

Temporal and Spatial Expression of Podocyte-Associated Molecules Are Accompanied by Proteinuria in IgA Nephropathy Rat Model

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Summary

We used a rat model to assess the role of nephrin, podocin, and desmin in the pathogenesis of IgA nephropathy (IgAN). A rat IgAN model was established by administration of BSA, CCl₄, and lipopolysaccharide (LPS) and compared with healthy control rats. Urinary protein, urine red blood cells, and biochemical parameters were measured for 12 weeks. Renal morphology and ultrastructure were examined by light and electron microscopy. Immunofluorescence was used to assess IgA deposition in the glomeruli and to measure expression of nephrin, podocin, and desmin. Real-time quantitative PCR was used to measure expression of nephrin, podocin, and desmin mRNAs. IgAN rats developed proteinuria at week-6 and this worsened over time. Pathological changes were evident under light microscopy at week-8 and under electron microscopy at week-4. Immunofluorescence analysis showed deposition of IgA in the kidneys of IgAN rats, but not control rats. IgAN rats had increased expression of glomerular podocin, nephrin, and desmin mRNAs and proteins at week-4. The expression of nephrin, podocin and desmin proteins and the expression of podocin and desmin mRNAs preceded the increase in urinary protein. Taken together, our study of a rat model of IgAN indicates that changes in the expression and distribution of nephrin, podocin, and desmin precede and may cause foot process fusion and proteinuria.

Key words

IgA nephropathy • Proteinuria • Podocyte • Nephrin • Podocin • Desmin

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Introduction

Immunoglobulin A nephropathy (IgAN) is characterized by abnormal deposition of IgA in the renal glomeruli, and is the most common primary glomerulonephritis (Cai and Chen 2009, Kiryluk *et al.* 2010). IgAN patients with proteinuria are generally considered to have increased risk for progression to chronic kidney disease (CKD) and renal failure (Barratt and Feehally 2005). Although most IgAN patients develop proteinuria, remission of proteinuria is associated with improved prognosis (Reich *et al.* 2007). Thus, slowing the progression of IgAN may help to control the development of severe proteinuria. The pathogenesis of proteinuria in IgAN is largely unknown. Elucidation of the pathogenesis of proteinuria in IgAN may help to identify therapeutic targets and allow prompt treatment, thereby delaying the progression to CKD. Therefore, several suitable animal models of IAGN have been established in order to examine the initiation and progression of this disease (Rifai *et al.* 1979, Emancipator 2011).

Recent studies of hereditary renal diseases in humans and of experimentally induced renal diseases in animals have indicated that numerous proteins produced by the podocytes are critical for maintenance of the structure and function of the slit diaphragm (SD), a region of the foot processes of podocytes through which blood is filtered. Disruption of the SD may allow passage of large molecules into the urine (Mundel and Shankland

2002, Lai *et al.* 2009). These podocyte-associated proteins include nephrin, podocin, and desmin. However, previous studies of the relationship between IgAN and nephrin, podocin, and desmin have produced conflicting results (Gagliardini *et al.* 2003, Koop *et al.* 2003, Szeto *et al.* 2005, Mao *et al.* 2006). Thus, it is necessary to clarify the dynamic changes in these proteins during the course of IgAN and the roles of these proteins in IgAN proteinuria.

In this study, we focused on three proteins that appear to have important roles in IAGN: nephrin, podocin, and desmin. Mundel and Shankland (2002) classified podocyte-related molecules into four groups: (i) slit diaphragm complex proteins, including nephrin (a 135 kDa transmembrane glycoprotein in the immunoglobulin adhesion molecule superfamily) and podocin (a 42 kDa transmembrane protein in the stomatin family); (ii) podocyte cytoskeletal proteins, including desmin (a 53 kDa cytoskeletal intermediate filament protein that is a marker of podocyte injury); (iii) basement membrane-podocyte junction proteins; and (iv) podocyte top membrane proteins.

In the present study, we established a rat model of IgAN to investigate the dynamic changes in the distribution of nephrin, podocin, and desmin in the glomeruli, assess the association of these proteins with IgAN proteinuria, and elucidate the pathogenesis of proteinuria in IgAN.

Materials and Methods

Animals and grouping

Ninety-six 6-week-old male Sprague-Dawley rats (specific pathogen free) weighing 150 ± 10 g were purchased from the Experimental Animal Center of the Medical College of Sun Yat-Sen University (Guangzhou, China). Rats were given *ad libitum* access to food and water. All rats were active and had glossy hair before the start of the experiments.

Establishment of IgAN model

The rats were randomly assigned to a control group (n=48) and an IgAN group (n=48). The rat IgAN model was established by administration of bovine serum albumin (BSA), lipopolysaccharide (LPS), and carbon tetrachloride (CCl₄) as reported previously (Emancipator 2001, Tang *et al.* 2006, Peng and Liu 2008), with slight modification (Fig. 1). In brief, immunogen BSA (MBCHEM, USA) was intragastrically administered at 400 mg/kg once every two days for 8 consecutive weeks and 0.1 ml of CCl₄ in 0.3 ml of castor oil was given once weekly for 9 weeks. At the 6th week, 0.05 mg of LPS (Sigma, USA) was injected through the tail vein. For the control group, 4 ml/kg distilled water was used to replace the BSA, 0.4 ml of castor oil was used to replace castor oil and CCl₄, and saline was used to replace LPS at the 6th week. The administration methods were the same as in the model group.

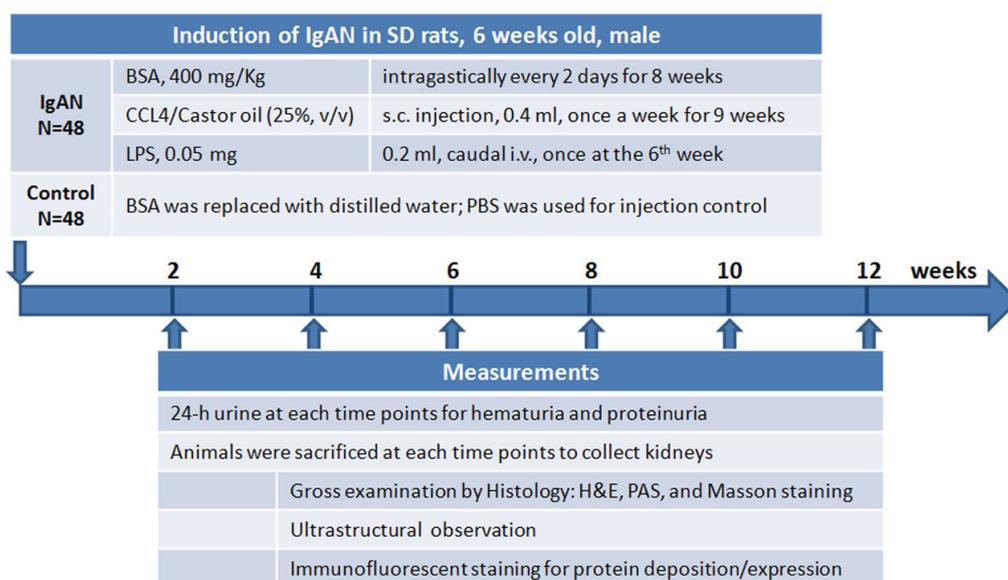


Fig. 1. Induction of IgAN in rats and timing of measurements.

Table 1. Primers for real-time fluorescence quantitative PCR.

Gene		Primer Sequences	Length (bp)
Nephrin	forward	-CCCTCCGGGACCCTACTG	82
	reverse	-TCTGGGAGGATGGGATTGG	
Podocin	forward	-TGGAAGCTGAGGCACAAAGA	97
	reverse	-AGAATCTCAGCCGCCATCCT	
Desmin	forward	-GACGTGGAGCGTGACAACCT	106
	reverse	-GGAAGGCAGCCAAGTTGTTC	
GAPDH	forward	-TGGTCTACATGTTCCAGTATGACT	134
	reverse	-CCATTTGATGTTAGCGGGATCTC	

The main advantages of our modified model are: (i) oral administration of BSA with doubled dosage and elongated administration time was safer; (ii) as an immune adjuvant, only one intravenous injection of LPS at the 6th week is less likely to have toxic effects; and (iii) the dosage of CCl₄ was reduced to one third of the dosage used to induce hepatic fibrosis and a subcutaneous, rather than intraperitoneal, injection was used to minimize liver damage and reduce risk of peritoneal infection or animal death.

Sample collection and preparation

At the end of 2, 4, 6, 8, 10, and 12 weeks, the 24-h urine was collected with metabolic cages, and urine color was observed. The presence of gross hematuria was recorded and Coomassie Brilliant Blue was used to measure the urinary protein. In addition, 8 rats in each group were sacrificed at different times and their kidneys were collected for histological examination. Each kidney was divided into 4 parts. The first one fourth was fixed in 40 g/l formaldehyde, embedded in paraffin, cut into sections, and then stained by hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), or Masson's trichrome for light microscopy. The second quarter of kidney tissues was fixed in 25 ml/l glutaraldehyde + 20 ml/l paraformaldehyde followed by embedding, sectioning and staining with uranyl acetate and lead citrate for electron microscopy. The third portion was embedded in OTC and stored at -70 °C followed by frozen sectioning for immunofluorescence staining. The last quarter of kidney tissue was stored at -70 °C for real-time quantitative fluorescence PCR.

Measurement of IgA deposition

Direct immunofluorescence (FITC-conjugated

rabbit anti-rats IgA antibody from Dako, USA) was used to visualize the IgA deposition in the glomeruli and was graded by a 5-stage semi-quantitative method (- to ++++): (-), no staining under low magnification and possible staining under high magnification; (+), possible staining under low magnification and staining under high magnification; (++) , staining under low magnification and clear staining under high magnification; (+++) clear staining under low magnification and bright staining under high magnification; (++++), very intense staining under high magnification. In this study, most of the staining in the IAGN mode group was ++ to +++.

Expression and distribution of nephrin, podocin and desmin in the glomeruli

Indirect immunofluorescence kits were used to detect the expression and distribution of nephrin, podocin, and desmin according to manufacturer's instructions (Abcam, UK). The primary antibodies were rabbit anti-rat nephrin antibody, rabbit anti-rat podocin antibody, and rabbit anti-rat desmin antibody (Abcam, UK). The secondary antibody was goat anti-rabbit antibody (Jackson, USA).

Blinded observers viewed the sections under a fluorescence microscope (AXIOPLAN 2, Carl Zeiss, Oberkochen, Germany). Representative photographs were captured using a digital camera for measuring the expression and distribution of nephrin, podocin, and desmin based on green fluorescence. A total of 5 glomeruli were selected from each section and the sum integrated optical density (IOD SUM) and sum area (area) were measured using Imagic-Pro Plus 6.0 software, followed by calculation of mean density (IOD SUM/area). The means of five glomeruli were used to quantify the expression of each protein.

Table 2. Biochemical parameters of the control and IAGN rats at the end of week-12.

Parameter	Controls	IgA nephropathy	P-value
ALT (U/l)	47.25±19.51	59.48±20.82	0.246
AST (U/l)	103.61±25.95	129.44±39.03	0.141
ALB (g/l)	36.76±1.49	36.04±1.81	0.398
TP (g/l)	61.08±6.83	62.59±3.96	0.597
CHOL (mmol/l)	1.65±0.49	1.52±0.20	0.521
TG (mmol/l)	0.60±0.77	0.70±0.15	0.718
Cr (μmol/l)	32.00±6.26	34.88±11.90	0.555
BUN (mmol/l)	6.81±0.72	6.40±0.24	0.161
CK-MB (U/l)	1674.25±590.51	1602.88±527.23	0.802

Comparisons were performed by Student's *t*-test. Data indicate means and standard deviations. Abbreviations: ALT, alanine amino transferase; AST, aspartate amino transferase; ALB, serum albumin; TP, total phosphate; CHOL, total cholesterol; TG, triglycerides; Cr, creatinine; BUN, blood urea nitrogen; CK-MB, creatinine kinase-myoglobin

Expression of nephrin, podocin, and desmin mRNAs in the renal cortex

RNA was extracted from the renal cortex after the kidney was separated into two parts along the sagittal plane. The cortex was separated along the medulla and stored directly at -70°C before real time PCR.

Real-time quantitative fluorescence PCR with SYBR Green was used to measure expression of nephrin, podocin, and desmin mRNAs in the renal cortex, with GAPDH as an internal reference. The gene sequences were identified in GeneBank for the design of specific primers (Table 1). Total RNA of the renal cortex was extracted using Trizol (Invitrogen, USA) according to the manufacturer's instructions. Then, 4 μl of total RNA were subjected to reverse transcription with random primers, and M-MLV reverse transcriptase (Da'an Gene Co. Ltd., Guangzhou, China) in 20 μl of reaction mixture using a PCR instrument (Model 9700, Applied Biosystems, city/state, USA). The reaction conditions were 37 °C for 1 h and then at 95 °C for 3 min. Then, 5 μl of cDNA were added to the 50 μl reaction mixture, followed by amplification in an automatic quantitative fluorescent PCR instrument (Model 7500, Applied Biosystems, USA). The PCR conditions were: pre-denaturation at 93 °C for 3 min, 40 cycles of denaturation at 93 °C for 30 sec, annealing at 55 °C for 45 sec, and extension at 72 °C for 45 sec. Expression of each target gene was normalized to that of GAPDH.

Statistical analysis

All data are expressed as means and standard deviations and statistical analysis was performed using

SPSS version 16.0. A *t*-test was used to compare two groups at different time points. Correlations were assessed by calculation of Pearson's correlation coefficient (R). A *p*-value less than 0.05 was considered statistically significant.

Results

Hematuria, proteinuria, urine red blood cell count and biochemical parameters

Control rats had no evidence of gross or microscopic hematuria throughout the experiment. IgAN rats had no evidence of hematuria before treatment, but all of these rats developed microscopic hematuria at week-6, and 60 % of the IgAN rats ultimately developed gross hematuria (data not shown). Measurement of 9 biochemical parameters indicated no significant difference between the control and IgAN rats (Table 2, $p>0.05$ for all). The control and IgAN rats had similar levels of urinary protein before treatment, but at week-6, the IgAN rats had significantly more urinary protein, and their urinary protein continued to increase until week-12 (Table 3). The IgAN rats had significantly more urinary protein than control rats at week-6, -8, -10 and -12 ($p<0.001$ for all). Similarly, there were significantly more urinary red blood cells in IgAN rats at week-6 and the urinary red blood cell count in IgAN rats continued to increased to week-12 (Table 3). There were no animal deaths and no evidence of liver or cardiac damage during the entire study period. Taken together, these results indicate successful establishment of our IAGN model.

Table 3. Daily urinary protein and daily urinary red blood cell count (mean ± SD) in control and IAGN rats from week-2 to week-12.

	Groups	Week					
		2	4	6	8	10	12
Urinary protein (mg/day)	Control	2.72±1.04	3.95±0.97	4.28±0.73	4.47±0.61	5.10±0.52	5.08±0.55
	IgAN	3.59±1.00	3.97±0.53	9.30±1.22*	9.98±0.91*	10.55±0.75*	11.43±1.78*
Urinary red blood cell count (cells/μl/day)	Control	5.0±1.3	4.9±1.4	4.8±1.3	4.9±1.2	5.1±1.2	5.1±1.4
	IgAN	5.2±1.28	5.6±1.3	196.3±11.7*	499.4±27.3*	851.5±20.6*	901.1±20.2*

Comparisons were conducted by Student's *t*-test. Eight rats were in each group at each time point. * P<0.001, compared to control group.

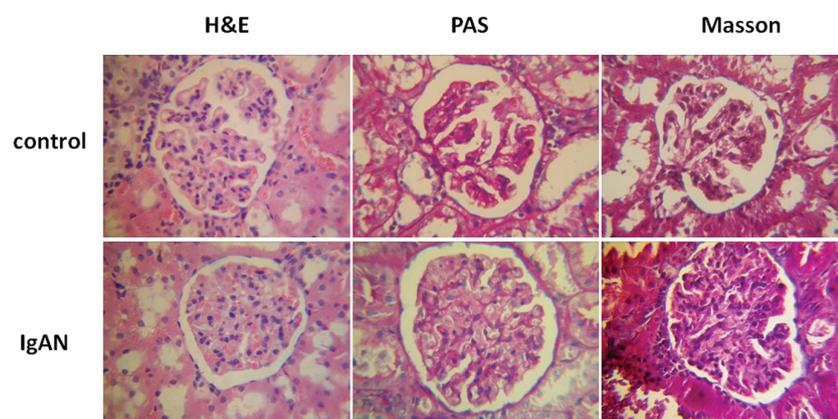
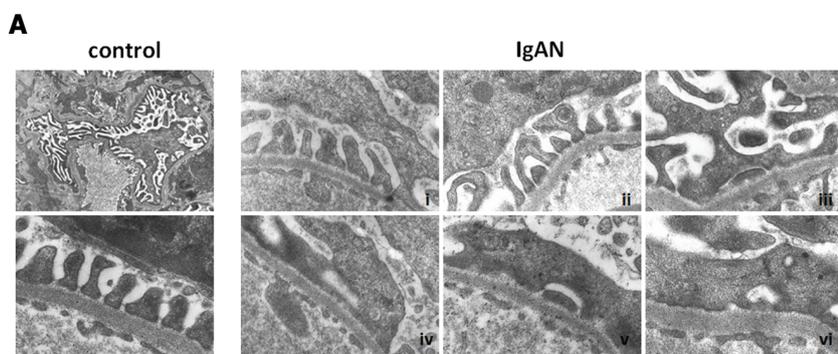
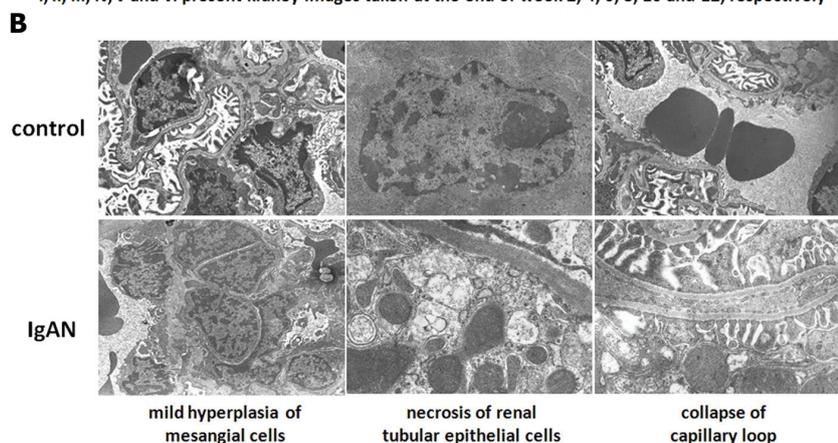


Fig. 2. Glomerular morphology of control and IgAN rats visualized by staining with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and Masson's trichrome at week-12.



i, ii, iii, iv, v and vi present kidney images taken at the end of week 2, 4, 6, 8, 10 and 12, respectively



mild hyperplasia of mesangial cells necrosis of renal tubular epithelial cells collapse of capillary loop

Fig. 3. A. Transmission electron microscopy of the kidneys of control and IgAN rats (×12000). **B.** Transmission electron microscopy of the kidneys of control rats and IgAN rats (×5000).

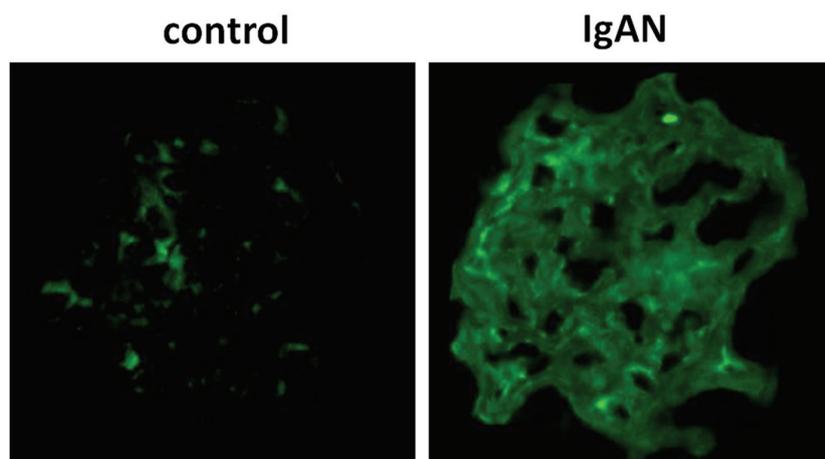


Fig. 4. Immunofluorescence staining for IgA in the glomeruli of control rats and IgAN rats at week 12 ($\times 200$).

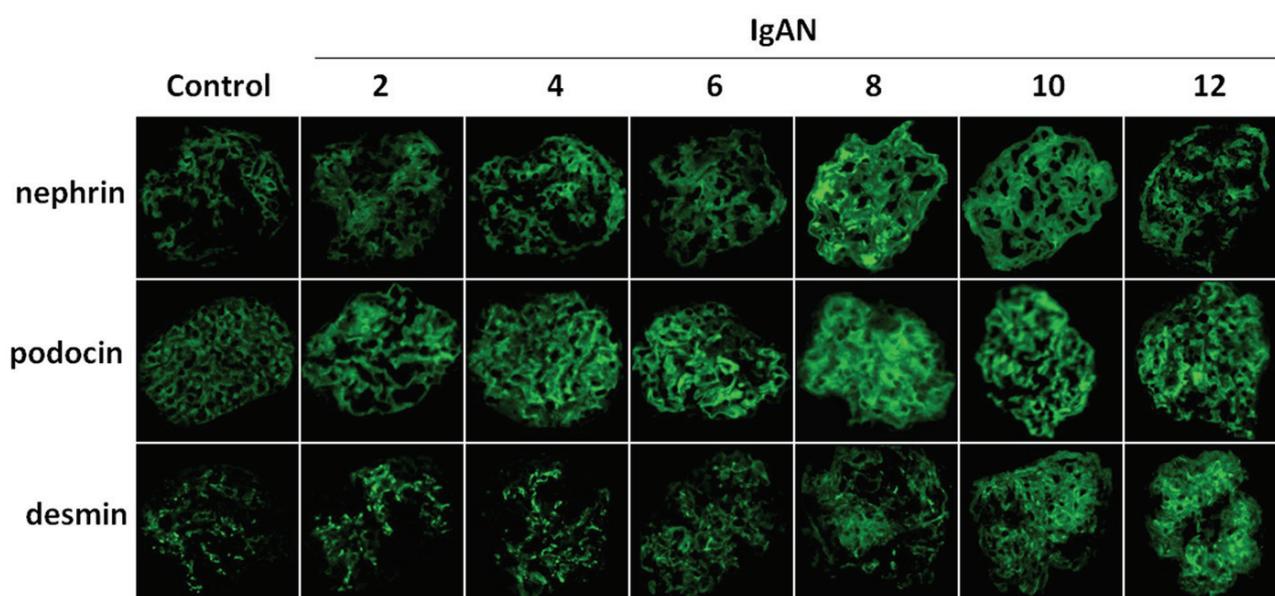


Fig. 5. Immunofluorescence staining of nephrin, podocin, and desmin in the glomeruli of control and IgAN rats ($\times 200$).

Light microscopy of the kidneys

Light microscopy indicated that pathological changes were first evident in the IgAN rats at the end of week-2, and these became worse over time (Fig. 2). At the end of week-8, the pathological changes were very obvious and characterized by glomerular swelling, increased glomerular volume, increased number of cells in the glomeruli, and moderate to severe hyperplasia of mesangial matrix and cells. In some of the renal tubules, there was necrosis of epithelial cells and infiltration of inflammatory cells. These same features are evident in pathological specimens of humans with IgAN.

Electron microscopy of the kidneys

Electron microscopy indicated that IgAN rats developed deformation and partial fusion of foot processes at the end of week-4 and that these changes

became more evident over time (Fig. 3). The foot processes were flat at the end of week-6, and diffuse fusion of foot processes was obvious at the end of week-10 and week-12 (Fig. 3), although effacement was not visible at this magnification. Again, these same features are evident in pathological specimens of humans with IgAN. In addition, IgAN rats developed mild hyperplasia of mesangial cells, collapse of the capillary loops, and necrosis of renal tubular epithelial cells (Fig. 3B).

Immunofluorescence of IgA

Immunofluorescence staining of IgA indicated that control rats had very little deposition of IgA in the glomeruli, and the fluorescence intensity was scored as “(-)” to “(+)” for all samples (Fig. 4). In contrast, IgAN rats had significant mass- or granule-like green fluorescence which was scored as “++” to “+++” for all

samples, although these images do not provide evidence for increased deposition in the thick loop region. Most of

this fluorescence was in the glomeruli. Again, these results indicate successful establishment of IgAN.

Table 4. Relative concentration (mean \pm SD) of nephrin, podocin and desmin in the glomeruli of control and IAGN rats from week-2 to week-12.

Protein expression	Week						
	2	4	6	8	10	12	
Nephrin	Control	0.095 \pm 0.017	0.096 \pm 0.008	0.097 \pm 0.006	0.098 \pm 0.006	0.099 \pm 0.006	0.099 \pm 0.007
	IgAN	0.104 \pm 0.006	0.129 \pm 0.014 ^{***}	0.148 \pm 0.017 ^{***}	0.187 \pm 0.010 ^{***}	0.148 \pm 0.010 ^{***}	0.124 \pm 0.006 ^{***}
Podocin	Control	0.105 \pm 0.002	0.104 \pm 0.016	0.106 \pm 0.009	0.103 \pm 0.011	0.102 \pm 0.013	0.102 \pm 0.004
	IgAN	0.120 \pm 0.016 [*]	0.123 \pm 0.019	0.132 \pm 0.008 ^{***}	0.154 \pm 0.007 ^{***}	0.130 \pm 0.020 ^{**}	0.116 \pm 0.012 [*]
Desmin	Control	0.095 \pm 0.009	0.098 \pm 0.007	0.099 \pm 0.008	0.100 \pm 0.004	0.101 \pm 0.007	0.101 \pm 0.007
	IgAN	0.110 \pm 0.007 ^{**}	0.121 \pm 0.017 ^{**}	0.137 \pm 0.009 ^{***}	0.149 \pm 0.007 ^{***}	0.158 \pm 0.006 ^{***}	0.170 \pm 0.008 ^{***}

The comparisons were conducted by Student's *t*-test. Eight rats were in each group at each time point. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to control group.

Glomerular expression of nephrin, podocin, and desmin proteins

Immunofluorescence staining for nephrin, podocin, and desmin indicated that the control rats had moderate expression of all three proteins, and that expression of each protein remained relatively constant over the 12 week experiment (Table 4). However, IgAN rats had increased expression of nephrin at week-4 ($p < 0.001$), maximal expression at week-8, and then a gradual decline (Fig. 5). Nephrin expression was significantly greater in IgAN rats than control rats from week-4 to week-12 ($p < 0.001$ for all time points). In control rats, nephrin had a continuous distribution along the capillary loops, but IgAN rats had much greater expression of nephrin at week-2, before the up-regulation (Fig. 5). Interestingly, changes in the expression and distribution of nephrin preceded the onset of proteinuria (Tables 3 and 4, Fig. 5).

IgAN rats had increased expression of podocin at week-2 ($p < 0.05$), maximal expression at week-8, and then a gradual decline (Fig. 5, Table 4). Podocin expression remained significantly higher than control rats at week-12 ($p < 0.05$). In control rats, podocin had a continuous distribution along the capillary loops, but IgAN rats had much greater expression of podocin at week-2. As with nephrin, changes in the expression and distribution of podocin preceded the onset of proteinuria (Tables 3 and 4, Fig. 5).

IgAN rats had increased expression of desmin at

week-2 ($p < 0.01$) and desmin expression continued to increase up to week-12. As with nephrin and podocin, changes in the expression and distribution of desmin preceded the onset of proteinuria (Tables 3 and 4, Fig. 5).

Expression of nephrin, podocin, and desmin mRNAs in the renal cortex

IgAN rats had increased expression of nephrin and podocin mRNAs in the renal cortex at week-2 ($p < 0.01$ for both), maximal expression at week-8, and then a decline to the level of control rats at week-12 (Table 5). IgAN rats had increased expression of desmin mRNA at week-8 ($p < 0.01$) and this remained higher than the control rats at week-12 ($p < 0.05$) (Table 5).

Correlation of expression of podocyte proteins and proteinuria

Finally, we used all of our data on IgAN rats ($n = 48$) and performed Pearson correlation analysis to assess the relationship between nephrin, podocin, and desmin proteins with 24-h urinary protein in the glomeruli of IgAN rats (Table 6). The results indicate that expression of nephrin, podocin, and desmin proteins were positively correlated with 24-h urinary protein ($p < 0.001$ for all). The relationship between nephrin, podocin and desmin mRNA in cortex of IgAN rats were also positively correlated with the 24-h urinary protein level ($p < 0.001$ for all).

Table 5. Relative expression of nephrin, podocin and desmin mRNAs (mean \pm SD) in the renal cortex of control and IAGN rats from week-2 to week-12.

mRNA expression	Week					
	2	4	6	8	10	12
<i>Nephrin Control</i>	0.176 \pm 0.021	0.186 \pm 0.024	0.186 \pm 0.029	0.180 \pm 0.016	0.182 \pm 0.011	0.184 \pm 0.015
<i>IgAN</i>	0.212 \pm 0.026**	0.323 \pm 0.022***	0.479 \pm 0.052***	0.694 \pm 0.061***	0.225 \pm 0.018***	0.192 \pm 0.012
<i>Podocin Control</i>	0.028 \pm 0.003	0.027 \pm 0.002	0.028 \pm 0.002	0.027 \pm 0.003	0.028 \pm 0.002	0.028 \pm 0.002
<i>IgAN</i>	0.043 \pm 0.002***	0.094 \pm 0.023***	0.138 \pm 0.005***	0.181 \pm 0.018***	0.122 \pm 0.009***	0.028 \pm 0.001
<i>Desmin Control</i>	0.150 \pm 0.015	0.154 \pm 0.020	0.154 \pm 0.019	0.150 \pm 0.009	0.153 \pm 0.011	0.154 \pm 0.018
<i>IgAN</i>	0.153 \pm 0.008	0.153 \pm 0.012	0.155 \pm 0.027	0.333 \pm 0.083***	0.242 \pm 0.036***	0.177 \pm 0.016*

The comparisons were conducted by Student's *t*-test. Eight rats were in each group at each time point. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to control group.

Table 6. Pearson's correlation coefficients of the relationship of urinary protein, nephrin, podocin and desmin.

mRNA	Urine protein	Nephrin Podocin Desmin		
		Nephrin	Podocin	Desmin
<i>Urine protein</i>	–	0.708*	0.460*	0.864*
<i>Nephrin</i>	0.460*	–	–	–
<i>Podocin</i>	0.577*	–	–	–
<i>Desmin</i>	0.527*	–	–	–

Data were expressed by Pearson's correlation coefficient. * $P < 0.001$, correlation coefficient was significantly different from zero.

Discussion

We used an established methodology to create rats with IgAN and then used this model to assess the roles of 3 podocyte proteins – nephrin, podocin, and desmin – in the pathogenesis of IgAN. We found the expression and distribution of nephrin preceded the onset of proteinuria and the expression of nephrin, podocin, and desmin proteins were positively correlated with 24-h urinary protein.

Interestingly, many of our model rats developed gross hematuria, yet had no evidence of segmental lesions, in contrast to the situation in humans. This may have been due to inherent biological differences of rats and humans or because the rapid induction of IgAN in our model did not allow time for the development of these pathological changes. Previous studies of congenital and hereditary kidney diseases, acquired kidney diseases, and animal models of kidney diseases have identified

changes in numerous podocyte-associated molecules (Luimula *et al.* 2000, Forbes *et al.* 2002, Guan *et al.* 2002, Luimula *et al.* 2002, Koop *et al.* 2003, Guan *et al.* 2004). These studies indicate that the SD complex appears to be regulated by nephrin, podocin, desmin, and CD2 associated protein (CD2AP) *via* their interactions with the podocyte cytoskeleton. However, there is currently no consensus on the role of these podocyte-associated proteins in the pathogenesis of proteinuria and kidney diseases (Ortmann *et al.* 2004, Jalanko 2003). Although the molecular mechanisms underlying proteinuria are not fully understood, animal and clinical studies suggest that changes in the expression and distribution of podocyte-associated molecules may damage the structure and function of the podocyte SD, and thereby disrupt glomerular filtration and cause proteinuria (Jalanko 2003, Ortmann *et al.* 2004, Li *et al.* 2008, Xu *et al.* 2010).

Previous studies of IgAN have produced conflicting results regarding the expression and distribution of podocyte-associated molecules. Patrakka *et al.* (2001) employed immunohistochemistry and *in situ* hybridization to measure expression of nephrin in the kidneys of patients with IgAN and reported no apparent changes. However, Gagliardini *et al.* (2003), using the same methods, reported up-regulation of nephrin. Koop *et al.* (2003) used immunohistochemistry and real-time quantitative PCR to measure expression of nephrin and podocin in the kidneys of IgAN patients and reported no apparent changes. Mao *et al.* (2006) performed immunohistochemistry, immunofluorescence microscopy, and real-time quantitative PCR to investigate the kidneys of children with IgAN. They reported that desmin protein was down-regulated, but that desmin

mRNA, podocin protein, and podocin mRNAs all had stable expression. They also reported that nephrin expression was positively associated with serum albumin in children with IgAN. Taken together, these studies seem to indicate an inconsistent relationship between disrupted expression of these proteins and IgAN. However, none of these studies investigated the association between proteinuria in IgAN with disrupted expression of podocyte-associated proteins and with pathological changes in foot process morphology.

The immunofluorescence results of the present study provide semi-quantitative evidence that changes in the expression and distribution of nephrin, podocin, and desmin preceded the deformation of foot processes and proteinuria, and that expression of these proteins were positively correlated with the concentration of urinary protein. These findings support our hypothesis that alterations in the expression and distribution of podocyte-associated proteins contribute to proteinuria in IgAN. Changes in the expression of podocyte proteins may disrupt the structure and function of podocyte SDs, promote the rearrangement of the podocyte cytoskeleton, and thereby disrupt glomerular filtration and cause proteinuria (Li *et al.* 2008). In addition, we also observed increased levels of glomerular nephrin and podocin in IgAN rats and that these changes occurred at nearly the same time as the increased expression (week-2). Guan *et al.* (2004) previously reported that abnormal distribution of nephrin and podocin preceded changes in the expression of both proteins in rats with polyarteritis nodosa (PAN), and speculated that abnormal distribution of these proteins was due to their elevated expression.

In our IgAN model, podocin expression changed before nephrin expression, consistent with previous reports (Schwarz *et al.* 2001, Guan *et al.* 2004, Xin *et al.* 2006). This finding suggests that up-regulation of podocin might be an initial process in the pathogenesis of proteinuria, and that podocin, as a component of the podocyte SD, plays a crucial role in maintaining the stability of nephrin in the SD complex (Schwarz *et al.* 2001). Our results also indicated that up-regulation of nephrin and podocin mRNAs occurred before up-regulation of desmin mRNA. Thus, the initial up-regulation of nephrin and podocin may change the SD complex and induce rearrangement of the podocyte cytoskeleton (Xin *et al.* 2006), resulting in alteration of desmin expression. The changes that we observed may result from a "chain reaction" that is triggered by podocyte injury, but we have no direct evidence for this

at present. Fan *et al.* (2005) previously reported that the interactions and associations of these proteins were dynamic and complex.

In our IgAN rats, expression of nephrin and podocin were up-regulated early and then decreased. This up-regulation may be compensatory and the down-regulation may be a result of podocyte injury, as previously suggested by Koop *et al.* (2003). Several lines of evidence support this hypothesis. Podocytes are mature cells which are well differentiated and have limited ability to proliferate (Hernandez *et al.* 2004). Thus, when they are injured, the expression and activities of cyclin-dependent kinase inhibitors (e.g. P21 and P27) increase, and podocytes have limited ability to compensate for this injury (Wang *et al.* 2004). Thus, in the present study, the increased expression of podocyte-associated proteins is due to increased synthesis within each cell, not from podocyte proliferation. Xin *et al.* (2006) and Peng *et al.* (2009) reported increased expression of nephrin and podocin in the kidneys of rats with adriamycin (ADR)-induced cardiomyopathy, but that the number of podocytes remained unchanged. Thus, podocytes may have a mechanism for protection against injury at the early stages of IgAN, but this mechanism may not be able to compensate for extensive injury. The mechanisms involved in the compensation of podocyte injury and the effects of compensation on renal function are still poorly understood and require further studies.

Our results also indicate that nephrin and podocin had abnormal cellular distributions in IgAN rats. Thus, the increased production of podocyte-associated proteins did not result in their accumulation in the plasma membrane, but was presumably due to accumulation in other organelles, such as endoplasmic reticulum. We hypothesize that these up-regulated proteins have altered biological function, leading to an unstable SD complex and an interruption of the connection between these proteins and the cytoskeleton, culminating in proteinuria (Roselli *et al.* 2004).

Our results also indicated that some of the changes in protein expression in IgAN rats were inconsistent with the changes in mRNA expression: (i) at week-2, nephrin and podocin mRNAs were markedly up-regulated, but the proteins were up-regulated at week-4; (ii) at week-12, nephrin and podocin mRNAs of IgAN rats were similar to those of control rats, but the proteins were significantly higher in model than control rats; (iii) at week-2, desmin mRNA expression was up-regulated and increased progressively up to week-12, but

expression of desmin protein was maximal at week-8, and then declined. Taken together, these results indicate that dysregulation of the expression of these genes is due to translational and post-translational effects. Luimula *et al.* (2002) and Koop *et al.* (2003) also reported disparate changes in the expression of nephrin and podocin proteins and mRNAs in rats with PAN. The mechanism underlying the dysregulation of these genes should be further investigated.

In conclusion, our study of a rat model of IgAN indicated that changes in the expression and distribution of nephrin, podocin, and desmin precede foot process fusion and proteinuria. Although our results do not prove a causal relationship between these events, our results and those of previous studies suggest that disruption in the

expression and distribution of these proteins is a crucial event in the pathogenesis of IgAN.

Conflict of Interest

There is no conflict of interest.

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