Acta Medica et Biologica
Vol. 39, (Suppl.) 47-52, 1991

Proteinuria-Inducing Monoclonal Antibodies

Hiroshi KAWACHI

Department of Immunology, Institute of Nephrology, Niigata University School of Medicine, Asahimachi, Niigata 951, Japan

Summary. **Two kinds of monoclonal antibodies (mAbs) capable of inducing massive proteinuria in rats were produced in BALB/c mice with collagenase-treated Wistar rat glomeruli. The first mAb (mAb 5-1-6) reacts with the surface of glomerular epithelial cell foot processes, mainly to slit diaphragms. Protein excretion was initiated immediately following an injection of 2 mg mAb 5-1-6, and reached an average value of 138.5 mg/24 h on day 5. No histological abnormalities were noted except for partial retraction of epithelial foot processes at the peak of proteinuria. The minimum dose of mAb 5-1-6 required to induce proteinuria is 0.125 mg as injected dose. This dose corresponds to 12.8 μg as the amount of kidney binding antibody 1 h after injection, and 0.34 μg at 5 days. The second mAb (mAb 1-22-3) is capable of inducing not only proteinuria but also morphological changes in the mesangial region. mAb 1-22-3 binds *in vitro* to the limited mesangial cell surface facing endothelial cells. Reactivity of mAb 1-22-3 toward any other site in the kidney except the mesangial cell surface could not be detected. These mAbs may possibly serve as a means for clarifying the mechanism regulating the macromolecule permeability of glomerular capillary wall.**

INTRODUCTION

Most cases of primary glomerulonephropathy are considered to be caused by an immune mechanism. Masugi reported proliferative glomerulonephritis induction by heterologous antisera containing various antibodies having specificity for the constituents of the glomerular capillary wall (GCW).[1] Since then, the roles of individual antigenic determinants in the pathogenesis of this injury have been investigated, but still remain incompletely understood. Heymann nephritis, an experimental membranous nephropathy, has been demonstrated to be induced by an antibody to glycoprotein of 330 kd (gp 330) located on the surface of the glomerular epithelial cell.[2] The exact antigenic determinant of Heymann nephritis, however, has not yet been reported.

To determine the epitopes involved in the induction of various types of glomerulonephritis, we produced monoclonal antibodies (mAbs) with specificity for various constituents of rat glomerulus.[3] We have already reported on two mAbs which are capable of inducing significant proteinuria in rats.[4,5] mAbs were produced in mice by immunization with collagenase-treated fresh rat glomeruli. In this paper, we will discuss these mAbs more comprehensively and introduce results of quantitative analysis.

MATERIALS AND METHODS

Preparation of mAbs

All experiments were performed using female Wistar rats weighing 150-200 g and purchased from Charles River Japan, Inc. (Atsugi, Japan). mAbs were prepared according to the method of Orikasa et al.[4] Briefly, BALB/c mice were immunized with collagenase-treated rat glomeruli using Bordetella pertussis. Spleen cells were fused with the myeloma cell line X63-Ag8653 by polyethylene glycol 4000 (Sigma Chemical Co., St. Louis, MO, USA). Supernatants of growing hybridomas were tested for anti-kidney activity by indirect immunofluorescence (IF) on cryostat sections of normal rat kidney. Selected hybridomas were cloned by limiting dilution and re-tested by indirect IF. Murine IgG1 mAb, RVG1 and IgG3 mAb, TS-11m (against rotavirus) were prepared as controls. RVG1 and TS-11m were confirmed not to be reactive toward rat kidney.

Characteristics of mAbs

The class and subclass were determined with an Ig kit purchased from Miles Laboratories Inc. (Elkhart,

Indiana, USA). Isoelectric focusing was carried out with Phast system (Pharmacia, Uppsala, Sweden). The *in vitro* binding activity of mAbs to rat kidney was assessed by indirect IF and immunoelectron microscopy. Its reactivity with other rat tissues (thymus, brain, intestine, liver, lung, skin, heart) and with kidney and other tissues from mice, guinea pigs, rabbits, and humans was also tested by indirect IF.

Animal experiment

mAb 5-1-6 Each of a total of 18 rats was injected with 2.0 mg of ^{125}I-labeled affinity purified mAb 5-1-6. and 3 rats were sacrificed following each of the following survival periods: 1 h, 24 h, 48 h, 5 day, 10 day and 15 day. The animals were perfused through the heart with PBS. The kidneys were removed and washed in PBS. Radioactivity of the kidneys was then counted with an autogamma scintillation photometer (model 5260, Packard). Blood samples were collected from the heart prior to perfusion with PBS. The radioactivity of 100 μl of blood specimen was counted. The kidneys were processed for light microscopy (LM), electron microscopy (EM), and IF, after measuring radioactivity. The rats were individually housed in metabolic cages with free access to water for collection of 24-h urine specimens. The amount of urinary protein was determined by the biuret method.[6] In the following experiment, thirty-six rats divided into experimental groups of 6 rats each were injected via the tail vein with 2.0, 1.0, 0.5, 0.25, 0.125, 0.063 mg of labeled mAb 5-1-6. At 1 h after injection 3 rats of each group were sacrificed, and the remaining were similarly sacrificed at 5 day after injection. 24-h urine specimens from the latter group were collected. The kidney sections from rats were observed by IF with FITC-conjugated anti-mouse Ig. The titres of antibody activity of mAb 5-1-6 remaining in the blood specimens from rats were compared with indirect IF as follows. Normal rat kidney sections were incubated with serum samples diluted with PBS to various degrees (×10, ×20, ×40, ×80, ×160, ×320) and stained with FITC-conjugated anti-mouse Ig absorbed with normal rat serum. Fluorescence intensity in 10 full sized glomeruli from two sections was measured by an auto-exposure system (Axiophot, Zeiss). The amount of exposure time as indicated by the auto-exposure system was considered to reciprocally reflect staining intensity.

mAb 1-22-3 Female Wistar rats were intravenously injected with 1.0 ml of saline containing 500 μg of ascitis-derived ammonium sulphate-precipitated mAb 1-22-3. Kidneys of the rats were studied by sacrificing groups of three rats at 30 min, 2, 24, 48 h, 4, 6, d, 2 wk and 4 wk after iv injection of mAb. Urinary protein was measured every other day for the first 14 day. For the next two weeks, protein excretion was measured every four days. The kidney material from each rat was examined by direct IF with FITC-conjugated anti mouse Ig or FITC-conjugated anti-rat C3, LM, EM and IEM with peroxidase-conjugated anti-mouse Ig.

RESULTS

Characteristics of mAbs

The characteristics of mAb 5-1-6 and mAb 1-22-3 are summarized in Table 1. The *in vitro* binding pattern of mAb 5-1-6 was linear to fine granular along the capillary wall in indirect IF (Fig. 1). The binding pattern at the ultrastructural level is shown in Fig. 2. In the immunoperoxidase technique, a reaction product was seen along the surface of foot processes and relatively heavy deposits of reaction product were observed around slit diaphragms. mAb 5-1-6 was highly organ and species specific and did

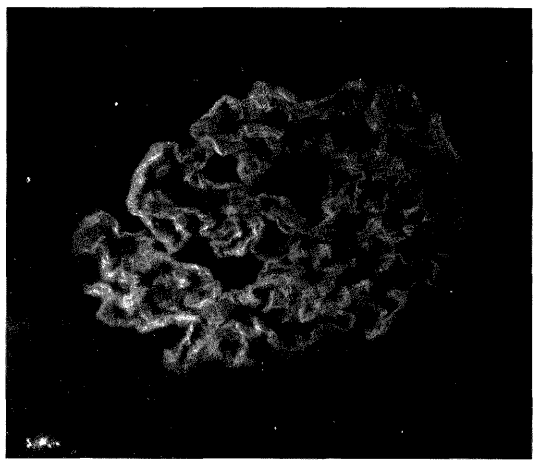
Table 1. Characteristics of proteinuria-inducing mAbs

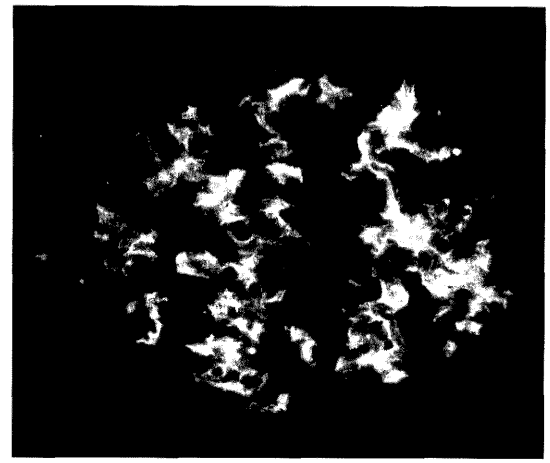
	mAb 5-1-6	mAb 1-22-3
reactive site:	kidney (glomerular epithelial cell)	kidney (mesangial cell) thymus brain intestine
subclass:	IgG1 (k chain)	IgG3 (k chain)
pI:	5.7	8.3
complement binding:	−	+

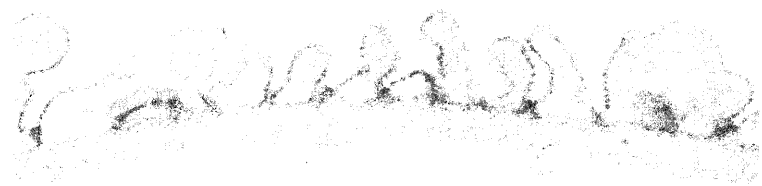
not bind to any of epitopes present in other rat tissues or cells examined. mAb 1-22-3 showed a fine granular *in vitro* binding pattern in the mesangial area by indirect IF, as shown in Fig. 3. It is shown at the ultrastructural level in Fig. 4. Reaction products were present on the limited mesangial cell surface facing the endothelial cell. Those were detected neither on endothelial cells, epithelial cells nor along GBM. Reactivity of this mAb toward thymus, brain and intestine, in addition to the kidney could be observed.

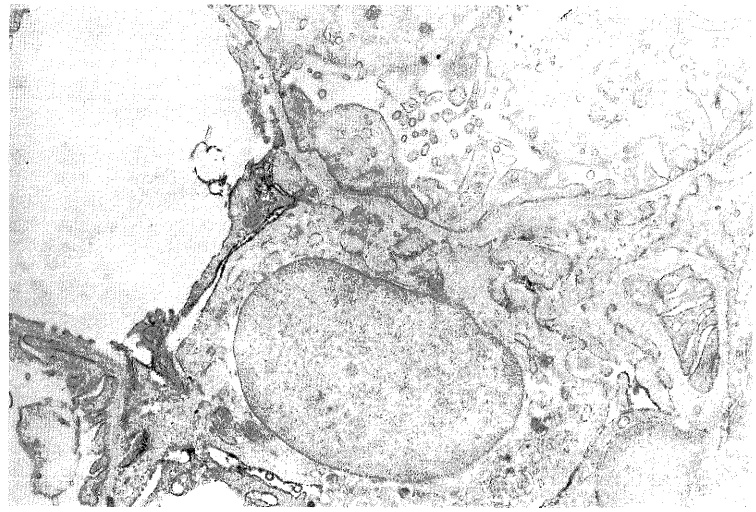
Animal experiment

mAb 5-1-6 Protein excretion started immediately following mAb injection and attained an average value of 138.5 mg/24 h on day 5. This was followed by a gradual decline, so that normalization was virtually resumed by day 15. Rat IgG and C3 could not be detected in glomeruli through the period observed. No histological abnormalities could be detected by light microscopy even on day 5 when proteinuria was most severe. No marked ultrastructural changes were evident except for partial retraction of glomerular epithelial foot processes as indicated by electron microscopy. The *in vivo* localization of administered mAb changed with time. Linear binding along the glomerular capillary wall was observed 1 h after injection by IF study. However, 2 days later, it partially shifted to a fine granular pattern. The linear pattern disappeared and the size as well as intensity









of fluorescent granules decreased on day 15. These results of animal experiments with mAb 5-1-6 were basically similar to those reported previously.[3] The amount of total kidney binding antibody (TKAb) 1 h after 2 mg injection was 50.8 ± 10.4 μg IgG/2 kidneys and TKAb declined to 1.9±0.4 at day 15. The minimum dose of mAb 5-1-6 required to induce abnormal proteinuria is 0.125 mg as injected dose. This dose corresponds to 12.8 μg of TKAb at 1 h and 0.34 μg of TKAb at day 5. An mAb 5-1-6 specific IF pattern was found in kidney section from rats 1 h after the injection of each dose of mAb. However, 5 days after injection, fluorescence decreased, becoming very faint with a 250 μg injection and a completely undetectable with a 125 μg injection. TKAb at 1 h for rats injected with 125 μg of mAb 5-1-6 was 12.8 μg. In contrast, 5 days after the administration of 2 mg mAb, it was only 4.65 μg, although the titre of remaining antibody in the blood was confirmed to exceed that in a rat 1 h after a 125 μg mAb injection. These data show that the number of the antigenic molecule recognized by mAb 5-1-6 decreases in glomeruli at 5 days after injection.

mAb 1-22-3 Abnormal proteinuria started immediately following injection of 500 μg of mAb 1-22-3 and peaked on day 5. All the animals showed mouse IgG in their mesangium at 30 min or 2 h after injection of mAb 1-22-3. Staining intensity decreased and 24 h after injection mouse IgG could no longer be detected. Rat C3 deposition was noted. IEM revealed that the binding pattern of *in vivo* administered mAb 1-22-3 after 30 min or 2 h was basically similar to that obtained *in vitro*. The reaction products were observed on the surface of the fragment of the altered mesangial cells. These were detected neither on endothelial cells, epithelial cells nor along GBM. In the early phase (30 min or 2 h after mAb 1-22-3 injection), the infiltration of polymorphonuclear cells was observed. Degenerative signs of mesangial cells were already very frequently observed in this stage. The mesangiolytic change, which was characterized by the large capillary dilatation and balooning of lumens filled plasma proteins, erythrocytes and leukocytes, occurred in almost all glomeruli in the second phase (24 or 48 h after injection). In the third phase (4 or 6 day after injection), nearly all the glomeruli showed an increased number of cells in the mesangial area. In a more advanced phase (2 or 4 wk after injection), cell proliferation in the mesangial area was still evident, and an increase of mesangial matrix was observed. Some glomeruli showed a crescentic change.

DISCUSSION

The detailed mechanism of proteinuria induced by these mAbs remains to be clarified. The abnormality induced by mAb 5-1-6 has been confirmed to occur without activation of the complement system, or participation of inflammatory cells, since neither rat C3, leukocytes nor macrophages could be detected in the glomeruli of the proteinuric rats. The mechanism of proteinuria induced by mAb 5-1-6 may be explained from our data as follows: 1) First, mAb reacts immediately with the surface of glomerular

epithelial cells at a very limited critical site, 2) antigenic molecules are metabolized together with Ab from the cell surface and then 3) proteinuria ensues from the disappearance of this molecule from the cell surface. Thus, the mAb 5-1-6 antigenic molecule itself may principally function to regulate the permeability of glomerular capillary wall, and may be the active site of receptors, an enzyme or a molecule involved in transcellular solute exchanges, as discussed by Mendrick et al. in their report on the mAb-induced proteinuric state.[7]

The morphological changes induced by mAb 1-22-3 are similar to those induced by ATS[8,9,10] or ER4.[11] Proteinuria is induced by ER4 due to reactivity with GBM, according to Bagchus et al.[11] The binding of mAb to GBM contribute to the increased permeability of GCW. Yamamoto et al. also described in the discussion section of their paper on antibody induced mesangial cell damage[8] such an explanation for the mechanism of proteinuria induced by ER4. mAb 1-22-3 did not bind to GBM and endothelial cells *in vivo* and *in vitro,* which indicated that the binding of mAb to limited sites on the mesangial cell surface can lead to increase of GCW permeability. This reaction at limited sites of the mesangial cell surface may trigger a chain reaction leading to proteinuria and mesangiolysis with subsequent cell proliferation and increase in mesangial matrix. The epitope recognized by mAb 1-22-3 appears to be a very critical site for maintaining the physiological constant stability of mesangial cells. Stimulation of this site by mAb binding may change the mesangial cell structure and function.

The minimum dose of mAb 5-1-6 required to induce abnormal proteinuria is 0.125 mg as injected dose. In our preliminary experiment, we obtained data that showed the minimum dose of mAb 1-22-3 required to induce proteinuria is 0.025 mg as injected dose. These amounts are smaller than the minimum dose required to induce nephrotoxic serum nephritis, passive Heymann nephritis or *in-situ* immune complex nephritis. These results also support the our hypothesis that the antigenic determinants recognized by these mAbs are the critical sites which regulate the permeability of GCW.

The clinical and morphological features of the disorder induced by mAb 5-1-6 resemble those of human minimal change-type nephropathy, the pathogenesis of which is still unknown. The abnormality induced by mAb 1-22-3 is considered to have some characteristics in common with the abnormal proteinuria with mesangial proliferative changes observed in human glomerulonephritis. The author is confident that the mAbs doccumented here will serve as a valuable means for clarifying the common mechanism of abnormal proteinuria of various etiologies.

Acknowledgments. This work was supported by research grant (01480163) from the Ministry of Education, Science and Culture and in part by a grant from the Ministry of Health and Welfare, Japan (1989).

Portions of this work were presented at the 1988 and 1989 annual meetings of the American Society of Nephrology and were published in abstract (Kidney Int. vol. 35, p. 351, 1989 and vol. 37, p. 418, 1990).

The author would like to express his gratitude to Prof. F. Shimizu, Niigata University, for his guidance and encouragement throughout this work, and Drs. T. Oite, M. Orikasa, K. Matsui for their helpful discussions. The author also thanks Mrs Takako Hioka for her excellent technical assistance.

REFERENCES

1) Masugi M: Über die experimentelle Glomerulonephritis durch das spezifische Antinierenserum. Ein Beitrag zur Pathogenese der diffusen Glomerulonephritis. *Beitr Path Anat Allg Pathol* **92**: 429, 1934.
2) Kerjaschki D, Farquhar MG: The pathogenic antigen of Heymann nephritis is a membrane glycoprotein of the renal proximal tubular brush border. *Proc Natl Acad Sci USA* **79**: 5557, 1982.
3) Shimizu F, Orikasa M, Sato K, Oite T: Monoclonal antibodies to rat renal antigens. *Immunology* **52**: 319, 1984.
4) Orikasa M, Matsui K, Oite T, Shimizu F: Massive proteinuria induced in rats by a single intravenous injection of a monoclonal antibody. *J Immunol* **141**: 807, 1988.
5) Kawachi H, Orikasa M, Matsui K, Morioka T, Oite T, Shimizu F: Induction of proteinuria and morphological changes in the rat by a monoclonal antibody 1-22-3. *Kidney Int* **37**: 418 (abstract), 1990.
6) Weichselbaum TE: An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *Amer J Clin Pathol* **10**: 40, 1946.
7) Mendrick DL, Rennke HG: II. Epitope specific induction of proteinuria by monoclonal antibodies. *Kidney Int* **33**: 831, 1988.
8) Yamamoto T, Wilson CB: Quantitative and qualitative studies of antibody-induced mesangial cell damage in the rat. *Kidney Int* **32**: 514, 1987.
9) Ishizaki M, Masuda Y, Fukuda Y, Sugisaki Y, Yamanaka N, Masugi Y: Experimental mesangioproliferative glomerulonephritis in rats induced by intravenous administration of anti-thymocyte serum. *Acta Pathol Jap* **36**: 1191, 1986.

10) Yamamoto T, Yamamoto K, Kawasaki K, Yaoita E, Shimizu F, Kihara I: Immunoelectron microscopic demonstration of Thy-1 antigen on the surfaces of mesangial cells in the rat glomerulus. *Nephron* **43**: 293, 1986.
11) Bagchus WM, Hoedemaeker PhJ, Rozing J, Bakker WW: Glomerulonephritis induced by monoclonal anti-Thy 1.1 antibodies. *Lab Invest* **55**: 680, 1986.