Immunology 1989 **67** 531–533

Rat mesangial cells actively produce phosphatidylinositol-anchored Thy-1

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Accepted for publication 11 April 1989

SUMMARY

We provide evidence that the mesangial cells of rat kidney glomeruli express Thy-1 as a phosphatidylinositol-anchored protein. Both the mesangial area of kidney, examined in tissue sections, and mesangial cells maintained in culture for more than 3 months, showed prominent immunofluorescence staining with an anti-Thy-1 monoclonal antibody (OX7); this staining was almost completely abolished by pretreating kidney sections or mesangial cells with the phosphatidylinositol-specific enzyme, phospholipase C. By Northern blotting, mesangial cells were shown to express mRNA of an appropriate size, hybridizing to a mouse Thy-1.1-specific probe.

The Thy-1 antigen as the putative primordial immunoglobulin domain (Williams & Gagnon, 1982) has been reported to show unusual patterns of tissue distribution within a species and between species. Thy-1 is a major constituent of the brain cell membrane in all species studied (Reif & Allen, 1964; Douglas, 1972; Dalchau & Fabre, 1979). In lymphoid tissues, Thy-1 is the most abundant surface molecule of rodent thymocytes (Acton, Morris & Williams, 1974), and is found in reduced amounts in murine mature T lymphocytes (Raff, 1971), and rat (Hunt, Mason & Williams, 1977) and human (Retter, Sauvage & Delia, 1983) early T and B lymphocytes. Furthermore, it has been suggested that Thy-1 or Thy-1-like antigen exists in other tissues including canine and human kidneys (Dalchau & Fabre, 1979). Thy-1 or Thy-1-like antigen was also demonstrated in the mesangial cells (MC) of glomeruli of the rat kidney by immunofluorescence (Ishizaki *et al.*, 1980) and immunoelectron microscopy (Yamamoto *et al.*, 1986). However, the possibility has not been ruled out that the serological demonstration of Thy-1 specificity on rat MC is due to cross-reaction at the determinant level or to trapping by MC of Thy-1 produced by other tissues.

Recently Thy-1 genes have been cloned and sequenced (Moriuchi *et al.*, 1983; Giguere, Isobe & Grosveld, 1985) and the structures of Thy-1 molecules from thymocytes and brain tissues were defined (Williams & Gagnon, 1982). The Thy-1 molecule on rodent thymocytes has been shown to be phosphatidylinositol (PI)-anchored (Low & Kincade, 1985). By applying PI-specific phospholipase C (PI-PLC) treatment and Northern hybridization technique to cultured MC as well as the kidney tissue, we describe in this report that rat MC do produce Thy-1 as a PI-anchoring protein.

MC were cultured according to the procedure described and were shown to have the same morphological and biological properties as reported elsewhere (Lovett, Ryan & Sterzel, 1983). In the present study MC maintained *in vitro* for more than 3 months by serial passages were used. PI-PLC was prepared from the culture supernatant of *Bacillus thuringiensis* as described elsewhere (Ikezawa *et al.*, 1983). This preparation of PI-PLC was protease free and did not release MHC class I antigens from rodent thymocytes. As shown in Fig. 1, the kidney section examined showed prominent staining for Thy-1 predominantly in the mesangial area of glomeruli, with the use of OX7 (donated by Dr A. Williams, University of Oxford, U.K.) as the first antibody (Fig. 1b). MC also showed prominent fluorescence staining for Thy-1 (Fig. 1e). However, after the kidney section (Fig. 1c) and cultured MC (Fig. 1f) were pretreated with PI-PLC, definite Thy-1 staining was no longer observed.

We tried to demonstrate Thy-1 mRNA expression in the kidney and cultured MC by Northern blotting analysis according to the method of Maniatis, Frisch & Sambrook (1982) using the probe that contained the mouse Thy-1.1 coding region. The probe that contained the mouse Thy-1.1 exon hybridized not

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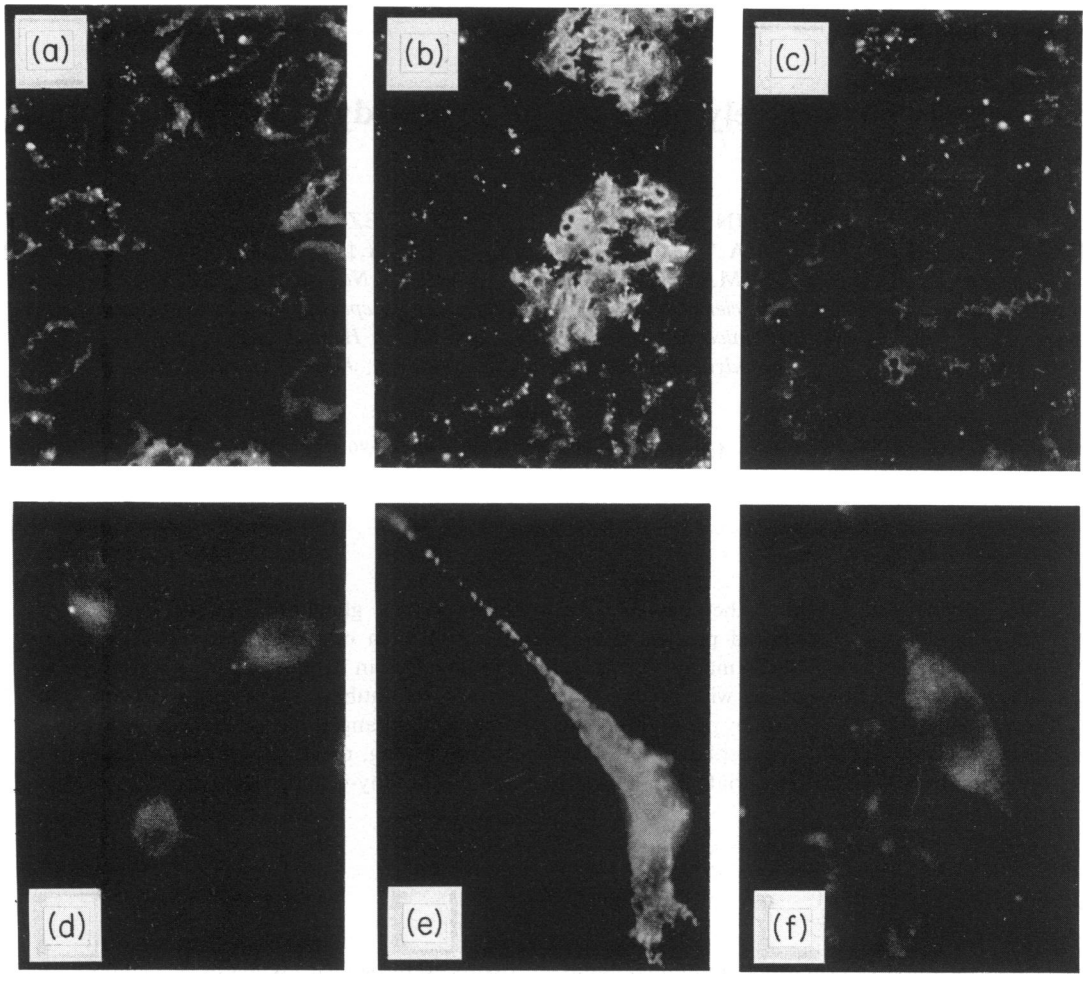

Figure 1. Immunofluorescence staining of rat kidney sections and cultured rat MC. Rat kidney cryosections and cultured rat MC were reacted first with: (a, d) phosphate-buffered saline (PBS) (control); (b, e) monoclonal anti-Thy-1 antibody (OX7; 1 : 100 dilution) as the first antibody (no pretreatment with PI-PLC); or (c, f) OX7 after treatment with 150 mU/ml of PI-PLC in PBS of pH 7·2 at 37° for 1 hr. After washing with PBS, they were stained with FITC-conjugated goat anti-mouse IgG antiserum (1 : 40; Tago Inc., Burlingame, CA) as the second antibody. Original magnification; (a, b, c) × 100; (d, e, f) × 200.

only to Thy-1 mRNA of AKR mouse thymocytes but also to Thy-1 mRNA of rat thymocytes, providing a band of about 2 kb. The degree of hybridization to the latter was, however, about one-fourth of that to the former. The Thy-1 probe also hybridized to the mRNA from cultured MC at the same position, showing that the MC express Thy-1 mRNA of normal size. The level of Thy-1 mRNA expression in rat MC was about a half of that in rat thymocytes. Thy-1 mRNA was not detectable in the rat kidney tissue that contains MC as a minor element, or in the rat liver as a negative control (Fig. 2).

By immunohistochemistry and Northern hybridization this study shows that rat MC actively produce Thy-1. The Thy-1 antigen was almost completely released from MC surface with PI-PLC treatment. The size of Thy-1 mRNA of rat MC was the same as that of rat or mouse thymocytes.

What would be the biological significance of the presence of Thy-1 as a PI-anchoring protein on MC? The function of Thy-1 in general has not been determined, but it is suggested that Thy-1 is a signal transducing molecule during cell interaction (Gunter *et al.*, 1987) or a molecule mediating activation of the cell seeing it (Isobe *et al.*, 1984). MC have been shown to be involved in the proliferative form of glomerulonephritis, and administration of anti-thymocyte serum or Thy-1-specific monoclonal antibody into rats induced MC damage (Yamamoto & Wilson, 1987; Bagchus *et al.*, 1987). Therefore, it is likely though not proven that Thy-1 is involved in one or more physiological functions or disorders of glomeruli of the kidney as a cell interaction molecule.

ACKNOWLEDGMENTS

We are grateful to Dr A. F. Williams, University of Oxford, U.K., for donating OX7. We are thankful to members of the Institute for Laboratory Animal Research, Nagoya University School of Medicine, for providing Lewis rats.

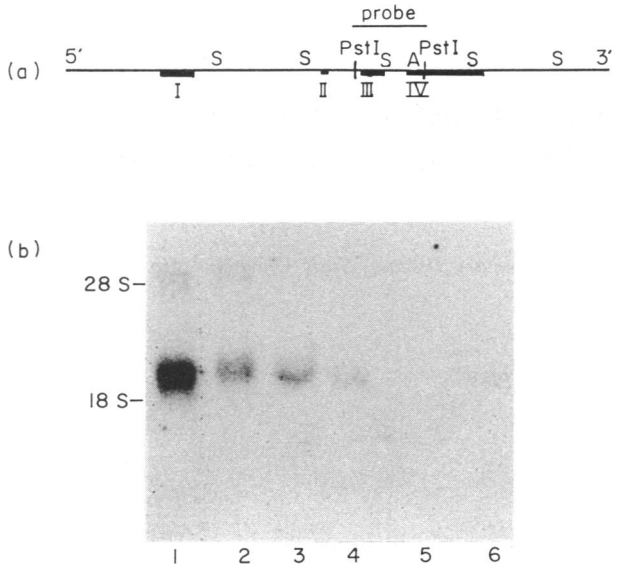

Figure 2. Probe of the mouse Thy-1.1 gene (a) and Northern blotting analysis (b). The exons (I, II, III, IV) and introns are indicated (a). Exon III codes for the extracellular Ig-like domain. S, Sac I; A, Apa I; Pst I, Pst I restriction fragment was used as a probe. RNA (10 μg for lanes 1, 2, 4, 5, 6, and 15 μg for lane 3) from different tissues was electrophorated on agarose gel, was transferred to the nylon membrane, and was hybridized with a radioactively labelled probe. Lane 1, AKR thymus RNA; lane 2, Lewis rat thymus RNA; lanes 3, 4, cultured MC RNA; lane 5, Lewis rat kidney RNA; lane 6, Lewis rat liver RNA. The numbers indicate the position of 18S and 28S ribosomal RNA. The level of RNA expression was scored by densitometry. The score of density is: lane 1, 3·40; lane 2, 1·00; lane 3, 0·73; lane 4, 0·40; lanes 5, 6, almost none.

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