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Role of Dipeptidyl Peptidase IV (gp108) in Passive Heymann Nephritis

Use of Dipeptidyl Peptidase IV-Deficient Rats

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Injection of antibodies to renal tubular membrane (Fx1A) into Lewis rats induces granular deposits of IgG in glomeruli and proteinuria (passive Heymann nephritis, PHN), and similar lesions are also induced by antibody to one of the antigens in Fx1A, dipeptidyl peptidase IV (DPP IV, gp108). In this study, the role of DPP IV in PHN was investigated using DPP IV-deficient F344 rats. The amount of DPP IV found in F344 rat kidneys was less than 0.05% of that present in Wistar rats, and injection of anti-DPP IV antibody into F344 rats did not induce proteinuria. Injection of anti-F344 Fx1A rabbit antibodies that contain no detectable anti-DPP IV antibody into Lewis or F344 rats induced PHN, characterized by granular deposits of rabbit IgG in glomeruli and massive proteinuria, although the appearance of proteinuria was delayed in comparison with that occurring in response to injection of anti-Wistar Fx1A antibodies. These results indicate that DPP IV may contribute to, but is not essential for, the induction of PHN. (Am J Pathol 1989, 134: 405–410)

Renal tubular membrane fraction (Fx1A) contains the antigens responsible for the induction of experimental membranous glomerulonephritis; administration of anti-Fx1A antibodies results in formation of granular deposits of IgG in glomeruli and proteinuria (passive Heymann nephritis, PHN).[1–4] Two glycoproteins, gp330 and dipeptidyl peptidase IV (DPP IV), have been identified as the major antigens in rat Fx1A using monoclonal or polyclonal antibodies. gp330, a glycoprotein with a molecular weight (MW) of 330 kd, is located in the coated pits of glomerular and proximal tubular epithelial cells and is considered to be the main pathogenic antigen of Heymann nephritis (HN).[5–12] DPP IV, initially reported as a 90 kd antigen by Ronco et al,[13,14] or a gp108 antigen by us,[15,16] is the other major immunogen in Fx1A in heterologous animals,[15,17] although it shows low immunogenicity in homologous animals.[15] We have demonstrated previously that injection of anti-gp108 (DPP IV) rabbit antibody into Lewis rats induces acute and severe proteinuria and granular deposits of rabbit IgG along the glomerular capillary walls.[15] Ronco et al[13] also have shown that anti-DPP IV monoclonal antibody induces transient glomerulopathy in rats; however, it is not known whether DPP IV contributes to PHN induced by anti-Fx1A antibodies.

Recently, Watanabe and coworkers[18] reported that Fischer 344 (F344)-strain rats are deficient in DPP IV with respect to both enzyme and antigenic activities. In the present study, we attempted to confirm the DPP IV deficiency of F344 rats, and to assess the role of DPP IV in PHN.

Materials and Methods

Preparation of Antigens

Lewis and F344-strain rats were purchased from Charles River Japan Inc. (Atsugi, Japan) and Wistar-strain rats were from Imamichi Institute for Animal Reproduction (Omiya, Japan). Kidneys of these strains of rats were homogenized and the membrane fractions were collected by centrifugation at 105,000*g* for 1 hour and washed with phosphate-buffered saline (PBS), pH 7.2. Fx1A was prepared from kidneys of Wistar or F344 rats by the method of Edgington et al[2] and was solubilized with PBS containing 1% Triton X-100. DPP IV was isolated from Wistar Fx1A as described previously[15]; briefly, Fx1A was solubilized with 1% Triton X-100 and fractionated using columns of DEAE-cellulose and Bio-Gel A-1.5m in the absence of detergent.

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Table 1. *Content of DPP IV in Kidneys of Three Rat Strains*

Strains	Enzyme activity (units/mg)	Antigenic activity (μg/mg)
Wistar	0.50 ± 0.09	14.3 ± 2.4
Lewis	0.47 ± 0.04	11.0 ± 0.9
F344	0.0012 ± 0.0001 (0.24%)*	0.0048 ± 0.0013 (0.03%)*

The values are mean ± SEM (N = 4).
* Percent of Wistar rats.

Analytic Methods

Enzyme activity of DPP IV was determined using the photometric method of Nagatsu et al.[19] The reaction mixture contained 50 mM TRIS-HCl (pH 8.0), 1% Triton X-100, 1.5 mM Gly-Pro-*p*-nitroanilide tosylate (Sigma Chemical Co., St. Louis, MO), and the enzymes of Triton-solubilized kidney membranes in a total volume of 0.25 ml.[16] For studies to determine the optimal pH of the enzyme reaction, either 50 mM sodium acetate (pH 4–5) or 50 mM sodium citrate (pH 5–6) buffer was used instead of TRIS-HCl buffer.

Gel permeation high-performance liquid chromatography (HPLC) was performed on TSK G3000SW (7.5 × 600 mm, Tosoh, Japan) with a mobile phase of 0.1 M phosphate buffer (pH 7.0) containing 1% sodium cholate. The flow rate was 0.5 ml/min and the column effluent was monitored continuously by absorbance at 280 nm with an ultraviolet detector (Waters 490, Millipore Corporation, Milford, MA). Fractions of 0.5 ml were collected and the enzyme activity of each fraction was measured as described above. The column was calibrated with the following proteins: human gammaglobulin dimer (MW 320 kd), human gammaglobulin monomer (MW 160 kd), bovine serum albumin (MW 67 kd), and chymotrypsinogen A (MW 25 kd).

Immunologic Methods

Antisera to Fx1A or DPP IV (gp108) were produced in rabbits as described previously[15] and the gammaglobulin fractions of the antisera were prepared by repeated 33% ammonium sulfate precipitation. Antigenic activity of DPP IV was measured quantitatively by a sandwich enzyme immunoassay, developed specifically for the measurement of gp108 and capable of detecting 0.1 ng of the antigen.[20] Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed on 5–15% gradient slab gels. Immunoblotting analysis was performed after electrophoretic transfer of proteins from the gels to nitrocellulose papers.[15] Myosin (MW, 200 kd), β-galactosidase (MW, 116 kd), phosphorylase B (MW, 92.5 kd), bovine serum albumin, and ovalbumin (MW, 45 kd) were used as markers. Direct immunofluorescence was used for the detection of rabbit IgG in kidneys of rats; 2-μ cryostat sections of unfixed kidneys were stained with FITC-conjugated goat anti-rabbit IgG (Cappel Laboratories Inc., Cochranville, PA).

Animal Experiments

Normal male 6-week-old rats of Lewis (130–140 g) or F344 (100–110 g) strain were injected intraperitoneally with 60 mg of gammaglobulin fraction of anti-Wistar or anti-F344 Fx1A antiserum, or with 50 mg of gammaglobulin fraction of anti-DPP IV antiserum. Urinary protein excretion was measured by the biuret method after trichloroacetic acid precipitation. The kidneys were used for direct immunofluorescence, 4 hours or 6 weeks after the injection of anti-Fx1A antibodies, or 2 days after the injection of anti-DPP IV antibody.

Results

DPP IV in F344 Rats

The content of DPP IV in the kidneys of F344, Wistar, or Lewis rats was determined by two methods, ie, an assay

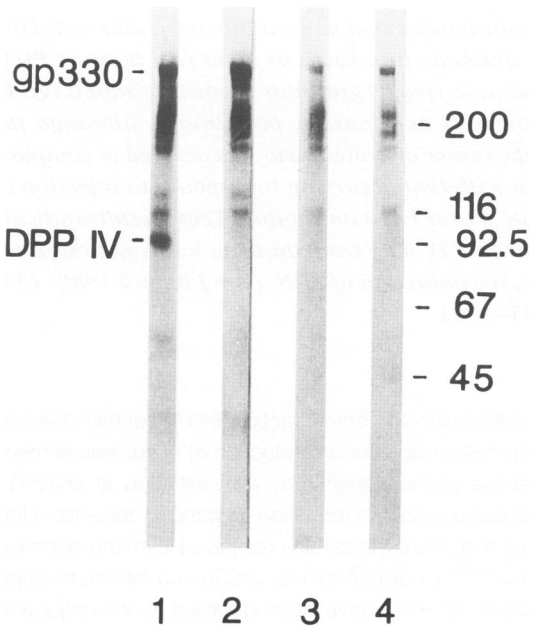

Figure 1. *Immunoblotting analysis of antigens in Fx1A and of anti-Fx1A antibodies. Antigens: lanes 1 and 2, 14 μg of Wistar Fx1A, lanes 3 and 4, 14 μg of F344 Fx1A. Antibodies: lanes 1 and 3, anti-Wistar Fx1A antiserum, lanes 2 and 4, anti-F344 Fx1A antiserum. Numbers on the right side indicate the positions and MW (kd) of marker proteins. Positions of gp330 and DPP IV are shown on the left side.*

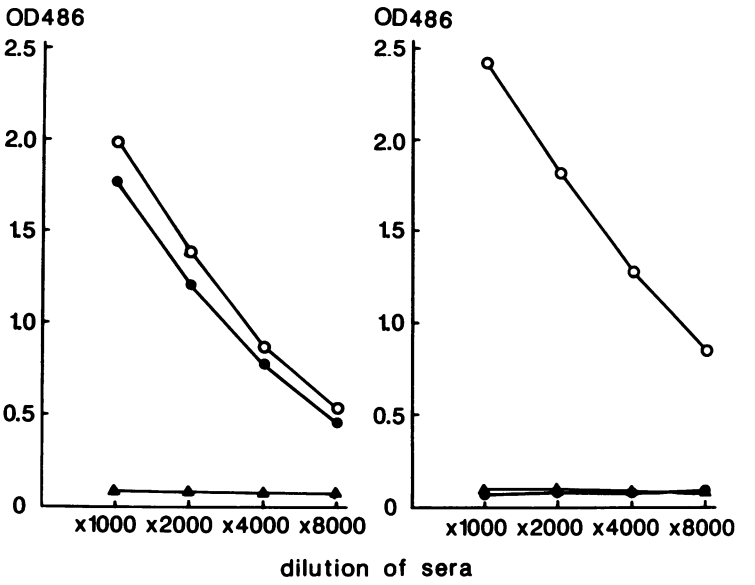

Figure 2. *Reactivity of anti-Wistar Fx1A or anti-F344 Fx1A antibodies on enzyme immunoassay. Wistar Fx1A (left) or purified DPP IV (right) was used as coated antigen.* ●——● *Anti-F344 Fx1A antiserum;* ○——○ *anti-Wistar Fx1A antiserum;* ▲——▲ *control rabbit serum.*

for activity of the enzyme itself and an assay for antigenic activity. Although homogenates of Wistar and Lewis rat kidneys showed similar DPP IV enzyme activity, only about 0.2% of this enzyme activity was found in F344 rat kidneys using Gly-Pro-*p*-nitroanilide tosylate (Table 1). These results compare favorably with those reported by Watanabe and coworkers[18]; however, the optimal pH of the weak enzyme activity in F344 rat kidneys was around 5 and the apparent MW of the enzyme measured by gel permeation HPLC was near 130 kd (data not shown). This indicates that the hydrolyzing activity measured against this substrate comes from contamination from dipeptidyl peptidase II, a lysosomal enzyme.[21] Only 0.03% of the antigenic activity of DPP IV in Lewis and Wistar rats was found in F344 rat kidneys on a sandwich enzyme immunoassay (Table 1).

The pathogenicity of anti-DPP IV antibody to F344 rats was investigated. When anti-DPP IV antibody was injected into F344 rats, proteinuria did not develop for 7 days (<10 mg/24 hours, N=4) and deposits of rabbit IgG were not observed in glomeruli by immunofluorescence on day 2 (N=2). The same amount of the antibody caused acute proteinuria (>100 mg/24 hours on day 2) and glomerular deposits of rabbit IgG in Lewis rats.[15] The fact that development of proteinuria depended on the presence of DPP IV confirms the potential of the anti-DPP IV antibody itself to induce proteinuria.

Characterization of Anti-F344 Fx1A and Anti-Wistar Fx1A Antibodies

Anti-F344 Fx1A and anti-Wistar Fx1A antibodies raised in rabbits were characterized. On an immunoblotting assay using Wistar Fx1A as antigen, both antibodies showed the same profile except for anti-DPP IV antibody; that is, when anti-F344 Fx1A antibodies were used, DPP IV was not stained (Figure 1, lanes 1 and 2). When F344 Fx1A was used as the antigen source, both antibodies showed virtually the same pattern (lanes 3 and 4). Furthermore, anti-F344 Fx1A antibodies did not react with purified DPP IV on a microplate enzyme immunoassay, whereas both showed similar reactivity to Fx1A (Figure 2). Therefore, anti-F344 Fx1A antibodies can be used as anti-Fx1A antibodies lacking the anti-DPP IV antibody.

PHN Induced by Anti-F344 Fx1A or Anti-Wistar Fx1A Antibodies

When anti-F344 Fx1A antibodies were injected into Lewis rats, proteinuria developed a little more slowly than in

Table 2. *Proteinuria in Rats Injected with Anti-Fx1A Antibodies*

Strains	Antibodies to	No. of rats	Urinary protein excretion (mg/24 hr)				
			Day 0	Day 3	Day 7	Day 10	Day 14
Lewis	Wistar Fx1A	4	4.9 ± 0.8	9.9 ± 3.2	164 ± 92	223 ± 61*	260 ± 127
Lewis	F344 Fx1A	7	6.6 ± 1.2	9.7 ± 1.5	30 ± 11	76 ± 25*	285 ± 118
F344	Wistar Fx1A	4	5.1 ± 0.5	8.1 ± 1.6	61 ± 20	125 ± 40	176 ± 72

* $P < 0.01$ by Student's *t*-test.

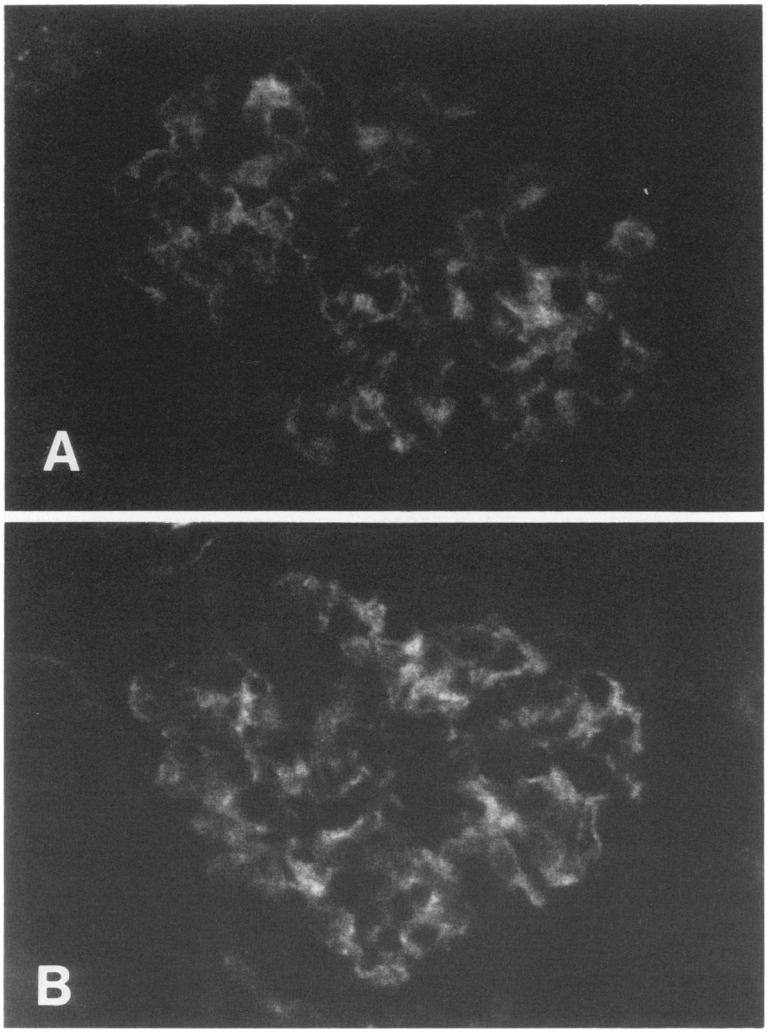

Figure 3. *Direct immunofluorescence of a glomerulus obtained from a Lewis rat 4 hours after the injection of anti-F344 Fx1A (A) or anti-Wistar Fx1A (B) antibodies. Original magnification, ×410.*

Lewis rats injected with anti-Wistar Fx1A antibodies, and reached over 100 mg/24 hours on day 14 (Table 2). The deposits of rabbit IgG observed in glomeruli of Lewis rats 4 hours after the injection of one or the other antibody preparations were similar (Figure 3). Likewise, similar deposits of rat IgG and rabbit IgG were seen after 6 weeks (data not shown). When anti-Wistar Fx1A antibodies were injected into F344 rats immunopathologic aspects characteristic of PHN also were present.

Discussion

It is widely accepted that glomerular immune deposits seen in many types of glomerulonephritis are formed not only by trapping of circulating immune complex but also by *in situ* binding of antibody to intrinsic or planted glomerular antigens.[22] In animal models, *in situ* immune complex formation occurs by antibody binding to glomerular endothelial,[23] epithelial,[24] and mesangial antigens[25–28] as well as to basement membrane components.[29]

Immune deposits in rats with HN are known to be produced mainly by *in situ* binding of antibodies.[22,30,31] The main pathogenic antigen of HN is considered to be brush border glycoprotein gp330,[5,10,12] which is also located on the foot processes of podocytes.[7–9] It has been demonstrated that injected anti-gp330 antibody directly binds to the glomerular epithelial antigen and forms immune deposits *in situ*.[7,32] However, it is still unknown whether another antigen or antigens of Fx1A contribute to HN, because proteinuria is absent or minimal[10–12] and only fine glomerular granular deposits of IgG[11] are observed in rats immunized with gp330 alone.

In our previous studies we have shown that gp108, one of the major antigens when Fx1A is injected into heterologous animals, is detectable in glomeruli by immunofluorescence, and that anti-gp108 antibody has the ability to induce proteinuria when injected into Lewis rats.[15] Sub-

sequently gp108 was shown to be identical to DPP IV on the basis of biochemical and immunologic characteristics.[16] The evidence in this study that DPP IV-deficient rats also lack gp108 antigenicity (<0.03%) confirms this identity. DPP IV is expressed on the endothelial and epithelial cell membranes of rat glomeruli,[9] so that the antigen on either or both types of cell might contribute to *in situ* immune complex formation in PHN and to the induction of proteinuria.

The present study demonstrated that DPP IV is not an essential component for PHN; the disease is induced in DPP IV-deficient F344 rats, and also in Lewis rats by anti-F344 Fx1A antibodies, which lack anti-DPP IV antibody, although proteinuria developed a little more slowly than that in rats injected with anti-Wistar Fx1A antibodies, which have anti-DPP IV activity. Jeraj *et al*[33] have shown that when rats are injected with anti-Fx1A antibodies, binding of antibody along the endothelial cell membranes is observed at an early stage (within 20 minutes). Because DPP IV is expressed on endothelial cell membranes, in contrast to gp330,[9] and an intraperitoneal injection of anti-DPP IV antibody causes binding of IgG to endothelial cell membranes within 4 hours (Natori Y, Shibata S, in preparation), this antigen might be the target of an early antigen-antibody interaction in PHN. However, deposits of rabbit IgG were seen in the glomerular capillary walls as soon as 4 hours after the injection of anti-F344 Fx1A antibodies. Therefore, it can be speculated that several antigens besides DPP IV are involved in the formation of immune complexes on endothelial cells and in the development of proteinuria induced by anti-Fx1A antibodies. Studies on the contribution of other endothelial and epithelial antigens to the formation of immune deposits in PHN are necessary to clarify the precise mechanism of tissue injury in this experimental model.

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