescence in a 1:8,000 dilution of serum.

Fig. 4 Immunoelectron microscopy was carried out with peroxidase-labeled antibody to study the details of the distribution of Thy-1·1 antigen in the mesangium. The antigen (arrow) was found on the surface of the mesangial cell. Immunoelectron microscopy was performed by a modification of the method of Nakane *et al.* (7). The normal rat kidney was fixed in periodate-lysine-paraformaldehyde. Then it was cut into frozen sections 4 μ m thick. Anti-Thy-1·1 antibody was allowed to react with these sections. As a secondary antibody, peroxidase-labeled anti-mouse IgG was used. For the detection of peroxidase activity the sections were incubated in the medium of Graham and Karnovsky (4). Glutaraldehyde and OsO_4 were used for fixation. Epon embedding was done by the conventional method. The resulting block was cut into ultrathin sections, which were subjected to single staining with uranium acetate, observed, and photographed with the electron microscope. MES: mesangial cell

insisted that it was localized in the glomerular basement membrane (GBM) rather than in mesangial cells. We could not find any evidence of such GBM localization of Thy-1·1 by either immunofluorescence or immunoelectron microscopy.

In 1933, Zimmerman (14) reported the presence of mesangial cells in the glomeruli. Recently, these mesangial cells have been studied by many workers from various points of view. It has been demonstrated immunohistologically that such substances as actomyosin (1) and fibronectin (12) were present in relation to the mesangial cells. In the present experiment we have shown that Thy-1·1 antigen, which is found on the surface of the thymocytes of rats and mice, is also present in the mesangial cells in rats. If the role of Thy-1·1 antigen in the mesangial cell is clarified, it will be possible to obtain some information about the nature of the mesangial cell. There are also still many things to learn about the role of the Thy-1 antigen contained in thymocytes and T-cell. Studies on

the biological significance of Thy-1·1 antigen in the mesangial cell may throw some light on the role of the Thy-1·1 antigen in thymocytes.

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REFERENCES

1. BECKER C. G. (1972) Demonstration of actomyosin in mesangial cells of the renal glomerulus. *Am. J. Pathol.* **66**, 97-110
2. DOUGLAS T. C. (1972) Occurrence of a theta-like antigen in rats. *J. Exp. Med.* **136**, 1054-1062
3. GOLUB E. S. (1971) Brain-associated theta antigen: reactivity of rabbit anti-mouse brain with mouse lymphoid cells. *Cell. Immunol.* **2**, 353-361
4. GRAHAM R. C. and KARNOVSKY M. J. (1966) The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* **14**, 291-302
5. KÖHLER G. and MILSTEIN C. (1975) Continuous

- cultures of fused cells secreting antibody of predefined specificity. *Nature* **256**, 495–497
6. MORRIS R. J. and RITTER M. J. (1980) Association of Thy-1 cell surface differentiation antigen with certain connective tissues *in vivo*. *Cell Tissue Res.* **206**, 459–475
 7. NAKANE P. K. and PIERCE G. B. (1967) Enzyme-labeled antibodies for the light and electron microscopic localization of tissue antigens. *J. Cell Biol.* **33**, 307–318
 8. REIF A. E. and ALLEN J. M. V. (1964) The AKR thymic antigen and its distribution in leukemias and nervous tissues. *J. Exp. Med.* **120**, 413–433
 9. SCHEID M., BOYSE E. A., CARSWELL E. A. and OLD L. J. (1972) Serologically demonstrable alloantigens of mouse epidermal cells. *J. Exp. Med.* **135**, 938–955
 10. STERN P. L. (1973) Theta alloantigen on mouse and rat fibroblasts. *Nature New Biol.* **246**, 76–78
 11. SUTTHIWAN P., SHORTER R. G., HALLENBECK G. A. and ELVEBACK L. R. (1969) Comparison of rabbit anti-mouse thymus sera produced after different numbers of injections of lymphoid cells. *Transplantation* **8**, 249–257
 12. WEISS M. A., OOI B. S., OOI Y. M., ENGVALL E. and RUOSLAHTI E. (1979) Immunofluorescent localization of fibronectin in the human kidney. *Lab. Invest.* **41**, 340–347
 13. WILLIAMS A. F., BARCLAY A. N., LETARTE-MUIRHEAD M. and MORRIS R. J. (1976) Rat Thy-1 antigens from thymus and brain: their tissue distribution, purification and chemical composition. *Cold Spring Harbor Symp. Quant. Biol.* **41**, 51–61
 14. ZIMMERMAN K. W. (1933) Über den Bau des Glomerulus der Säugenierte. *Ztschrft. f. mikr-anat. Forsch.* **32**, 176–278