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Isolation and characterization of rat nephritogenic and non nephritogenic brush border antigens

H. KAWAI, S. YANO & T. NARUSE *The Third Department of Internal Medicine, Gunma University School of Medicine, Maebashi 371, Japan*

SUMMARY

Two kinds of brush border antigens were isolated from pronase-treated rat tubular material by gel filtration, DEAE-chromatography and disc-electrophoresis, successively. One, a 0·05 M antigen which was eluted from DEAE-column with 0·05 mol of NaCl solution, has no nephritogenic ability when inoculated into homologous rats. The other, a 0·30 M antigen eluted with 0·30 mol of NaCl solution, induces membranous nephritis when injected into rats. Immunoprecipitation studies show no common factor between these two antigens. SDS-polyacrylamide electrophoresis shows the molecular size of 0·05 M and 0·30 M antigens to be respectively over 200 kD and about 90 kD. Rabbit antiserum against the 0·05 M antigen fixed to the GBM in a diffuse granular fashion as well as to the brush border by immunofluorescence when incubated *in vitro* with normal rat kidney section. Rabbit antiserum to the 0·30 M antigen, however, fixed exclusively *in vitro* to the brush border. Passive transfer of nephritis was studied with these rabbit antisera. When antiserum to 0·05 M antigen was injected into normal rat, diffuse granular deposition of rabbit IgG was observed in the GBM within 2 h of the injection, but the deposits became negative 1 week later. Rats injected with antiserum to the 0·30 M antigen showed no glomerular deposition within 2 days but diffuse granular deposits of rabbit IgG were observed within 1 week and increased until 2 weeks after the injection. These facts should be considered in the studies on passive Heymann nephritis and its pathogenesis.

Keywords experimental glomerulonephritis experimental membranous nephritis immune complex-mediated nephritis brush border antigens Heymann nephritis

INTRODUCTION

Although others have not confirmed it (Zager *et al.*, 1979), nephritogenicity of renal tubular brush border antigen (Tub-Ag) has been well established in human glomerulonephritis (Naruse *et al.*, 1973; Strauss *et al.*, 1975; Douglas *et al.*, 1981; Ozawa *et al.*, 1976) and experimental membranous glomerulonephritis (Heymann nephritis) in rats (Edgington *et al.*, 1967; 1968). Heymann nephritis induced by the Tub-Ag–antibody complex has the unusual immunological characteristic feature of being an immune complex-mediated nephropathy, in that exclusive subepithelial deposition of immune complexes in the glomerular basement membrane (GBM) developed in animals with antibody excess but not in those with antigen excess (Grupe & Kaplan, 1969; Fleuren *et al.*, 1978; Naruse *et al.*, 1978). Many investigations on the pathogenesis of this experimental Heymann nephritis have been published, and recent studies on its passive form with heterologous antibody (Barabas & Lannigan, 1974; Van Damme *et al.*, 1978; Couser *et al.*, 1978) or homologous antibody

Correspondence: Takuji Naruse MD, The Third Department of Internal Medicine, Gunma University School of Medicine, Maebashi 371, Japan.

(Fleuren, Grond & Hoedemaeker, 1980; Makker & Moorthy, 1981; Neale & Wilson, 1982; Madio *et al.*, 1983) to rat Tub-Ag, as well as perfusion experiments (Van Damme *et al.*, 1978; Couser *et al.*, 1978; Makker & Moorthy, 1981) have indicated that direct binding of circulating anti-Tub-Ag antibody to glomerular fixed antigen could develop, that is, *in situ* immune complex formation in the GBM.

The results of isolation and characterization of the pathogenic Tub-Ag, on the other hand, have provided conflicting data regarding the molecular size and localization in the glomerulus (Edgington *et al.*, 1968; Naruse *et al.*, 1976; Miettinen *et al.*, 1980; Kerjaschki *et al.*, 1983; Makker & Singh, 1984; Ronco *et al.*, 1984).

We isolated two kinds of Tub-Ag differing in nephritogenic characters when injected into homologous rats and in their ability to produce antibody when injected into heterologous rabbits (Kawai & Naruse, 1984). The characterization of these two antigens and passive transfer of nephritis with heterologous antibodies against them are reported here in detail.

MATERIALS AND METHODS

Solubilization of Tub-Ag. Tubular segments of rat kidney were prepared by a modification (Naruse *et al.*, 1976) of the method originally described by Edgington *et al.* (1967). Tub-Ag was solubilized from the segments by pronase digestion and then separated through gel filtration on a Bio-Gel A-1·5 m column as previously described (Naruse *et al.*, 1976), and a protein peak with potent nephritogenic activity was obtained.

DEAE-chromatography. Sephadex A-25 column with approximately 70 ml of bed volume was equilibrated with 0·005 M phosphate buffer, pH 7·0. Nephritogenic material obtained from gel filtration which was predialysed in the same buffer for 48 h with three renewals of the dialysing buffer, was applied on the column and washed with 100 ml of the same buffer. A step-wise salt elution method was performed. One hundred millilitres of the phosphate buffer containing respectively 0·05 M, 0·10 M, 0·20 M, 0·30 M, 0·40 M and 1·0 M sodium chloride, were used as the eluting solutions.

Eluates were automatically monitored by absorbance at 280 nm, and each peak was concentrated by negative pressure ultrafiltration and designated as 0·05 M, 0·10 M, 0·20 M, 0·30 M, 0·40 M and 1·0 M fractions, respectively.

Polyacrylamide disc-electrophoresis. For the further separation of Tub-Ag, polyacrylamide disc-electrophoresis was performed according to the method of Davis (1964). Gel column, each containing 7·5% of polyacrylamide, 8 × 75 mm were used. After electrophoresis the columns were cut with a razor to separate the protein bands relevant to a particular gel, and this was stained with Coomassie Blue R. The cut gels with identical locations were suspended in physiological saline and homogenized. After refrigeration overnight, the gel homogenate was filtered and the process was repeated once more. The filtrate was passed through a millipore filter of 0·45 μm pore size and concentrated with negative pressure utrafiltration. Separation of the protein bands in each fraction was again checked by electrophoresis.

SDS-polyacrylamide disc-electrophoresis was performed using the method of Neville (1971) in 7·5% polyacrylamide gel. For standards of molecular size, a mixture of ovalbumin, bovine serum albumin, phosphorylase B, β-galactosidase and myoglobulin (from Bio-Rad Co.) was used.

Evaluation of immunogenic activity of the separated antigens. Wistar male rats, weighing 150 g, were injected in the rear footpads with each material which was emulsified with the same volume of complete Freund's adjuvant. Protein excretions in urine were estimated weekly until 12 weeks after the inoculation by the method of Kingsbury *et al.* (1926). Rats were killed 12 weeks after the inoculation and immunofluorescent, light microscopic and electron microscopic studies of the renal tissues were performed as previously reported (Naruse *et al.*, 1976).

Albino rabbits, weighing about 3 kg, were immunized with each antigenic material emulsified with the same volume of Freund's complete adjuvant, and then one-fifth of the initial amount of antigen without adjuvant was given subcutaneously as a booster injection 4 weeks after the initial injection. The animals were bled 1 week after the booster injection and the antisera were collected.

Induction of passive Heymann nephritis. Wistar male rats, weighing 150 g, were injected intraperitoneally with 2 to 3 ml of the antiserum. Kidneys from the injected rats were examined on fixing the rabbit antibody to renal tissues by immunofluorescent and electron microscopy 2 h, 24 h, 3 days, 5 days, 1 week, 2 weeks, 3 weeks and 4 weeks sequentially after the antiserum injection. The rats were bled each time, and were examined for the presence of the injected rabbit antibody in their circulation by the indirect immunofluorescent method on frozen normal kidney sections.

Localization of the isolated antigens in the renal tissues. Cryostat kidney sections from normal rat, 4 μm thick, were incubated first with rabbit antiserum to the separated antigen and then stained with FITC-labelled anti-rabbit IgG reagent.

For electron microscopic immunoperoxidase study, rat kidney was fixed by perfusion for 5 min with Karnovsky's fixative. Forty to 50 μm Vibratome sections were prepared for the peroxidase-anti-peroxidase (PAP) method (Sternberger *et al.*, 1970). Sections were incubated in the first antibody to rat Tub-Ag for 48 h, after which they were washed with PBS containing 0·4% of Triton X and incubated in the second antibody to rabbit IgG for 2 h. They were then incubated in PAP complex for 3 h and further incubated in 0·05% diaminobenzidine in 0·01% hydrogen peroxide in PBS for 3 min. The sections were washed and fixed in 1% of OsO_4. Ultrathin sections were examined by electron microscopy.

RESULTS

The elution pattern of the nephritogenic material from gel filtration in DEAE-chromatography is shown in Fig. 1. The absorbance at 280 nm was found high at the first peak, the 0·05 M fraction, which was eluted with buffer containing 0·05 M of sodium chloride and accounted for 62·4% of the total absorbance of eluates. Subsequent peaks decreased in absorbance despite an increase in the salt concentration of the eluting buffer. For the evaluation of the nephritogenic activity of these fractions, eight rats were injected respectively with these fractions emulsified with adjuvant.

All rats injected with the 0·30 M fraction developed severe membranous glomerulonephritis and brush border-fixing antibody in their blood. Some rats injected with the 0·20 M fraction also developed nephritis, but none developed experimental membranous nephritis, pathological proteinuria or brush border-fixing antibody in their blood with injections of the other fractions, even in large amounts.

Production of heterologous antiserum to each fraction from DEAE-chromatography is studied. All six fractions could induce antibodies in rabbits against brush border. The 0·05 M fraction, which has no ability to induce membranous nephritis or kidney-fixing antibody in homologous rats, however, could produce antibody which could fix on the GBM as well as brush border.

When normal rat kidney cryostat section was incubated with rabbit antiserum to the 0·05 M fraction and then with FITC-labelled anti-rabbit IgG reagent, fluorescence with a diffuse granular

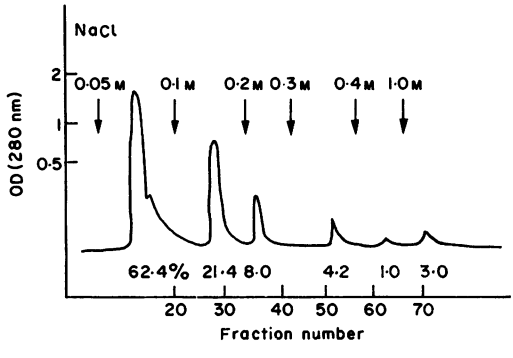

Fig. 1. Elution pattern of the nephritogenic brush border fraction from gel filtration in DEAE-chromatography

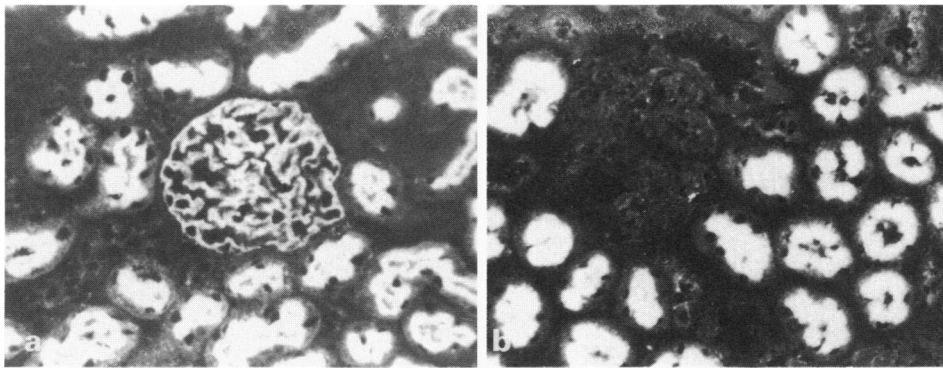

Fig. 2. Normal rat kidney cryostat section incubated with rabbit antisera to the 0·05 M antigen (A) and to the 0·30 M antigen (B) and then stained with FITC-labelled anti-rabbit IgG reagent. (A) Antibody to the 0·05 M antigen fixed on the glomerular capillary walls as well as the luminal layer of the proximal tubules. (B) With antibody to the 0·30 M antigen, the luminal layer of the proximal tubules was exclusively stained (× 300).

pattern was found on the GBM as well as on the brush border (Fig. 2A). On the contrary, rabbit antiserum to the 0·30 M fraction, which has a potent nephritogenic activity in rats, fixed exclusively on the luminal layer of the proximal tubules (Fig. 2B).

These two fractions from DEAE-chromatography, 0·05 M and 0·30 M fractions, were further purified by disc-electrophoresis. These fractions were separated in polyacrylamide gels into one main and several small protein bands, respectively. The gels were cut and each protein band was isolated. Nephritogenic activity and production of heterologous antiserum in rabbits were studied on each band as was done on the DEAE-fractions. Two brush border antigens, designated as 0·05 M and 0·30 M antigens, with the same immunological properties as each starting material but with a single band in SDS-electrophoresis (Fig. 4) were isolated. It is primarily these two characteristic antigens which are studied in the following experiments.

Immunoprecipitation test. A double diffusion immunoprecipitation test of these antisera was performed in 1·5% agar plates. There were no precipitation lines common to 0·05 M and 0·30 M antibodies, suggesting that they have different antigenic epitopes.

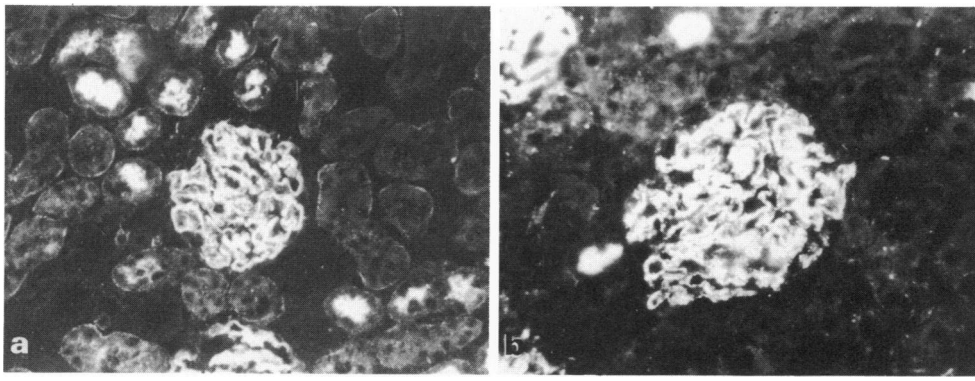

Fig. 3. (A) Frozen kidney section from rats injected with rabbit antiserum to 0·05 M antigen and killed 24 h after the injection, stained with the same reagent as in Fig. 2. Diffuse granular deposition of the injected rabbit antibody is seen along the GBM and the luminal layer of some proximal tubules is also stained. (× 300). (B) Frozen kidney section from rats injected with rabbit antiserum to 0·30 M antigen and killed 7 days after the injection, stained with the same reagent as in Fig. 2. Diffuse granular deposition of rabbit antibody is seen along the GBM. Some tubules are also stained (× 300).

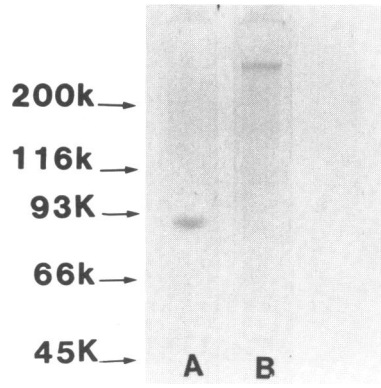

Fig. 4. SDS-polyacrylamide disc electrophoresis of 0·30 M (A) and 0·05 M (B) antigens. The numbers on the left show the molecular weight markers as kD.

SDS-polyacrylamide disc-electrophoresis. The molecular sizes of these 0·05 M and 0·30 M antigens were estimated by SDS-polyacrylamide disc-electrophoresis. As shown in Fig. 4, the molecular size of 0·05 M antigen was over 200 kD, whereas that of 0·30 M antigen was approximately 90 kD.

Passive Heymann nephritis. Two millilitres of rabbit antiserum against 0·05 M or 0·30 M antigen were injected intraperitoneally into normal rats. Table 1 shows the results of the glomerular deposition and amount of the injected rabbit antibody remaining in circulation at various intervals after the injection.

With the injection of anti-0·05 M antigen serum to a normal rat, diffuse granular deposition of rabbit IgG was observed in the GBM, even 2 h after the injection, suggesting *in situ* immune

Table 1. Passive Heymann nephritis with rabbit antisera to 0·05 M and 0·30 M antigens

Time after injection	Granular deposition of injected rabbit IgG in the GBM		Presence of injected rabbit antibody in circulation	
	Anti-0·05 M	Anti-0·30 M	Anti-0·05 M	Anti-0·30 M
2 h	++ (13)*	− (9)*		
24 h	++ (9)	− (7)	$\times 2^{6-7}$ (3)*	$\times 2^{7}$ (3)*
3 days	+ (4)	± (7)	$\times 2^{2}$ (3)	$\times 2^{6-7}$ (3)
5 days	± (5)	± (3)		$\times 2^{6}$ (3)
7 days	− (12)	+ (13)	Negative (3)	$\times 2^{5}$ (3)
14 days	− (3)	++ (16)		$\times 2^{3}$ (3)
21 days	− (3)	++ (13)		Negative (3)
28 days		+ (4)		

* Numbers in parenthesis are number of rats studied.

† Titre of antibody remaining in circulation is presented with maximum serum dilutions which show positive staining of brush border by indirect immunofluorescence. The staining titres of original antiserum were $\times 2^{11}$ in anti-0·05 M and $\times 2^{12}$ in anti-0·30 M antisera.

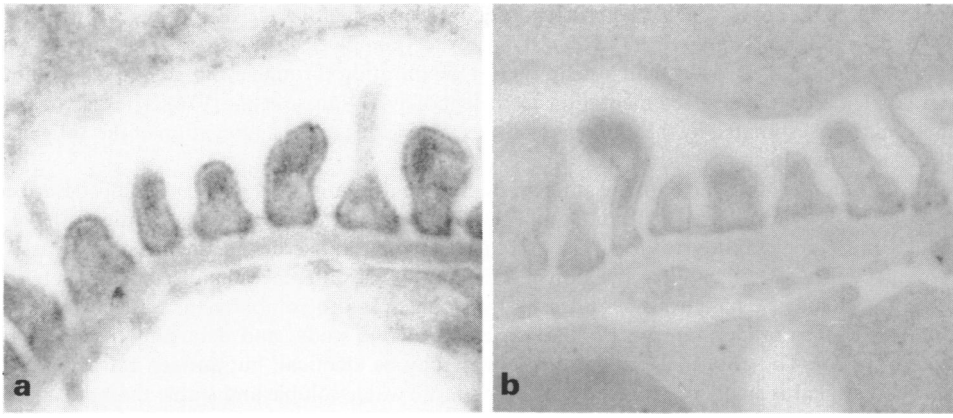

Fig. 5. Indirect immunoperoxidase staining of normal rat kidney sections using rabbit antisera against 0·05 M antigen (A), and 0·30 M antigen (B) as the first antibody, respectively. (A): With anti-0·05 M antigen antiserum, the cell membrane and the base of the footprocesses are stained. (A: ×36,000). (B): With anti-0·30 M antigen antiserum, the GBM and the footprocesses are not stained (B: ×36,000).

complex formation in the GBM. Electron microscopy, however, showed no electron dense deposits in the GBM. The luminal layer of the proximal tubules was also stained with anti-rabbit IgG reagent and transient proteinuria developed 24 h after the antiserum injection (Fig. 3A). The rabbit IgG deposition in the GBM decreased with time and could hardly be found 1 week after injection.

On the contrary, rats injected with anti-0·30 M antigen serum did not develop rabbit IgG deposition in the GBM until 3 days after the injection. Definite rabbit IgG deposition in the GBM appeared 7 days after the injection (Fig. 3b) and increased thereafter. The luminal layer of some proximal tubules was also stained. A faint but significant proteinuria developed in rats 10 to 14 days after the injection. Sub-epithelial electron-dense deposits in the GBM were also observed in these kidney sections, indicating the development of passive Heymann nephritis. Autologous rat IgG deposition was detected along the GBM 2 weeks after the antiserum injection.

Rabbit antibody to 0·05 M antigen could be detected in relatively high concentrations in the circulation 24 h after the injection, but had almost disappeared 1 week later, while antibody to 0·30 M antigen remained high in concentration 1 week after the injection and was gone 3 weeks thereafter (Table 1).

Localization of the 0·05 M and 0·30 M antigens in the glomerulus. Antibody to 0·05 M antigen fixed to the glomerular capillary walls and luminal layer of proximal tubules of normal rat kidney cryostat section by immunofluorescent microscopy. The antibody fixed on the cell membranes of the epithelial foot processes and also at the base of the foot processes in the GBM by immunoperoxidase electron microscopy as shown in Fig. 5a. Antibody to 0·30 M antigen fixed exclusively to the luminal layer of the proximal tubules by immunofluorescent study. No definite glomerular fixation of the antibody was observed even by immunoelectron microscopy (Fig. 5b).

DISCUSSION

Heymann nephritis is an experimental membranous glomerulonephritis with glomerular subepithelial immune deposits induced by the autologous antibody against renal brush border antigens (Edgington *et al.*, 1967; 1968).

Characterization of the pathogenic brush border antigen and its localization in the glomerulus have been extensively studied. The isolated nephritogenic brush border antigens are described as glycoproteins with a molecular size of over 28*s* (Edgington *et al.*, 1968), 8·4*s* (Naruse *et al.*, 1976), 600 kD (Makker & Singh, 1984), 330 kD (Kerjaschki & Farquhar, 1983) and 80–200 kD (Miettinen *et al.*, 1980), respectively.

We isolated the nephritogenic rat brush border antigen designated as 0·30 M antigen which has a molecular size of approximately 90 kD by SDS-polyacrylamide electrophoresis. This antigen was previously eluted a little faster than human IgG by gel filtration through a Bio-gel A 1·5 M column and was calculated to have a sedimentation coefficient of 8·4s (Naruse *et al.*, 1976). The discrepancy in molecular size estimated by the different methods may be due to the aggregation of the antigen in the eluting buffer during gel filtration.

The immunogenic character of the antigen is similar to the antigen of gp330 originally described by Kerjaschki & Farquhar (1983) and confirmed by Ronco *et al.* (1984); these antigens have a potent nephritogenic ability to homologous rats and rabbit antiserum to them induced passive Heymann nephritis 3–5 days after injection in normal rats. The difference in molecular size between these antigens may be based on the different procedures used to solubilize the antigen from the brush border membrane, namely, pronase digestion in our study, and detergent treatment by Kerjaschki *et al.* The antigenic site of each moiety may be identical, but antigen extracted with pronase treatment is smaller in molecular size and more water-soluble and stable than the gp330.

The localization of the 0·30 M antigen in normal glomerulus is not demonstrated by either immunoperoxidase electron microscopy or immunofluorescence. This finding differs from those reported by others that nephritogenic brush border antigens localize in the GBM (Van Damme *et al.*, 1978), the subepithelial aspect of the GBM (Couser *et al.*, 1978; Fleuren, Grond & Hoedemaeker, 1980), or the surface of the epithelial cells (Kerjaschki & Farquhar, 1984). Differences in localization of the nephritogenic antigen may be the result of differences of rat strains or antigens used, or technical problems.

If present at all, the nephritogenic brush border antigen occurs only a very small amount in the normal rat glomerulus according to our study, a result which contradicts the theory of *in situ* immune complex formation in the GBM.

We have isolated another brush border antigen designated as 0·05 M antigen which has a molecular size of over 200 kD, does not share a common antigenic site with the 0·30 M antigen by immunodiffusion test and has no nephritogenic ability when injected to homologous rats, yet produces antibody when inoculated into rabbits. The rabbit antiserum to 0·05 M antigen reacts to the GBM in a diffuse granular fashion as well as brush border of normal frozen kidney section, suggesting that these tissues share the same antigen. Immunoelectron microscopy showed the antigen to be localized at cell surface of foot processes and the subepithelial aspect of the GBM; this finding is similar to those described by others (Couser *et al.*, 1978; Fleuren, Grond & Hoedemaeker, 1980).

Passive transfer of Heymann nephritis has been studied with both heterologous and homologous antibodies to the brush border antigens (Barabas & Lannigan, 1974; Van Damme *et al.*, 1978; Couser *et al.*, 1978; Fleuren, Grond & Hoedemaeker, 1980; Makker & Moorthy, 1981; Neale & Wilson, 1982; Madio *et al.*, 1983).

Passive transfer was further tried by blood-free perfusion with heterologous antibody and succeeded within 1 h after the injection (Van Damme *et al.*, 1978).

Couser *et al.* (1978) also perfused isolated normal rat kidney without antigen and with heterologous sheep antiserum to Fx-1A, and induced granular deposition of the sheep IgG in the GBM 10 min after addition of antibody to the perfusate.

From these results obtained in passive Heymann nephritis, it is now believed that circulating anti-brush border antibody reacts directly with the antigen present in the GBM, forming *in situ* immune complex formation in the GBM.

Passive Heymann nephritis was studied using two kinds of heterologous antisera to 0·05 M and 0·30 M antigens. Following injection of rabbit antiserum to 0·30 M antigen into normal rat, glomerular subepithelial deposition of rabbit IgG appeared 3–5 days after the injection although it was not seen during the first 2 days. The deposits in the GBM increased for 14 days in the presence of injected rabbit antibody in circulation, and then decreased when autologous rat antibody to the injected rabbit antiserum quickly eliminated the injected rabbit antibody. This finding is quite different from those reported by others (Van Damme *et al.*, 1978; Couser *et al.*, 1978).

On the contrary, with the injection of rabbit antiserum to 0·05 M antigen which reacted with the GBM as well as the brush border of normal frozen kidney sections *in vitro*, glomerular deposition of

rabbit IgG appeared within 2 h and became faint 7 days after the injection. Furthermore, injected rabbit antibody fixed on the luminal layer of the proximal tubules 24 h after the injection, and moderate proteinuria was observed. The serum level of rabbit antibody decreased quickly and had almost disappeared 1 week thereafter. These facts might indicate that injected rabbit antibody deposited in the GBM within 2 h and increased its permeability within 24 h thereafter, and that therefore rat serum protein, containing rabbit antibody, passed through the GBM and the excreted rabbit IgG fixed to the brush border. Injected rabbit antibody would be eliminated promptly into urine, subsequently deposits of rabbit IgG in the GBM disappeared within 1 week after the injection.

Passive transfer of nephritis with rabbit antibody to 0·05 M antigen is similar to that reported by others using anti-Fx1A heterologous antiserum (Van Damme *et al.*, 1978; Couser *et al.*, 1978). It is possible that previously reported passive Heymann nephritis, the antisera used contained antibody to non nephritogenic antigen in rats such as 0·05 M antigen, since kidney brush border antigens extracted by detergent contained many autoantigens, only one of which had a possibly nephritogenic ability (Edgington, Glassock & Dixon, 1968).

The possibility exists that a pathomechanism in addition to the *in situ* immune complex formation may play a role in the development of active Heymann nephritis, since the 0·30 M antigen although not present in the glomerulus in our study, can induce experimental membranous nephritis.

REFERENCES

BARABAS, A.Z. & LANNIGAN, R. (1974) Induction of an autologous immune complex glomerulonephritis in the rat by intravenous injection of heterologous anti-rat kidney tubular antibody: I. Production of chronic progressive immune complex glomerulonephritis. *Br. J. exp. Pathol.* **55,** 47.

COUSER, W.G., STEINMULLER, D.R., STILMANT, M.M., SALANT, D.J. & LOWENSTEIN L.M. (1978) Experimental glomerulonephritis in the isolated perfused rat kidney. *J. clin. Invest.* **62,** 1276.

DAVIS, B.J. (1964) Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* **121,** 404.

DOUGLAS, M.F.S., RABIDEAU, D.P., SCHWARTZ, M.M. & LEWIS, E.J. (1981) Evidence of autologous immune-complex nephritis. *N. Engl. J. Med.* **305,** 1326.

EDGINGTON, T.S., GLASSOCK, R.J., WATSON, J.I. & DIXON, F.J. (1967) Characterization and isolation of specific renal tubular epithelial antigens. *J. Immunol.* **99,** 1199.

EDGINGTON, T.S., GLASSOCK, R.J. & DIXON, F.J. (1968) Autologous immune complex nephritis induced with renal tubular antigen. I. Identification and isolation of the pathogenic antigen. *J. exp. Med.* **127,** 555.

FLEUREN, G.J., LEE, R.V.D., GREBEN, H.A., VAN DAMME, B.J.C. & HOEDEMAEKER, PH.J. (1978) Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens. IV. Investigations into the pathogenesis of the model. *Lab Invest.* **38,** 496.

FLEUREN, G.J., GROND, J. & HOEDEMAEKER, PH.J. (1980) The pathogenic role of free-circulating antibody in autologous immune complex glomerulonephritis. *Clin. exp. Immunol.* **41,** 205.

GRUPE, W.E. & KAPLAN, M.H. (1969) Demonstration of an antibody to proximal tubular antigen in the pathogenesis of experimental autoimmune nephritis in the rat. *J. Lab. clin. Med.* **74,** 400.

KAWAI, H. & NARUSE, T. (1984) Heterogeneity of rat tubular brush border antigens, in *Abstracts of IXth International Congress of Nephrology*. Los Angeles, p246A.

KERJASCHKI, D. & FARQUHAR, M.G. (1983) Immunocytochemical localization of the Heymann nephritis antigen (gp330) in glomerular epithelial cells of normal lewis rats. *J. exp. Med.* **157,** 667.

KINGSBURY, F.B., CLARK, C.C., WILLIAMS, G. & POST, A.L. (1926) The rapid determination of albumin in urine. *J. Lab. clin. Med.* **11,** 981.

MADIO, M.P., SALANT, D.J., COHEN, A.J., ADLER, S. & COUSER, W.G. (1983) Comparative study on *in situ* immune deposit formation in active and passive Heymann nephritis. *Kidney Int.* **23,** 498.

MAKKER, S.P. & MOORTHY, B. (1981) *In situ* immune complex formation in isolated perfused kidney using homologous antibody. *Lab. Invest.* **44,** 1.

MAKKER, S.P. & SINGH, A.K. (1984) Characterization of the antigen (gp 600) of Heymann nephritis. *Lab Invest.* **50,** 287.

MIETTINEN, A., TOERNROTH, T., TIKKANEN, I., VIRTANEN, I. & LINDER, E. (1980) Heymann nephritis induced by kidney brush border glycoproteins. *Lab. Invest.* **43,** 547.

NARUSE, T., KITAMURA, K., MIYAKAWA, Y. & SHIBATA, S. (1973) Deposition of renal tubular epithelial antigen along the glomerular capillary walls of patients with membranous glomerulonephritis. *J. Immunol.* **110,** 1163.

NARUSE, T., FUKASAWA, T., HIROKAWA, N., OIKE, S. & MIYAKAWA, Y. (1976) The pathogenesis of experimental membranous glomerulonephritis induced with homologous nephritogenic tubular antigen. *J. Exp. Med.* **114,** 1347.

NARUSE, T., FUKASAWA, T., UMEGAE, S., OIKE, S. &

MIYAKAWA, Y. (1978) Experimental membranous glomerulonephritis in rats. Correlation of ultrastructural changes with the serum level of autologous antibody against tubular antigen. *Lab. Invest.* **39,** 120.

NEALE, T.J. & WILSON, C.B. (1982) Glomerular antigen in Heymann's nephritis: reactivity of eluted and circulating antibody. *J. Immunol.* **128,** 323.

NEVILLE, D.M. JR (1971) Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. *J. Biol. Chem.* **246,** 6328.

OZAWA, T., BOEDECKER, E.A., SCHORR, W., GUGGENHEIM, S. & MCINTOSH, R.M. (1976) Immune-complex disease with unilateral renal vein thrombosis. *Arch. Pathol. Lab. Med.* **100,** 279.

RONCO, P., ALLEGRI, L., MELCION, C., PIROTSKY, E., APPAY, M.D., BARIETY, J., PONTILLON, F. & VERROUST, P. (1984) A monoclonal antibody to brush border and passive Heymann nephritis. *Clin. exp. Immunol.* **55,** 319.

STERNBERGER, L.A., HARDY, P.H. JR, CUCULIS, J.J. & MEYER, H.G. (1970) The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. *J. Histochem. Cytochem.* **18,** 315.

STRAUSS, J., PARDO, V., KOSS, M.N., GRISWOLD, W., MCINTOSH, R.M. (1975) Nephropathy associated with sickle cell anemia: an autologous immune complex nephritis. I. Studies of the nature of the glomerular bound antibody and antigen identification in a patient with sickle cell disease and immune deposit glomerulonophritis. *Am. J. Med.* **58,** 382.

VAN DAMME, B.J.C., FLEUREN, G.J., BAKKER, W.W., VERNEIR, R.L. & HOEDEMAEKER, PH.J. (1978) Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens: IV. Fixed glomerular antigens in the pathogenesis of heterologous immune complex glomerulonephritis. *Lab. Invest.* **38,** 502.

ZAGER, R.A., COUSER, W.G., ANDREWS, B.S., BOLTON, W.K. & POHL, M.A. Membranous nephropathy: a radioimmunologic search for anti-renal tubular epithelial antibodies and circulating immune complexes. *Nephron* **24,** 10.