THE PATHOGENESIS OF EXPERIMENTAL MEMBRANOUS GLOMERULONEPHRITIS INDUCED WITH HOMOLOGOUS NEPHRITOGENIC TUBULAR ANTIGEN*

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We have previously reported on patients with idiopathic membranous glomerulonephritis considered to be mediated by renal tubular epithelial antigen. Immunoglobulins and β1c, as well as the antigen that had antigenic determinants in common with the brush border of proximal tubular epithelia, were found in immune deposits along glomerular capillary walls by a fluorescent antibody technique (1, 2). The "nephritogenic" tubular antigen (Tub-Ag)[1] was purified after it had been solubilized from kidney tissue by digestion with a potent proteolytic enzyme, Pronase, and utilizing radiolabeled Tub-Ag, a sensitive radioimmunoassay was developed that enabled us to identify Tub-Ag activity in the serum, as well as in various organs of humans (3).

These findings prompted us to purify Tub-Ag of the rat with the same procedure employed to isolate the Tub-Ag of humans, in order to develop a laboratory model of membranous glomerulonephritis mediated by Tub-Ag-antibody complexes. The rats that received a single injection of partially purified Tub-Ag preparation with adjuvant developed a typical membranous glomerulonephritis with deposition of homologous immunoglobulin and Tub-Ag along the glomerular capillary walls (4).

In this paper, we report the distribution of rat Tub-Ag in the serum and various organs, and also, changes in the level of Tub-Ag and the appearance of antibodies against Tub-Ag in the serum of the rats with Tub-Ag-mediated membranous glomerulonephritis.

Materials and Methods

Solubilization and Isolation of Tub-Ag from the Kidney. Renal tubular epithelial segments were prepared from 100 *g* of cortices by a modified method (4) originally described by Edgington et al. (5). They were suspended in 60 ml of Tris-HCl buffer (0.02 M, pH 7.8) containing 0.13 M of NaCl, and digested by Pronase (0.5 mg/ml; *Streptomyces griseus* protease; Kaken Chemical Co., Tokyo) at 37°C for 12 h. The supernatant was concentrated to 3 ml by negative pressure ultrafiltration. 1 ml of concentrated sample containing Tub-Ag was applied to a Bio-Gel A-1.5 M column (bed

* This work was supported in part by a grant from the Japanese Ministry of Education.

[1] *Abbreviations used in this paper:* FITC, fluorescein isothiocyanate; RIA, radioimmunoassay; Tub-Ag, nephritogenic tubular epithelial antigen.

volume 110 ml; Bio-Rad Laboratories, Richmond, Calif.) and the elution was performed under a constant flow of 5 ml/h with the Tris-HCl buffer in the cold. Tub-Ag was recovered in a peak of optical density at 280 nm (OD_{280}), which appeared ahead of the position of γ-globulin (4). The fractions containing Tub-Ag were pooled and concentrated.

Tub-Ag was further purified by disc electrophoresis in acrylamide gel in a Pyrex glass tube (5 × 70 mm), according to the method of Davis (6). The partially purified Tub-Ag in buffer containing 40% (wt/vol) of sucrose was applied on spacer gel (2.5% acrylamide), layered on separating gel (7.5% acrylamide). A current of 4 mA/tube was applied at room temperature for 1 h. Bromphenol blue (50 μl, 0.01%) was added to the upper well as an indicator of migration. 10 tubes were run at a time, and one of them was fixed and stained in a solution of acetic acid (7%) containing 1% amido black 10B. By referring to the staining pattern of the gel, unstained gel columns were cut. The corresponding sections were pooled and extracted by an aliquot of physiological saline by homogenizing with a glass homogenizer and then centrifuging at 100,000 *g* for 30 min. The supernatant was concentrated by negative pressure ultrafiltration.

Solubilization of Tub-Ag from Tissues. Tissues of kidney, liver, lung, spleen, intestine, stomach, and heart were obtained from normal rats of Wistar strain. 20 g each of tissue were cut into small pieces with scissors, washed free of blood with sterile physiological saline, suspended in 60 ml of Tris-HCl buffer (Tris 0.02 M, NaCl 0.13 M, pH 7.8), and homogenized in a blender. 30 mg Pronase was added to the tissue homogenate and incubated at 37°C for 12 h with shaking. The Pronase digest was then centrifuged at 100,000 *g* for 60 min. The supernatant was collected and concentrated to 3 ml by negative pressure ultrafiltration.

Gel Filtration and Column Electrophoresis. 1 ml of the concentrated sample containing Tub-Ag was applied to a column of Bio-Gel A-1.5 M (bed volume 110) preequilibrated with Tris buffer, and 1-ml fractions were collected by eluting at a flow rate of 5 ml/h. Electrophoresis was carried out in a column of Bio-Gel P2 (bed volume 74 ml) in Tris-HCl buffer (Γ/2 0.05, pH 8.6, at 4°C). After 0.6 ml of the sample was applied, the column was eluted with 4 ml of the same buffer. A current of 3 mA/cm^2 was applied in the cold, and the column was eluted with the buffer at a flow rate of 2 ml/h.

Analytical Ultracentrifugation. Sedimentation coefficients were determined in a Spinco Model E analytical ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) spinning at 60,067 rpm and 20°C. Tris-HCl buffer, 0.02 M, pH 7.8, containing 0.13 M NaCl was used as a solvent. Observed sedimentation velocities were corrected for the viscosity and density of the buffer ($s_{20,w}$), but were not extrapolated to zero concentration.

Radioimmunassay of Tub-Ag and Anti-Tub-Ag. The Tub-Ag preparation, purified by acrylamide gel electrophoresis, was labeled with ^{125}I at a sp act of 10 μCi/μg (estimating the $E^{1\%}_{1\,cm,\,280\,nm}$ value at 10.0) by the chloramine-T method (7). Test antigen in 50 μl of buffer (Tris 0.02 M, NaCl 0.13 M, bovine serum albumin 200 μg/ml, NaN_3 0.1%, pH 8.0) was incubated at room temperature for 1 h with 5 μl of the same buffer containing 0.01 μl of rabbit antiserum against purified Tub-Ag plus 5 μl of normal rabbit serum. Then, 5 ng of ^{125}I-labeled Tub-Ag in 10 μl of the buffer was added and incubated further at 4°C for 18 h. 50 μl of goat anti-rabbit γ-globulin antiserum were added to each tube, the resulting precipitate was washed twice with 2.5 ml of Tris-HCl buffer (Tris 0.02 M, NaCl 0.13 M, pH 8.0), and the radioactivity was counted in a γ-counter. The control was set up without antigen, and percent inhibition [1 − (cpm of the sample/cpm of the control)] × 100, was calculated. Usually, three or more points of twofold antigen dilution were assayed, so as to contain 50% inhibition between two points. The amount to induce 50% inhibition was defined as 1 radioimmunoassay (RIA) U of Tub-Ag. Tub-Ag activity in column fractions was compared by determining the percent inhibition of an aliquot of samples, and the position of peak activity on column chromatographies was determined. Anti-Tub-Ag in the rat serum was determined by the following method. 5 μl of rat serum was incubated with 5 ng of ^{125}I-labeled Tub-Ag in 10 μl of buffer, and incubated at 4°C for 18 h. Then, 50 μl of rabbit anti-rat γ-globulin antiserum was added and the resulting precipitate was washed twice with Tris-HCl buffer, and the percent binding ([cpm in precipitate/total cpm added] × 100) was calculated.

Antisera. Male albino rabbits weighing 2.5–3.0 kg were immunized. Each received 1 OD_{280} U of the sample emulsified in Freund's incomplete adjuvant in footpads and intracutaneously; 1 OD_{280} U was defined as the amount of the material contained in 1 ml of the solution that exhibited an optical density at 280 nm of 1.0. 4 wk later, a booster injection was given with 0.2 OD_{280} U of the antigen. 10 days after the booster injection, rabbits were bled and sera were harvested and stored at −20°C until used.

Bioassay for Nephritogenicity. Male rats of Wistar strain weighing 150 g were used for the assay of nephritogenic activity of isolated antigens. Fractions containing antigen were emulsified in Freund's complete adjuvant. Each rat received 0.2 ml of emulsion containing 0.1 OD_{280} U of the sample in rear footpads. Urinary protein was determined by the method of Kingsbury et al. (8), with human albumin as a standard. Proteinuria of 200 mg/100 ml or more was considered as pathologic; control rats immunized with saline emulsified in adjuvant passes less than 100 mg/100 ml of protein into their urine. Blood samples were taken by venipuncture, and sera were collected. Kidneys were removed and examined morphologically and by fluorescent antibody technique. Renal tissues were fixed in 10% formalin and embedded in paraffin, cut at 2 μm thick, and stained with hematoxylin and eosin, periodic acid-Schiff, and periodic acid methenamine silver.

For immunofluorescent studies, the γ-globulin fraction of the antiserum was isolated by 33% ammonium sulfate precipitation method, and conjugated with fluorescein isothiocyanate (FITC) by the method of Wood et al. (9). Excess FITC was removed by passing the conjugate through a column of Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, N. J.) preequilibrated with phosphate buffer (0.005 M, pH 7.0) containing 0.1 M NaCl. The conjugate was further purified by a column of DEAE-cellulose, equilibrated with the phosphate buffer. Antiserum to rat γ-globulin was purchased from a commercial source (Behringwerke AG, Marburg-Lahn, W. Germany) and conjugated with FITC as described above. Kidney tissues were snapfrozen in *n*-hexane cooled in an acetone-dry ice bath and cut at 4 μm by a cryostat. Kidney sections were fixed in acetone for 3 min, dried in the air, stained with FITC-conjugated reagents, and observed under a fluorescence microscope. To expose binding sites of antigen deposited in the glomerular capillary walls, immunoglobulins were partially eluted by the treatment with acid (0.1 M glycine-HCl buffer, pH 2.4, at 0°C for 10 min) or with enzymatic digestion (trypsin 0.05 mg/ml in 0.02 M Tris-HCl buffer containing 0.13 M NaCl, pH 7.8, at room temperature for 2 min). After these procedure, sections were washed three times in phosphate-buffered saline and stained with fluoresceinated reagents.

Results

Isolation of Tub-Ag on Acrylamide Gel Electrophoresis. Tub-Ag was solubilized by Pronase from renal tubular epithelial segments, and partially separated by gel filtration chromatography (4). It was further purified by acrylamide gel electrophoresis. The partially purified Tub-Ag disclosed five protein bands (Fig. 1). These bands, designated as B-1 to B-5 in the increasing order of electrophoretic mobility, were tested for their nephritogenic activity in rats. Only the rats immunized with B-3 developed proteinuria after 5–6 wk of the injection (Table I). Pathological findings in the kidneys of these rats were those of a typical membranous glomerulonephritis, i.e., diffuse thickening of the glomerular basement membrane without cellular proliferation (Fig. 2*a* and *b*). Immunofluorescent study revealed the deposition of γ-globulin and Tub-Ag in diffuse granular fashion along glomerular capillary walls (Fig. 3*a* and *b*). Six rats were also immunized with 0.01 OD_{280} U of B-3, one-tenth of the amount of antigen used in Table I. Five of them developed proteinuria with all the immunopathological hallmarks of Tub-Ag-mediated membranous glomerulonephritis after a longer incubation period of 60–70 days.

When the purified Tub-Ag preparation (B-3) was tested on acrylamide gel electrophoresis for homogeneity, a single sharp protein band was obtained (Fig. 1). The protein band of Tub-Ag stained heavily with periodic acid-Schiff reagent, but not with Sudan III, indicating its glycoprotein nature. Analytical ultracentrifugation was performed on the purified Tub-Ag, and a single symmetrical peak with a $s_{20,w}$ value of 8.4 was obtained (Fig. 4). When purified Tub-Ag preparation and rabbit anti-Tub-Ag were tested by a double immunodiffusion, a single precipitin line was observed.

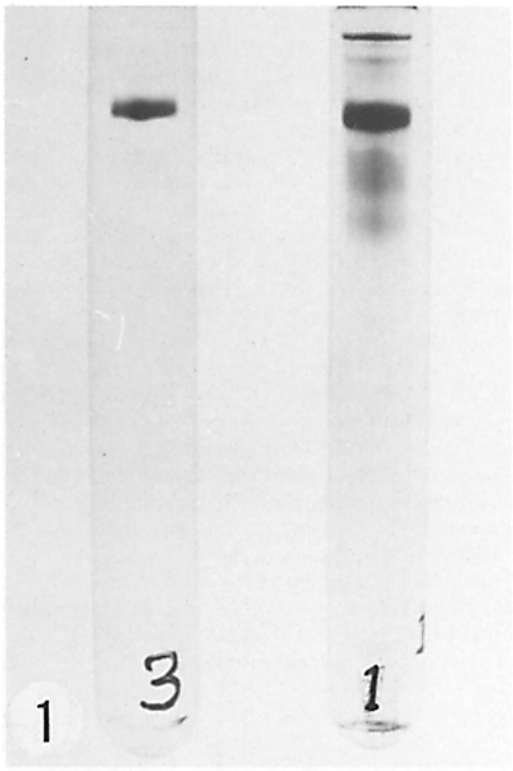

FIG. 1. Disc electrophoresis in acrylamide gel. (1) Tub-Ag preparation partially purified by Bio-Gel A-1.5 M column. (3) Tub-Ag purified by disc electrophoresis.

Tub-Ag Activity in the Serum and Tissue Extracts. Utilizing ^{125}I-labeled Tub-Ag, a sensitive radioimmunoassay was developed for the determination of Tub-Ag and homologous antibody. Fig. 5 shows a titration curve of Tub-Ag by the radioimmunoassay.

When the serum and Pronase extracts of tissues were tested by the radioimmunoassay, they were invariably found to contain Tub-Ag activity. The results are given in Table II. Tub-Ag activity extracted per gram of wet tissue by Pronase treatment was by far the highest for the kidney, followed by liver, lung, and spleen in the decreasing order. The activity of intestine, stomach, and heart were much lower, but they still exhibited up to several times as much activity as the untreated serum. The total extractable Tub-Ag activity, i.e., the specific activity multiplied by total weight of each organ, was equally high in kidneys and liver, followed by lungs. Fig. 6 shows gel filtration patterns of the serum and Pronase extracts of kidney and lung. Tub-Ag activity in the serum appeared as a single peak, in ahead of the position of γ-globulin. Tub-Ag activity in kidney and lung extracts appeared at the same position as in the serum. The positions of Tub-Ag activity in Pronase extracts of all the organs listed in Table II were the same.

Fig. 7 illustrates the electrophoretic pattern of rat serum. Tub-Ag activity in the serum appeared in three different peaks distributed in regions of α- and β-

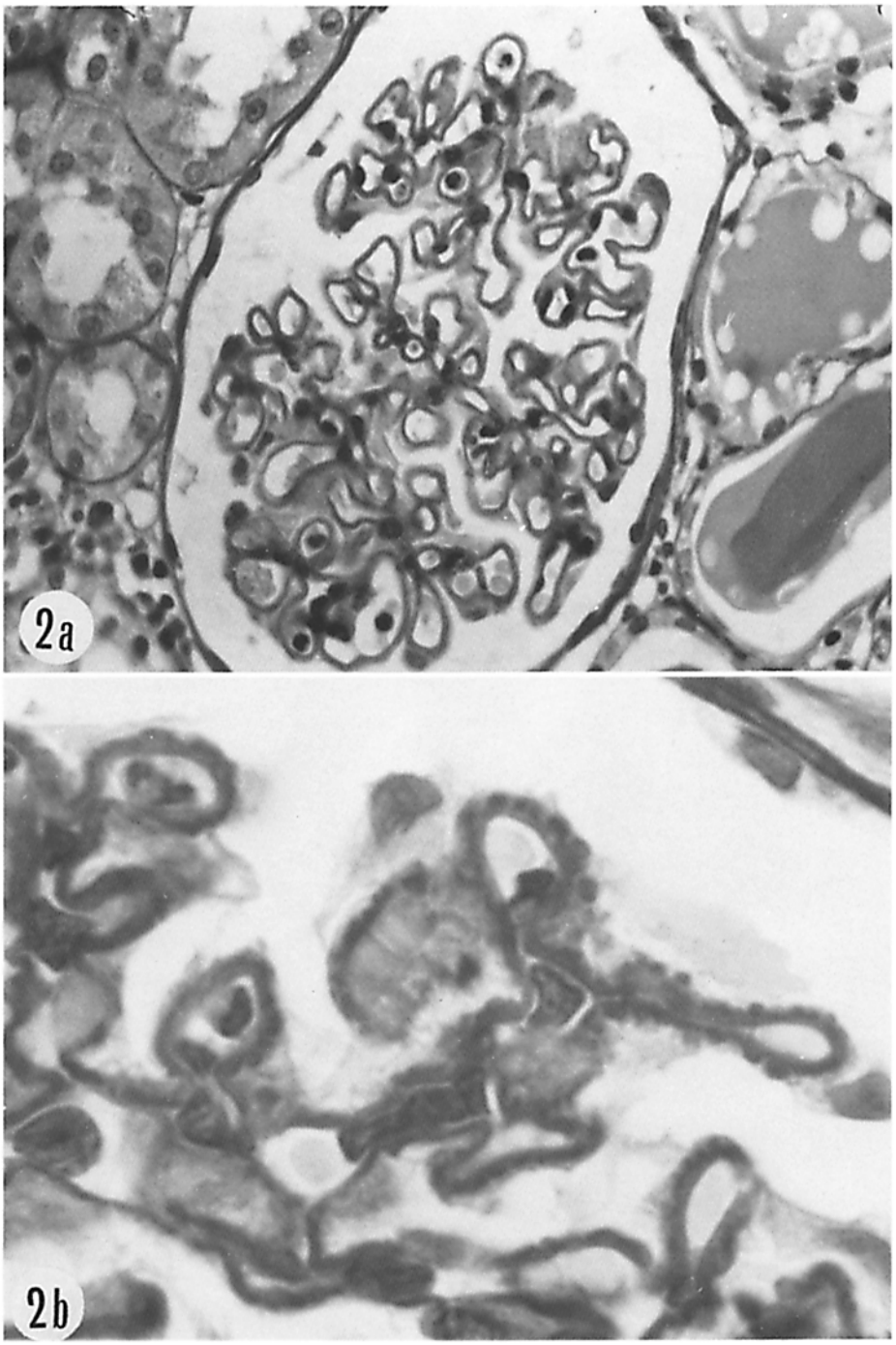

FIG. 2. A glomerulus from the nephritic rat killed 80 days after immunization. (*a*) Marked thickening of the glomerular basement membrane is seen without any noticeable cellular proliferation (periodic acid-Schiff stain × 200). (*b*) Typical tooth-comb appearance is observed (periodic acid-methenamine silver × 900).

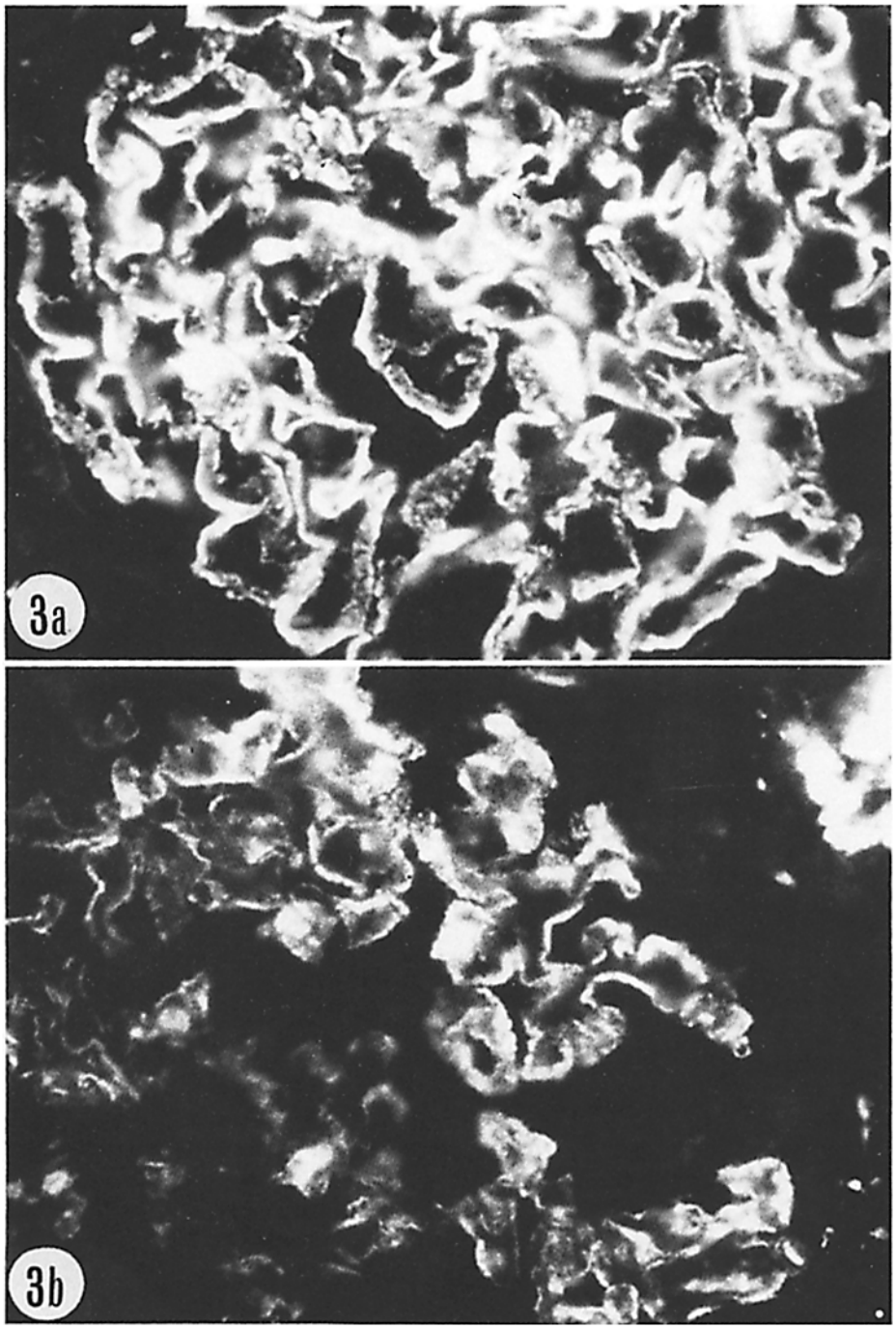

FIG. 3. Fluorescent micrograph of glomeruli from the same rat as in Fig. 2 (× 400). (*a*) Kidney section stained with fluorescinated anti-rat γ-globulin reagent. Diffuse granular deposits of host γ-globulin are found along the glomerular capillary walls. (*b*) Kidney section stained with FITC-labeled antibody against Tub-Ag. Note the specific fluor in the same pattern as that of host γ-globulin. The luminal layer of tubular epithelium is also stained in the right upper corner of the field.

TABLE I
Distribution of Nephritogenic Activity in Fractions of Disc Electrophoresis

Fractions	Distribution of OD_{280} values recovered	Nephritogenic activity: nephritic rats/immunized rats*
	%	
B-1	4.6	0/6
B-2	7.1	0/6
B-3	48.5	0/6
B-4	30.5	0/6
B-5	9.2	0/6

* Each rat received 0.1 OD_{280} U of antigen.

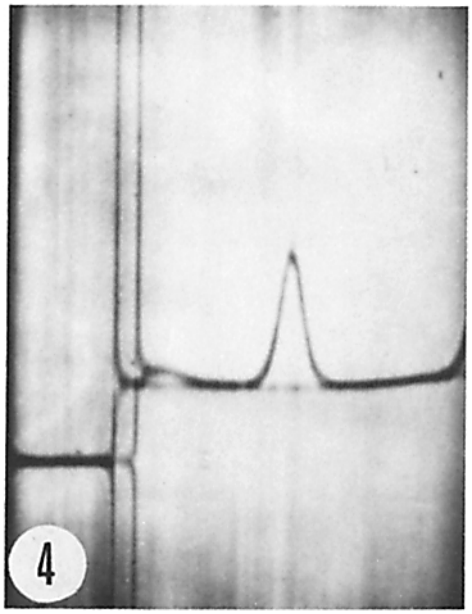

FIG. 4. Ultracentrifugation profile of isolated Tub-Ag (B-3). Photograph was taken at 32 min with a bar angle of 62°. Direction of the sedimentation is to the right. A single symmetrical peak is seen.

globulins. When the purified preparation isolated from renal tubular epithelia and tissue extracts of the other organs were tested, a single peak of activity appeared at the position of the one of the three peaks of serum with the slowest electrophoretic mobility.

Immunoserologic Studies of Rats with Tub-Ag-Mediated Membranous Glomerulonephritis. A group of 40 rats were immunized with 0.1 OD_{280} U of purified Tub-Ag emulsified with adjuvant. Four of them were sacrificed at a time at intervals, their serum samples were tested for Tub-Ag and anti-Tub-Ag activities, and kidney specimens were studied immunohistologically. Their urine samples were tested for protein concentration. The results are shown in Table III. Between 15 and 30 days after the immunization, Tub-Ag disappeared from the circulation of all the rats tested, and they invariably developed anti-

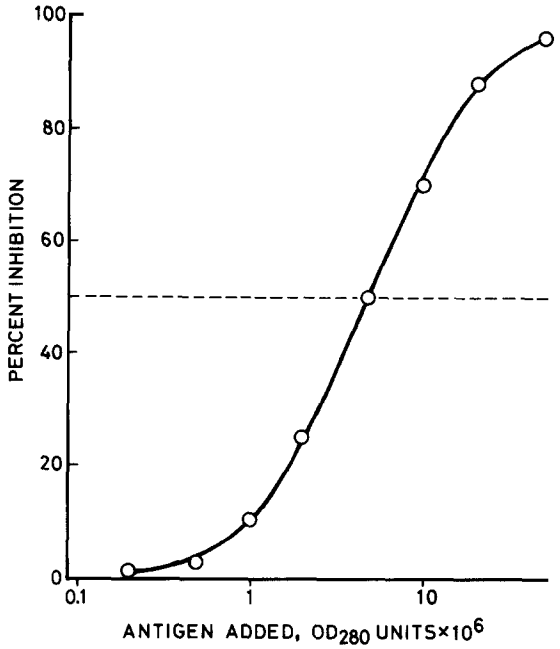

FIG. 5. Inhibition curve of purified nephritogenic tubular antigen.

Tub-Ag in the serum. At this period, however, proteinuria had still not appeared. In all the rats tested, proteinuria appeared as late as 35–40 days after the injection. Tub-Ag and anti-Tub-Ag in the serum varied after the appearance of proteinuria; in some Tub-Ag reappeared in the circulation without detectable free anti-Tub-Ag, while in others anti-Tub-Ag persisted in the serum. Pathological findings in the kidney of rats after the development of proteinuria were those of typical membranous glomerulonephritis, with deposition of γ-globulin and homologous Tub-Ag in a granular fashion along glomerular capillary walls (Fig. 3 *a* and *b*).

Discussion

Membranous glomerulonephritis has generally been accepted as a typical glomerular lesion mediated by immune complexes, since immunoglobulins and β1c are invariably found in diffuse granular fashion along the glomerular capillary walls (10, 11). Some of the antigens that form immune complexes have been identified. Except for DNA in systemic lupus erythematosus (12, 13), however, most of the responsible agents thus far identified are heterologous antigens such as malarial (14), syphilitic (15, 16), streptococcal (17), and viral (18) antigens. The glomerular injury mediated by these exogenous antigens is not self-perpetuating; it disappears when the pathogenic organism has been eradicated by the specific therapy. A continuous supply of the antigen seems to be required for the maintenance of pathologic process in this type of glomerulonephritis, as has been clearly shown by Dixon et al. in the experimental immune-complex nephritis mediated by bovine serum albumin-anti-bovine serum albumin complexes (19).

TABLE II
Tubular Antigenic Activity in Rat Tissue Extracts

Organ	Tub-Ag activity	Relative activity
	RIA U/g*	
Kidney	182,000	788
Liver	22,200	96
Lung	11,900	52
Spleen	3,260	14
Intestine	2,500	11
Stomach	758	3
Heart	520	2
Serum‡	231	1

* Radioimmunoassay units.
‡ Activity of untreated serum.

We have found deposition of renal tubular epithelial antigen together with immunoglobulins and β1c along the glomerular capillary walls of some patients with "idiopathic" membranous glomerulonephritis (1, 2). The proteinuria of the nephritic patients with the glomerular deposition of tubular antigen-antibody complexes persisted without episodes of remission (2). We have solubilized the *nephritogenic* tubular antigen (Tub-Ag) from renal tubular epithelia of humans with Pronase and isolated Tub-Ag that was physicochemically homogenous and antigenically pure (3). By radioimmunoassay, the Tub-Ag of humans was found to be widely distributed in the serum and various organs (3).

The experimental membranous glomerulonephritis in rats reported here is considered to be the laboratory counterpart of the human glomerulonephritis mediated by Tub-Ag-antibody complexes, which has been reported by us and confirmed by Strauss and co-workers (20), because (*a*) Tub-Ags of both humans and rats have been solubilized and purified by the same method and found to be glycoproteins associated with essentially the same physicochemical characters in terms of molecular size, electrophoretic mobility, and $s_{20,w}$ value; (*b*) in the kidney, Tub-Ag of both humans and rats localized exclusively in the brush border of proximal tubular epithelia; (*c*) Tub-Ag activities were identified in the sera of both humans and rats, and they were borne by a protein of the same size as the Tub-Ag purified from homologous renal tubular epithelia; and (*d*) the Tub-Ag activity was liberated by Pronase treatment not only from the kidney, but also from all the other organs tested including liver, lung, intestine and so forth, and the concentration of Tub-Ag in each human organ approximately paralleled that in the rat organ.

Tubular antigens have long been used to produce experimental glomerulonephritis. Heymann et al. first produced membranous glomerulonephritis by injecting rats repeatedly with homologous kidney homogenate emulsified in adjuvant (21). Edgington and collaborators refined this model by employing a single injection of purified tubular antigen designated as RTE α5 for immunization (22). RTE α5 was liberated from renal tubular epithelia by treatment with detergent and borne by a macromolecule of lipoprotein nature with a $s_{20,w}$ value of 28.6 and an electrophoretic mobility in α-globulin region. It was

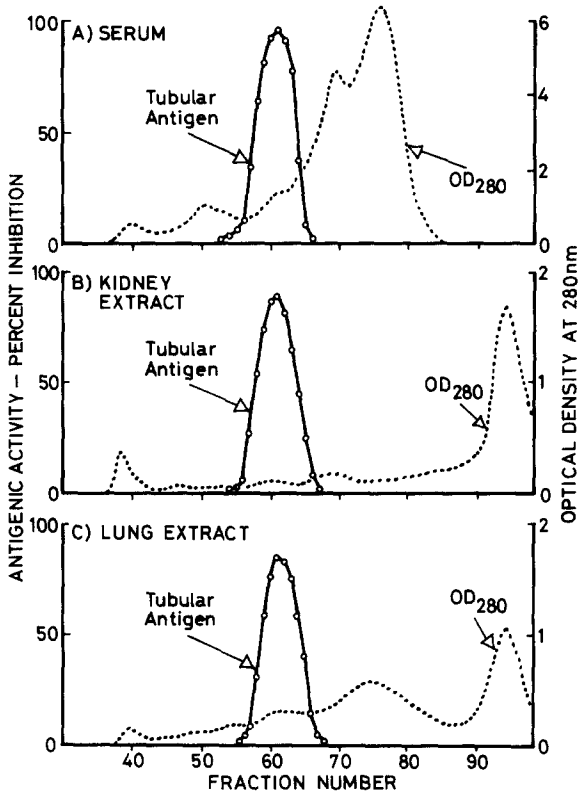

FIG. 6. Gel filtration patterns of serum (A), Pronase extracts of kidney (B) and lung (C) on Bio-Gel A-1.5 M. Open circles and solid lines represent Tub-Ag activity, and dotted lines represent optical density at 280 nm.

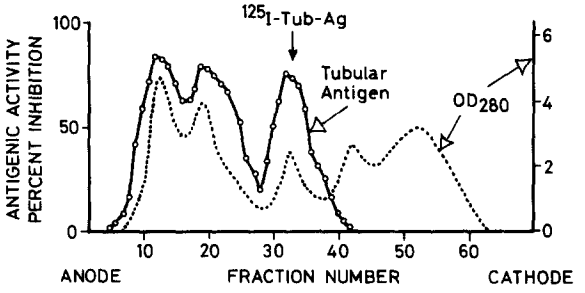

FIG. 7. Column electrophoresis of rat serum. Open circles and solid line represent Tub-Ag activity, dotted line represents optical density at 280 nm. The position of ^{125}I-labeled Tub-Ag is indicated.

apparently different from Tub-Ag, which was solubilized by Pronase digestion and borne by a glycoprotein with a $s_{20,w}$ value of 8.4 and an electrophoretic mobility in β-globulin region. In the kidney, however, both of them were localized in the brush border of proximal tubular epithelial cells, and they were equally potent in nephritogenicity. There is a possibility, therefore, that both of RTE α5 and Tub-Ag bear the same antigenic determinants present on the cellular membrane of proximal tubular epithelia. The essential difference

TABLE III

Followup of Nephritogenic Tubular Antigen and Homologous Antibody in the Sera of Immunized Rats

Rats	Days after injection	Tub-Ag	Antibody to Tub-Ag*	Proteinuria
		RIA U/ml	%	*mg/100 ml*
Normal	0	231 ± 22‡	<0.7	<30
Immunized				
1	10	104	6.2	50
2	10	125	4.6	26
3	10	250	3.5	30
4	15	29	17.2	26
5	15	42	46.1	38
6	20	26	28.9	65
7	20	43	21.4	34
8	25	36	29.6	30
9	25	54	39.5	46
10	25	80	68.1	28
11	25	25	27.2	34
12	30	44	68.2	40
13	30	32	58.2	36
14	30	83	29.1	20
15	30	29	77.5	50
16	35	114	6.9	175
17	35	217	6.1	540
18	35	95	16.2	1,700
19	35	175	6.2	170
20	40	100	13.1	1,400
21	40	122	7.2	2,300
22	40	50	23.6	2,100
23	40	95	41.6	1,800
24	45	33	61.0	1,900
25	45	37	68.7	2,300
26	45	222	4.7	1,400
27	45	172	5.6	970
28	50	95	74.8	650
29	50	95	61.0	2,000
30	50	222	4.5	1,800
31	60	125	26.4	1,200
32	60	169	38.9	2,100
33	60	217	7.0	980
34	80	217	10.7	2,300
35	80	120	6.7	800
36	80	238	1.9	1,100
37	90	87	24.2	2,000
38	90	161	13.9	1,800
39	90	215	1.9	2,000

* Percent of 5 ng of ^{125}I-labeled Tub-Ag bound with 5 μl of the serum.

‡ Mean ± SD of 10 normal rats.

between the method of Edgington et al. and ours is the technique employed to solubilize the antigen. They used a detergent, while we used a proteolytic enzyme. Different sites of membrane may have been cleaved by these two kinds of treatment to produce molecular fragments of dissimilar sizes, but containing the same antigenic configurations. Similar observations have been reported in the solubilization of HL-A antigens from cellular membranes. When cells were treated with detergents, the antigenic activity of HL-A was obtained in macromolecules with a molecular size of a few million daltons (23). In contrast, much smaller molecules with a size of 48,000 daltons, bearing the same antigenicity, were liberated by digesting cellular membranes with papain (24). Possibly Tub-Ag can be incorporated in RTE $\alpha 5$ in the same way. It would be of interest to digest RTE $\alpha 5$ with Pronase to see if the nephritogenic activity can be recovered in a smaller molecule. It may be justified to assume, however, that Tub-Ag is somewhat more physiologic than RTE $\alpha 5$ because the activity of Tub-Ag was found to occur naturally in the serum, associated with physicochemical properties identical to those of Tub-Ag purified from the kidney, and to those from all the other organs tested. Tub-Ag may exist ubiquitously as a component of cellular membrane in a number of organs, and be released into the circulation as a physiological metabolite. Since it is clear that a glycoprotein bearing the antigenicity of Tub-Ag is not specific to renal tubular epithelia, the terminology, by definition, is no longer justified. We are inclined to retain the term Tub-Ag, however, until a more appropriate nomenclature is available to characterize it, for example, as a distinct molecular component of cellular membranes.

Although Tub-Ag in the rat serum was homogeneous in size by a gel filtration criterion, it revealed three different electrophoretic mobilities distributed widely in α- and β-globulin regions. Such a heterogeneity in electrophoretic mobility was also observed for Tub-Ag in human serum (3). The electrophoretic mobility of Tub-Ag purified from the kidney was homogeneous and identical to one of the peaks of Tub-Ag in the serum that had the slowest mobility. The Tub-Ag activities in the serum with faster mobilities would probably be associated with additional molecules with negative charges, such as sialic acid.

Glassock et al. have found that radiolabeled rabbit anti-RTE $\alpha 5$ was cleared from circulation much faster than the pair-labeled normal rabbit γ-globulin, and suggested the presence of tubular antigen activities in the serum of rats (25). With radioimmunoassay, tubular antigen was directly demonstrated in the serum of rats, confirming their observations. The reason why the previous workers had difficulties in detecting tubular antigens in the serum, although their presence had been strongly suspected, was apparently their low concentration in the circulation. Circulating Tub-Ag would have been responsible for the experimental glomerulonephritis in rats induced by the passive transfer of heterologous as well as homologous antibodies against tubular antigens. Feenstra and his associates have injected rats with rabbit antibody to tubular epithelial antigen and demonstrated the deposition of immune complexes containing the antigen and heterologous antibody in the glomerular basement membrane as early as 3 h after the injection (26). Sugisaki et al. transferred the Heymann-type glomerulonephritis by the γ-globulin fraction of sera of

nephritic rats (27). Their findings were at variance with those of Hess et al., who failed to induce nephritis by the injection of sera of diseased animals (28). Circulating free antibodies against tubular antigen in the rats with Heymann-type glomerulonephritis have been a matter of controversy. Hunter and collaborators found the antibody by immunodiffusion in sera of only one-fourth of the nephritic rats (29). Glassock and his co-workers used the inhibition of staining of brush border by fluoresceinated antibody and demonstrated antibodies within the period of 3–8 wk after the immunization (25). Utilizing radioimmunoassay, we have shown that free antibody to Tub-Ag appeared in the serum of all the rats tested about 15–30 days after inoculation, earlier than the development of proteinuria. Thereafter, anti-Tub-Ag antibody persisted in some of the rats, while it disappeared from circulation in others. The reason why free antibody may or may not be detected in the nephritic rats without any noticeable difference in the degree of proteinuria or immunopathological changes in the kidney remains unexplained.

It is of interest that Tub-Ag was demonstrated not only in the kidney, but also universally in all the other organs tested. The antigenicity in common with the brush border of renal tubular epithelia has already been demonstrated in the intestine of rats by fluorescent antibody technique (5). A number of experiments have been undertaken to detect tubular antigen in organs other than kidney by inducing Heymann-type glomerulonephritis by the injection with extracts of various organ tissues, including liver, lung, intestine, and stomach, without any success (22, 27), except in the report of Heymann et al., who noted that 3 out of 21 rats immunized with liver homogenate emulsified in adjuvant developed glomerulonephritis (21). This would have been attributable to lower concentrations of the antigen contained in these organs. Although the kidney contained by far the highest Tub-Ag per gram of wet tissue among all the organs tested, the total antigenic activity in the organs such as liver and lung was comparable to that in kidneys, owing to their respective weight. Evidence that kidneys accounted for only a part of circulating Tub-Ag has already been obtained in humans. Tub-Ag activity was detected in the serum of patients on maintenance hemodialysis who received bilateral nephrectomy, although at a slightly lower level than normal individuals (60.8 ± 7.8 vs. 69.9 ± 10.6 RIA U/ml) (3). Taken altogether, organs other than kidney seem to be greater sources of circulating Tub-Ag.

There remains little doubt that circulating Tub-Ag maintains the pathologic process in the kidney both in humans and in rats by supplying the antigen continuously to form immune complexes. In experimental membranous glomerulonephritis in rats, antibody responses were initiated by immunizing rats with homologous Tub-Ag purified from the kidney. In the human version of this type of glomerulonephritis, however, the way in which the antibody response to Tub-Ag is triggered remains to be determined. The primary antibody response to Tub-Ag could have occurred against Tub-Ag in almost any organ. Just as the heterologous antibody raised against tissue homogenate of any organ always bound with the glomerular basement membrane and induced Masugi-type glomerulonephritis in rats (30), and the injury in the lung has been implicated for the production of nephrotoxic antibodies in Goodpasture's syndrome in humans (31, 32), the kidney could again be a

universal sufferer in Tub-Ag-mediated membranous glomerulonephritis. We are now immunizing rats with Tub-Ag preparations isolated from the other organs containing RIA units comparable to those from the kidney which were sufficient to induce membranous glomerulonephritis to determine the validity of this assumption.

Summary

The renal tubular epithelial antigen (Tub-Ag) of rats was solubilized by Pronase and purified by gel filtration and acrylamide gel electrophoresis. Purified Tub-Ag was a glycoprotein with $s_{20,w}$ value of 8.4. Utilizing radiolabeled Tub-Ag, a sensitive radioimmunoassay for Tub-Ag and homologous antibody (anti-Tub-Ag) was developed. Tub-Ag activity associated with a protein of the same molecular size was demonstrated in the serum, as well as in Pronase extracts of all the organs tested, including kidney, liver, lung, spleen, intestine, stomach, and heart. The physicochemical properties of the Tub-Ag of rats and its distribution were essentially the same as the Tub-Ag of humans, which had been found in immune deposits in the kidney of some patients with idiopathic membranous glomerulonephritis. Rats were immunized with the purified Tub-Ag emulsified in Freund's complete adjuvant and followed for Tub-Ag and anti-Tub-Ag in the serum, as well as for proteinuria and immunohistological changes in the kidney. Serum Tub-Ag dropped sharply after 20 days, when anti-Tub-Ag appeared in the circulation. Persistent, massive proteinuria appeared still later, more than 30 days after injection, when anti-Tub-Ag disappeared and Tub-Ag reappeared in the serum of some of those rats. In others, anti-Tub-Ag in the serum persisted throughout the observation period of 90 days. The pathology of the kidney of the rats with proteinuria was that of a typical membranous glomerulonephritis; thickening of glomerular capillary walls with granular deposits of γ-globulin and Tub-Ag was observed. On the basis of these results, Tub-Ag in the serum, probably released from cellular membranes of various organs as a physiological metabolite, is considered to maintain the pathological process in the kidney by providing the antigen continuously to form immune complexes.

The authors thank Professor Tadashi Maekawa of the Third Department of Internal Medicine, Gunma University, for his valuable discussion and criticism.

Received for publication 21 July 1976.

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