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Distribution of podocyte protein (44 KD) in different types of glomerular diseases

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Abstract The podocyte protein, 44 KD (pp44), is a podocyte-specific antigen that is selectively distributed in the cytoplasm of foot processes. It has been suggested that the pp44 antigen is associated with the cytoskeleton and helps to maintain the complex architecture of podocytes. To answer the question as to whether changes in pp44 expression are associated with changes in podocyte morphology , we investigated the distribution of the pp44 antigen in different kidney diseases. Twenty-one kidney biopsies and one nephrectomy specimen were studied by indirect immmunofluorescent technique and electron microscopy. The pp44-antigen is preserved in cases associated with foot process fusion. In contrast, the antigen could not be detected in areas of capillary wall necrosis, cellular crescents or early and advanced stages of focal segmental glomerulosclerosis – even in the presence of podocytes. Our results show that the pp44 antigen is preserved in diseases associated with reversible loss of foot processes (in cases with foot process fusion associated with proteinuria). In contrast, the pp44 antigen is not detectable in the area of FSGS and cellular crescents, suggesting that in these conditions, podocytes undergo irreversible injury even if they are still present on conventional light microscopy.

Key words Podocyte protein 44 KD · Focal segmental glomerulosclerosis · Podocyte damage

Introduction

The glomerular (visceral) epithelial cell layer of Bowman's capsule is composed of cells termed podocytes to describe the footlike appearance of numerous foot processes that arise from these cells. Glomerular podocytes cover the glomerular capillary wall and play an important part in the maintenance of the glomerular filtration barrier. Evidence from both pathological and experimental findings indicates that glomerular podocytes can undergo significant morphological changes both in the human and in experimental animals. With the development of proteinuria the foot processes are effaced and fused to form a continuous cytoplasmic compartment that covers the glomerular basement membrane (GBM) [1–3, 5, 9, 15, 20, 22]. Partial loss of foot processes has also been described in response to renal ischaemia [21]. In addition to loss of foot processes, podocytes can also show swelling, vacuolization and focal detachment from the GBM both in humans [7, 12, 24] and in experimental models [6, 11, 23] especially in focal segmental glomerulosclerosis (FSGS) associated with severe proteinuria [16].

A monoclonal antibody (mAb) that specifically recognizes the podocyte protein 44 KD (pp44) has been characterized by Mundel et al. [18]. Immuno-electron microscopy allowed localization of the pp44 antigen to the microfilament containing areas in the podocyte foot processes in both rat and human kidney. The cell body and the cell membrane of podocytes and other nephron structures were not labelled. In the newborn rat kidney, pp44 antigen positivity first appeared during the capillary loop stage, when the formation of podocyte foot processes commences. On the basis of these data it has been concluded that the podocyte-specific pp44 antigen is associated with the cytoskeleton and has a role in maintaining podocyte architecture and function.

Based on this hypothesis , we wanted to answer the question as to whether the distribution of pp44 antigen is altered in glomerular diseases associated with changes in podocyte morphology. The results show that pp44 is lost in cases of FSGS even if the podocytes are still present morphologically. In the case of podocyte foot process effacement pp44 is well preserved.

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Material and methods

The distribution of the pp44 antigen was studied by means of the indirect immunofluorescent technique in 5 normal kidney speci-

mens taken at some distance from tumour-bearing kidneys and in 21 kidney biopsies affected by different glomerular diseases (see Table 1). One transplant nephrectomy specimen with recurrent focal segmental glomerulosclerosis (FSGS) was also studied. The mAb against the pp44 antigen was kindly provided by Dr. P. Mundel and Professor W. Kriz, University of Heidelberg, Germany. It has been extensively characterized by Mundel et al. [18].

Samples of kidney specimens were snap frozen in liquid nitrogen. Frozen sections (5 um) were mounted onto glass slides, welded in plastic bags, and stored at –70°C. Before staining, the slides were thawed for 5 min, fixed in acetone for 10 min at room temperature and incubated with the appropriately diluted mouse mAb (1:5) for 30 min. After washing in PBS the sections were incubated with fluorescein (FITC)-labelled F(ab)2 fragments of anti-

Table 1 Distribution of pp44 antigen in different types of kidney diseases (*GN* glomerulonephritis, *TPL* kidney transplant, *N* normal, *FL* focal loss, * present, *NG* no glomeruli, – not significant, + focal, ++ partial, +++ complete)

No.	Diagnosis	pp44 Antigen distribution	Focal loss of detectable pp44 antigen positivity corresponds to:			Electronmicroscopic evaluation of podocytes with respect to foot processes	
			FSGS	Cresent	Synechia	Focal foot process destruction	Foot process fusion
1	TPL. Vascular rejection, normal glomeruli	N	0	0	0	*	–
2	Mesangioproliferative GN with crescents	FL	0	*	0	NG	NG
3	IgA GN: Mesangioproliferative GN with FSGS and crescents	FL	*	*	*	NG	NG
4	Exsudative GN with fibrin thrombi in glomeruli	FL	Protein droplets in the podocyte over loop necrosis	0	0	*	++
5	IgA: Minor glomerular abnormalities	N	0	0	0	0	–
6	Mesangioproliferative and epimembraneous GN, Lupus	FL	0	*	*	0	+++
7	TPL: Glomerulopathy and glomerulis	FL	0	0	0	NG	NG
8	Epimembraneous GN and segm. foc. proliferative and sclerosing GN	FL	0	0	*	0	+++
9	Mesangioproliferative GN	N	0	0	0	0	+
10	Mesangioproliferative GN with FSGS and crescents	FL	*	*	*	0	++
11	TPL: Glomerulitis and glomerulopathy	FL	0	0	*	NG	NG
12	TPL: Non-specific glomerulopathy	N	0	0	0	0	–
13	TPL: Micronodular glomerulosclerosis	N	0	0	0	NG	NG
14	TPL: Mesangioproliferative GN with crescent	FL	0	*	*	0	+
15	TPL: Diffuse diabetic nephrosclerosis	N	0	0	0	*	+
16	Diffuse focally segmentally necrotising GN with crescents	FL	0	*	*	0	+++
17	Unclassified GN with extensive protein depots	N	0	0	0	*	+++
18	IgA: minor glomerular abnormalities	N	0	0	0	0	–
19	TPL: Diffuse segmentally accentuated proliferative GN	FL	0	*	0	*	+
20	Glomerular minimal change	N	0	0	0	*	+++
21	Membranoproliferative GN; lobular type	N	0	0	0	0	+++
22	TPL: Idiopathic focal segmental glomerulosclerosis	FL	*	0	0	*	+++

mouse IgG + IgM (AN-DER-GRUB Biological Research, Vienna, Austria) for 30 min. Subsequently, the sections were stained with PAS to permit optimal correlation between regular histology and the immunofluorescence staining pattern.

Double staining experiments were performed in normal human kidney and the nephrectomy specimen with FSGS to confirm the localization of the antigen to the podocytes and to study the sequence of the noted loss of pp44 positivity in FSGS. Podocytes were labelled with a rabbit anti-vimentin antibody (1:20, Bio-Science-Product AG, Emmenbrücke, Switzerland) and visualized with a fluorescein (DTAF)-labelled goat anti-rabbit IgG (Jackson Immunoresearch, Baltimore, West Grove Pa.). The mAb binding was detected with Texas red-labelled goat anti-mouse immunoglobulin (1:80, Sigma). As controls, either the first or the second specific reagents were replaced by PBS, and the sequence of staining was reversed. The sections were viewed under a Zeiss UV microscope with epi-illumination utilizing band pass filter combinations for selective red and green fluorescence.

Transmission electron microscopy was also performed according to standard techniques.

Results

In normal human kidney the anti-pp44 antibody showed strong positivity at the periphery of glomerular capillary loops (Fig. 1). By means of double labelling with anti-vimentin antibody as a marker of podocytes, pp44 reactivity was localized to the base of these cells. The vascular pole of the glomerulus was not stained. In the small vessels a weak, uneven positivity was seen. Other renal structures were not stained with the antibody.

The results showing the distribution of pp44 antigen in different kidney diseases are summarized in Table 1.

Among the 22 cases studied the pp44 antigen distribution was similar to that seen in normal kidney specimens in 10 (Fig. 1) .These included cases of vascular rejection with no glomerular abnormality by light microscopy, IgA-nephropathy with minor glomerular abnormalities, mesangioproliferative glomerulonephritis, nonspecific glomerulopathy, diabetic diffuse or nodular glomerulosclerosis, nonproliferative glomerulonephritis with extensive protein deposits, glomerular minimal change with nephrotic syndrome (Fig. 2) and membrano-proliferative glomerulonephritis. In none of these cases was either FSGS or crescent and synechia formation present.

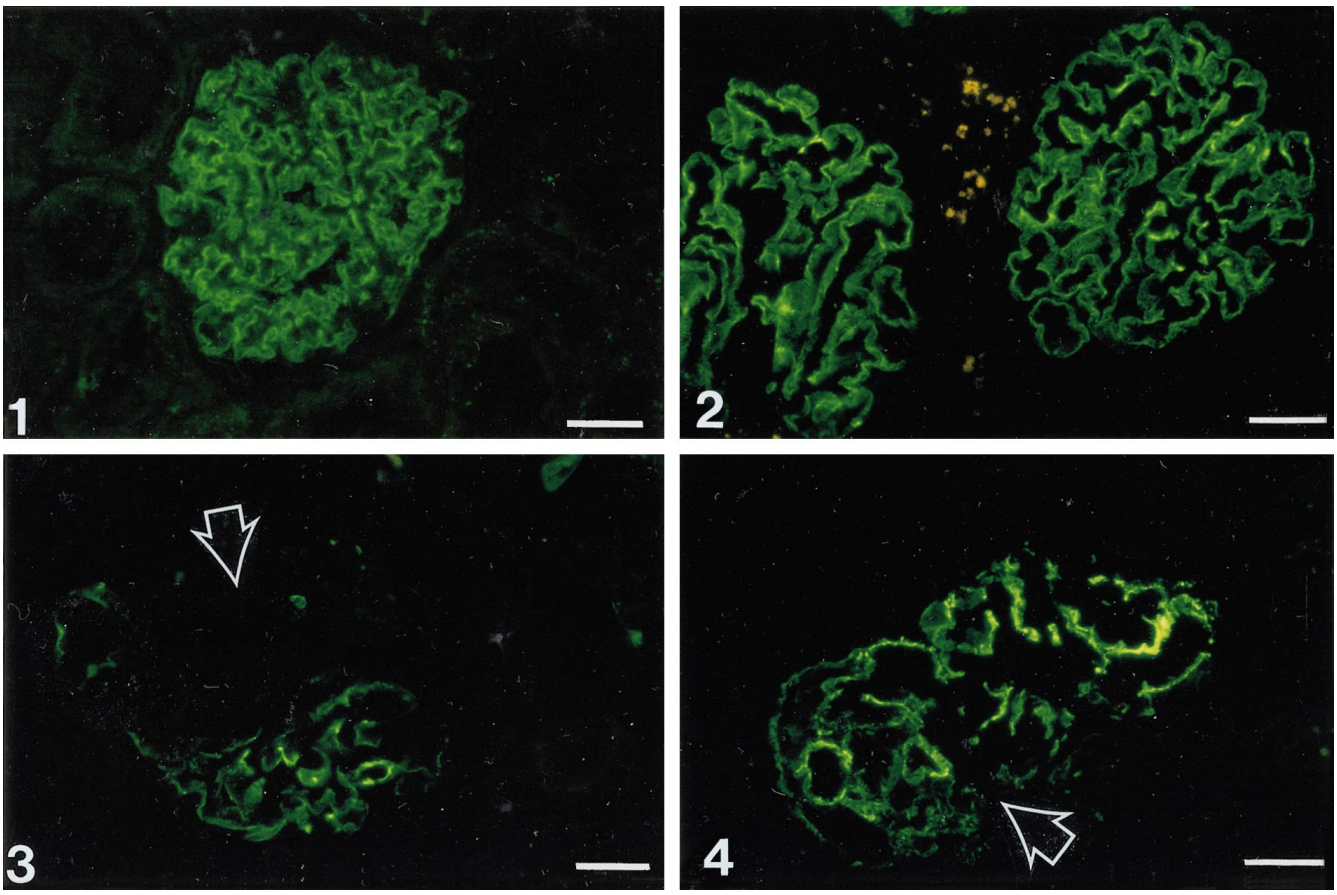
Focal loss of detectable pp44 positivity affecting the whole circumference of at least one capillary loop was

Fig. 1 Distribution of pp44 antigen in normal human kidney. Note the positivity at the periphery of the glomerular capillary loops corresponding to the foot processes of podocytes. ×250, *bar* 40 μm

Fig. 2 Glomerular minimal change. The distribution of the pp44 antigen is similar to that in normal glomeruli. ×250, *bar* 40 μm

Fig. 3 Diffuse focally and segmentally necrotizing glomerulonephritis with crescents. Cells in the area of segmental necrosis surrounded by the crescent (*arrow*) do not stain with the anti-pp44 antibody. ×250, *bar* 40 μm

Fig. 4 Lupus glomerulonephritis, type V. In the area of fibrotic crescent (*arrow*) no or only trace positivity for the pp44 antigen is visible. ×250, *bar* 40 μm



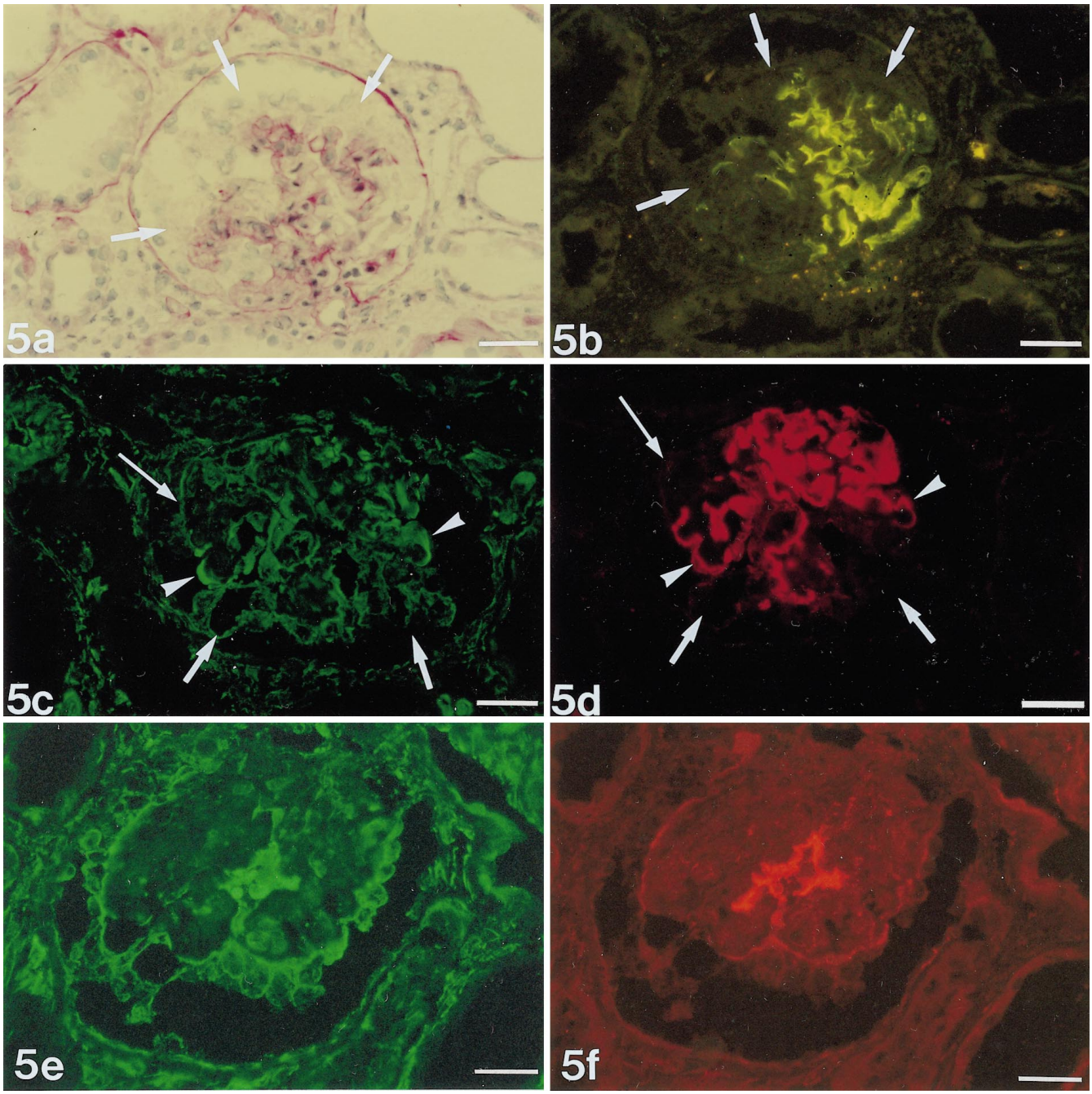


Fig. 5 **a–f** Focal segmental glomerulosclerosis (FSGS). **a**, **b** Early FSGS. The same glomerulus stained **a** by PAS and **b** for the pp44 antigen. Note that the antigen cannot be detected in the area of early FSGS lesions corresponding to the swollen podocytes, containing protein droplets and vacuoles (*arrows*). **c**, **d** Another glomerulus from the same case showing early (*short arrows*), and advanced (*long arrow*) stages of FSGS stained for **c** vimentin and **d** the pp44 antigen utilizing double immunofluorescence. Note the pp44 antigen is basally localized in the normal podocytes (*arrowheads*), as indicated by the pp44 positivity co-lined with the base of podocytes (*arrowheads*) labelled with the anti-vimentin antibody. By contrast, the pp44 antigen cannot be detected in the area of FSGS lesions. **e** (vimentin) and **f** (pp44 antigen) glomerulus from the same case, with global sclerosis capped by small rounded podocytes. Note the pp44 antigen cannot be detected in the morphologically altered podocytes in the periphery, whereas strong positivity is seen both for vimentin and for the pp44 antigen in the few well preserved podocytes present in the central portion of the glomerulus. ×250, *bars* 40 μm

seen in the remaining 12 cases. Based on the light microscopical analysis of the immunostained frozen sections, we found that the focal loss of pp44 positivity corresponded in 11 of the 12 cases, to focal segmental lesions, including podocyte injury over capillary loop necrosis (n = 1), FSGS (n = 2), crescents (n = 7) or synechiae (n = 7) in a variety of different forms of glomerulonephritis or transplant-related glomerular lesions. This is demonstrated in Fig. 3, where the loss of pp44 positivity corresponds to capillary wall necrosis associated with cellular crescent formation. The pp44 antigen is also undetectable in the area of a fibrotic crescent (Fig. 4). No positivity for the pp44 antigen was seen in the area of global glomerulosclerosis.

Based on the double labelling study and on the analysis of the PAS-stained frozen sections of the nephrecto-

my specimen with recurrent FSGS in a renal transplant, we found that loss of pp44 antigen positivity occurs early during the development of FSGS. Not only the small rounded podocytes capping advanced sclerotic lesions with capillary loop obliteration (Fig. 5c–f) proved to be negative, but also podocytes showing swelling, vacuolization and protein droplets (Fig. 5a and b) were also unreactive with the antibody.

On electron microscopy foot process fusion of various degrees (7 complete, 6 partial) was noted in 13 cases (Table 1). The pp44 antigen positivity was preserved in all cases except in glomeruli showing focal segmental lesions on light microscopy, in the form of capillary loop necrosis, FSGS, crescents and/or synechiae. In 3 of these cases focal destruction of podocyte foot processes was also noted in the glomerulus processed for electron microscopy and was associated with focal detachment of podocytes from the underlying GBM (Table 1). Focal foot process destruction was noted on electron microscopy in 4 other cases in which the pp44 antigen distribution pattern appeared to be normal according to light microscopy.

Discussion

We investigated the distribution of the pp44 antigen in normal human kidney and in different renal diseases. Our results are in agreement with those obtained by Mundel et al. [18]. Immunoelectron microscopy revealed that the positivity corresponds to the foot processes of podocytes [18]. We found that the antigen was preserved in cases with foot process fusion associated with proteinuria. Experimental findings and studies on human kidney biopsies clearly indicate that the loss of foot processes is a reversible morphological change [15, 19, 20, 22, 24].

In contrast to this, the pp44 antigen is focally and segmentally undetectable in the area of early and advanced stages of focal segmental glomerulo-sclerosis. Based on double staining experiments with vimentin as a marker for podocytes and on the light microscopical analysis of the immunostained frozen sections, it was clearly shown that the loss of pp44 antigen positivity was not due to the loss of podocytes themselves. The loss of pp44 antigen positivity did correspond to morphologically altered podocytes observed early during the development of FSGS lesions, and to podocytes capping advanced sclerotic loops. Other antigens, which are not limited to the foot processes but cover the whole podocytic cell surface (e.g. CALLA, endopeptidase [4, 8, 17] C3b receptor), are also lost in both early and advanced FSGS [13]. Alterations in integrin subunit expression are also found in these podocytes, depending on the stage of FSGS [14]. Fleming [10], reported that podocytes form desmosomes over permanent sclerosis, and express desmosomal components such as desmoglein I and desmoplakins. Our findings together with the observations cited above support the interpretation that podocytes in FSGS undergo a phenotypic change, reflecting irreversible injury.

Loss of pp44 antigen positivity was also noted in diseases associated with capillary wall necrosis, cellular or fibrotic crescents and synechiae. In the area of capillary wall necrosis associated with swollen podocytes and cellular crescents, the loss of pp44 positivity can be associated with podocytic injury similar to that seen in the injured podocytes in FSGS lesions. In fibrotic crescents and synechia formation the loss of pp44 positivity was obviously due to necrosis of podocytes replaced by fibrosis. On electron microscopy, focal destruction of podocyte foot processes associated with focal segmental detachment of podocytes from the GBM was seen in some of the cases with loss of pp44 antigen positivity. The immunohistochemical finding together with the ultrastructural observations support the assumption that podocytes suffer irreversible morphological change, including permanent loss of organized foot processes in all three conditions, in FSGS, in crescents formation and capillary wall necrosis.

In summary, our results show that the pp44 antigen is preserved in diseases associated with reversible loss of foot processes, that is to say in cases with foot process fusion associated with proteinuria. In contrast, the pp44 antigen is not detectable in the area of FSGS and cellular crescents, suggesting that in these conditions, podocytes undergo irreversible injury even if they are still seen to be present on conventional light microscopy.

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