http://www.kidney-international.org

see commentary on page 236

Urinary CD80 is elevated in minimal change disease but not in focal segmental glomerulosclerosis

Eduardo H. Garin[1], Wei Mu[2], John M. Arthur[3], Christopher J. Rivard[4], Carlos E. Araya[1], Michiko Shimada[2,4] and Richard J. Johnson[2,4]

[1]Department of Pediatrics, University of Florida, Gainesville, Florida, USA; [2]Department of Medicine, University of Florida, Gainesville, Florida, USA; [3]Department of Medicine, Medical University of South Carolina, Charleston, South Carolina, USA and [4]Division of Renal Diseases and Hypertension, Department of Medicine, University of Colorado, Denver, Colorado, USA

Controversy exists as to whether minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) represent different diseases or are manifestations within the same disease spectrum. Urinary excretion of CD80 (also known as B7.1) is elevated in patients with MCD and hence we tested whether urinary CD80 excretion might distinguish between patients with MCD from those with FSGS. Urinary CD80 was measured in 17 patients with biopsy-proven MCD and 22 with proven FSGS using a commercially available enzyme-linked immunosorbent assay and its molecular size determined by western blot analysis. A significant increase in urinary CD80, normalized to urinary creatinine, was found in patients with MCD in relapse compared to those in remission or those with FSGS. No significant differences were seen when CD80 urinary excretion from MCD patients in remission were compared to those with FSGS. In seven of eight MCD patients in relapse, CD80 was found in glomeruli by immunohistochemical analysis of their biopsy specimen. No CD80 was found in glomeruli of two patients with FSGS and another MCD patient in remission. Thus, our study supports the hypothesis that MCD and FSGS represent two different diseases rather than a continuum of one disease. Urinary CD80 excretion may be a useful marker to differentiate between MCD and FSGS.

Kidney International (2010) **78,** 296–302; doi:10.1038/ki.2010.143; published online 19 May 2010

KEYWORDS: CD80; minimal change disease; nephrotic syndrome

Correspondence: *Eduardo H. Garin, Department of Pediatrics, University of Florida, 1600 SW Archer Road, HD 216, Gainesville, Florida 32610, USA. E-mail: garineh@peds.ufl.edu*

Received 8 October 2009; revised 20 February 2010; accepted 16 March 2010; published online 19 May 2010

Minimal change disease (MCD) is the most common type of nephrotic syndrome in children and accounts for approximately 80–90% of cases in those <10 years and 50% of cases in children >10 years.[1] The second most common type of nephrotic syndrome in children is focal segmental glomerulosclerosis (FSGS), which accounts for 10% of all cases in children.[1] Most children presenting with nephrotic syndrome due to MCD respond to corticosteroid therapy but some are steroid-dependent or, rarely, steroid resistant.[2] In contrast, most patients with FSGS are relatively resistant to corticosteroid therapy.[2]

The relationship between MCD and primary FSGS has remained controversial, as some experts have considered the two entities to represent a continuum of one disease whereas others consider them separate entities.[3] The question is compounded by those patients who on initial renal biopsy have been considered to have MCD, but who undergo a repeat biopsy due to steroid dependence or resistance and are found to have a lesion that resembles FSGS, often with some tubular atrophy and interstitial fibrosis. Whether the initial renal biopsy findings were because of sampling error and may have missed glomeruli displaying segmental sclerosis remains open to question. Unfortunately, in most cases, this type of patient has a slowly progressive deterioration of renal function, often resulting in renal replacement therapy.[4]

Proteinuria in both MCD and FSGS appears to be due to a circulating factor.[5,6] Savin *et al.* have described the presence of a 'vascular permeability factor' in FSGS patients.[7] However, the same authors were unable to detect the presence of this factor in MCD patients.[7]

We recently reported significantly elevated levels of CD80 (also known as B7.1) in the urine of subjects with MCD with active nephrotic syndrome, when compared with the urinary CD80 levels in healthy subjects and in MCD patients in remission.[8] In our initial report, we also tested small number of patients with other glomerular diseases, including FSGS, and found urinary CD80 levels that were similar to those observed in healthy controls.[8] Given these preliminary data, we tested the hypothesis that urinary CD80 levels may be able to distinguish MCD from FSGS. We also examined renal biopsies in a limited number of cases to determine if CD80

Table 1 | Demographic, laboratory data, and therapy for MCD patients in remission

Patient	Age (years)	Gender	Urinary sCD80 (ng/g creat)	Serum albumin (g/dl)	Up/Uc ratio[a] (or dipstick)	Treatment
1	20	F	25	4	0.05	Prednisone 60 mg QD Tacrolimus 2 mg BID Mycophenolate mofetil 500 mg BID
2	3	M	BLD[b]	NA[c]	0.34	Prednisone 24 mg QOD
3	3	F	150	NA	0.22	None (off steroids for 3 weeks)
4	2	M	33	NA	Neg	Prednisone 36 mg QOD Cyclosporin 30 mg BID
5	13	M	12	NA	0.1	Prednisone 30 mg QOD
6	8	F	2	NA	0.17	Prednisone 15 mg BID
7	3	M	BLD[b]	NA	Neg	Prednisone 24 mg QD
8	5	F	BLD[b]	NA	Neg	Prednisone 15 mg QOD (tapering)
9	4	M	6	NA	Neg	Prednisone 6 mg QOD Cyclosporin 40 mg BID
10	3	F	12	2.7	Trace	Prednisone 12 mg QOD Mycophenolate mofetil 340 mg BID Tacrolimus 1 mg BID
11	10	M	65	NA	0.17	Prednisone 40 mg QD Cyclosporin 75 mg BID
12	1	M	48	NA	100 mg%	Prednisone 30 mg QOD
13	5	F	71	NA	Neg	Prednisone 8 ml QOD Cyclosporin 75 mg BID
14	3	M	10	NA	0.24	None
15	14	F	BLD[b]	NA		Cyclosporin 100 mg BID Mycophenolic acid 360 mg BID
Mean ± s.e.m.	6.5 ± 1.4		29 ± 11		0.18 ± 0.03	

[a]Up/Uc, urinary protein/urinary creatinine.
[b]BLD, below limit of detection.
[c]NA, not available.

expression is present in podocytes of subjects with MCD and active nephrotic syndrome.

RESULTS

Demographics, laboratory tests, and immunosuppressive therapy at the time the urine samples were collected are shown in Tables 1–3. All patients with MCD in relapse were studied at the time the diagnosis of relapse was made. All but three of these subjects were on a tapering dose of immunosuppressive therapy. For patients in remission, urinary CD80 was measured at different times after remission. All but two patients in remission were receiving immunosuppressive drugs at the time of testing. Five of the FSGS patients were on immunosuppressive therapy at the time of collection (Table 3).

Urinary CD80 excretion in MCD and FSGS

A significant increase in urinary CD80 excretion was observed in MCD patients in relapse (524 ± 86 ng/g creatinine, mean ± s.e.m.) when compared with MCD patients in remission (29 ± 11 ng/g creatinine) ($P<0.0001$) and to those with FSGS (57 ± 9 ng/g creatinine) ($P<0.001$). No statistical differences in urinary CD80 excretion between MCD patients in remission and FSGS was found ($P=0.64$) (Figure 1).

A receiver-operating characteristic curve for urinary CD80 levels comparing MCD in relapse with patients with FSGS and MCD patients in relapse with MCD in remission are shown on Figure 2a and b. For MCD in relapse vs FSGS, the area under the curve was 0.99 and for MCD in relapse vs MCD in remission, the area under the curve was 1.00.

CD80 is expressed in podocyte in MCD patients in relapse

A limited number of biopsies were available for study, including seven cases of MCD in relapse, one case of MCD in remission, and two subjects with FSGS. The only MCD patient studied during remission of his nephrotic syndrome was a patient with steroid-dependent nephrotic syndrome who having suffered multiple relapses underwent a renal biopsy done after remission was induced. CD80 was present in the glomeruli of 7 of 7 MCD patients in relapse, but was minimal or absent in 2 of 2 subjects with FSGS and in the one subject with MCD in remission. Of the seven MCD patients in relapse, none showed tubular staining for CD80 (Figure 3).

Figure 4 shows CD80 staining in glomerulus of a patient with MCD in relapse (Figure 4a), absence of CD80 staining in glomerulus from MCD patient in remission (Figure 4b) and minimal segmental CD80 staining in a glomerulus from an FSGS patient. This latter patient (patient no. 3, Table 3), had a concomitant urinary CD80 excretion of 75 ng/g creatinine. Figure 5a and b show two glomeruli from an MCD patient in relapse stained for CD80 in red (Figure 5a) and podocin in green (Figure 5b). Figure 5c and d shows a glomerulus from an MCD patient in relapse stained for CD80 with double immunostaining for CD80 and podocin that shows co-localization (Figure 4d).

Urinary CD80 is cell membrane-associated. Western blotting was performed of urine proteins in patients with MCD to determine if the CD80 was soluble CD80 (MW 23 kDa) or membrane-associated CD80 (MW 53 kDa). The molecular weight of the CD80 in patients with active or

Table 2 | Demographic, laboratory data, and therapy for MCD patients in relapse

Patient	Age (years)	Gender	Urinary sCD80 (ng/g creat)	Serum albumin (g/dl)	Up/Uc ratio[a] (or dipstick)	Treatment
1	19	F	214	2.6	16.2	Prednisone 60 mg QD
2	3	M	521	NA[b]	10	Prednisone 33 mg QD
3	4	F	1139	2.5	4.3	None
4	3	M	500	2.4	0.89	Prednisone 3 mg QOD Cyclosporin 20 mg BID
5	13	M	737	3.3	6.2	None
6	8	F	201	2.7	7.9	Prednisone 15 mg BID
7	3	M	984	NA	5.5	Prednisone 27 mg QD
8	5	F	825	NA	3+	None
9	4	M	193	NA	3+	Prednisone 12 mg QOD
10	4	F	882	1.3	3+	Prednisone 4.5 mg QOD Tacrolimus 1 mg QD
11	11	M	380	1.1	22.7	Prednisone 10 mg QOD Cyclosporin 100 mg BID
12	1	M	725	1	10.37	Prednisone 30 mg QOD
13	5	F	200	2	3+	Prednisone 36 mg QOD Cyclosporin 75 mg BID
14	6	M	158	1.5	3+	Prednisone 39 mg QD
15	3	M	198	NA	3+	Prednisone 20 mg QOD
Mean ± s.e.m.	6.1 ± 1.2		524 ± 86	2.2 ± 0.3	7.7 ± 2.2	

[a]Up/Uc, urinary protein/urinary creatinine.
[b]NA, not available.

relapsing MCD was 53 kDa, consistent with the cell membrane-associated CD80 (Figure 6).

DISCUSSION

We tested the hypothesis that urinary CD80 excretion can distinguish MCD from FSGS. The hypothesis was based on our previous study in which we had found elevated levels of CD80 in the urine of subjects with MCD in relapse.[8] This study confirms the preliminary finding of an increased CD80 urinary level in MCD patients in relapse.[8] In contrast, urinary CD80 was not increased in any of the FSGS subjects. These data suggest that urinary CD80 represents a robust marker that may be able to distinguish MCD in relapse from FSGS and therefore may be useful as a diagnostic marker.

The molecular weight of CD80 in the urine was shown to be 53 KDa, consistent with the CD80 being the whole cell membrane-associated CD80 (ref. 9) as opposed to the circulating 23 kDa soluble CD80 that is known to be secreted by circulating B cells.[10,11] In addition, while tubular cells and dendritic cells can express CD80, the immunofluorescence studies in subjects with active MCD documented that CD80 was almost exclusively localized to podocytes with negative staining outside the glomerulus. Thus, these studies suggest that the source of the urinary CD80 is the podocyte.

Recently a role for podocyte CD80 has been shown in several experimental models of proteinuria.[12] Increased expression of CD80 in podocytes has been found in genetic, drug-induced, immune-mediated, and bacterial toxin-induced experimental kidney diseases with nephrotic syndrome.[12] In turn, CD80 expression in cultured podocytes has been shown to result in a decreased expression of nephrin, which is critical to maintain the glomerular capillary barrier to protein.[13] Consistent with this observation, the injection of lipopolysaccharide (LPS) to mice results in proteinuria and podocyte CD80 expression, but proteinuria fails to develop if LPS is injected into CD80 knockout mice.[12]

The mechanism responsible for inducing CD80 in the podocyte of MCD patients is currently under active study. One potential candidate is IL-13, which is a cytokine expressed by activated T cells. IL-13 levels are elevated in the serum of subjects with MCD, and IL-13 expression is increased in T cells isolated from these patients.[14,15] IL-13 overexpressing rats also develop nephrotic syndrome associated with podocyte CD80 expression in which the histological features resemble MCD.[16]

We have also postulated that MCD may be due in part to a defect in the ability of the immune system to turn off podocyte CD80 expression.[8] In this regard, regulatory T cells produce factors, such as soluble CTLA-4, that can bind to dendritic cells expressing CD80 and which act to block T-cell activation.[17] Some studies suggest CTLA-4 may also alter dendritic cell function. We have recently shown that T regulatory cells are functionally deficient in MCD subjects,[18] and soluble CTLA-4 levels tend to be low in the serum and urine of subjects with MCD in relapse.[8] Hence, one could postulate that continued stimulation (such as by IL-13), or ineffective censoring of CD80 expression by T-regulatory cells may underlie the pathogenesis of MCD.

Although our studies do not prove that the pathogenesis of MCD and FSGS are different, the data are consistent with the hypothesis that expression of CD80 by the podocyte may identify a steroid-sensitive form of MCD and suggest this entity may be distinct from cases that are steroid resistant (and who may in effect have an early form of FSGS). The concept that MCD is distinct from FSGS is also supported by the studies of Savin *et al.*,[7] who have identified a specific

Table 3 | Demographic, laboratory data, and therapy for FSGS patients

Patient	Age (years)	Gender	Urinary sCD80 (ng/g creat)	Serum albumin (g/dl)	Up/Uc ratio[a] (or dipstick)	Treatment
1	18	F	52	4.2	0.56	None
2	15	M	24	NA[b]	1.5	None
3	14	F	75	3.5	2.5	Prednisone 20 mg QOD Cyclosporin 100 mg BID
4	40	F	72	3.6	3+	None
5	23	F	88	4.1	2.2	None
6	17	F	10	2.7	23.6	Prednisone 7 mg QD Mycophenolate mofetil 500 mg AM, 700 mg PM Tacrolimus 1 mg AM, 0.5 mg PM
7	34	F	21	1.6	5.9	Cyclosporin 50 mg AM, 25 mg PM Sirolimus 5 mg QD Prednisone 10 mg QD
8	26	M	9	2.6	9.8	None
9	25	F	81	1.5	5.4	Prednisone 60 mg QD
10	56	M	31	2.3	7.9	Prednisone 20 mg QD Sirolimus 4 mg QD Tacrolimus 0.5 mg QD
11	81	F	54	2.3	8.7	None
12	26	F	184	<1.0	30.5	None
13	59	F	65	3.6	4.7[c]	None
14	27	F	142	NA	7.2	None
15	49	F	50	<1.0	5.8	None
16	15	F	32	3.0	4.7	None
17	61	F	5	3.3	3+	None
18	59	F	66	3.8	8.5[c]	None
19	18	M	21	3.4	3+	None
20	30	F	43	3.4	5.3	None
21	48	F	119	2.2	3+	None
22	42	F	58	2.7	4.0	None
Mean ± s.e.m.	35.8 ± 3.8		57 ± 9	2.8 ± 0.2	7.9 ± 1.8	

[a]Up/Uc, urinary protein/urinary creatinine.
[b]NA, not available.
[c]These two patients were included among the nephrotic patients because of their rather elevated urinary protein/creatinine ratio.

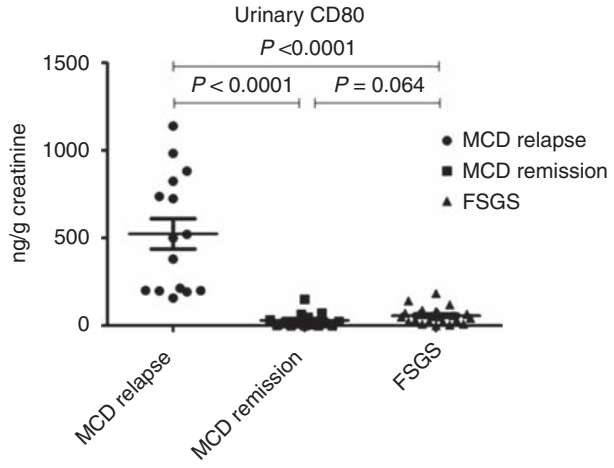

Figure 1 | Urinary CD80 levels in MCD patients in relapse, MCD patients in remission, and FSGS patients. FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease.

factor they believe may be responsible for some cases of FSGS as opposed to MCD.

Historically FSGS has been difficult to distinguish from MCD as early in its course FSGS may not be associated with glomerular sclerosis.[3] This has best been shown in subjects with FSGS who relapse immediately following transplantation, in which the renal biopsy shows a lesion that resembles MCD.[19] This should not necessarily be viewed as surprising, as the proteinuria in subjects with FSGS is known to be due to a generalized glomerular capillary wall defect as noted by the foot process fusion observed by electron microscopy. Indeed, the segmental sclerosing lesions are thought to be a consequence of the prolonged proteinuria, perhaps due to mesangial activation[20,21] or the development of synechiae with collapse of a segment of the glomerular tuft.[22] Our studies raise the possibility that urinary CD80 may provide a noninvasive means for distinguishing these two entities.

Sixteen of the FSGS were older than 21 years. Therefore, it may be argued that the low CD80 levels observed in these subjects could be the consequence of an increased urinary creatinine present in adults compared with children rather than a true low level of urinary CD80 excretion. However, we believe that the low urinary CD80/creatinine ratios in FSGS patients are not because of higher urinary creatinine levels because the urinary excretion of CD80 in MCD patients in remission (29 ± 11 ng/g creatinine (mean ± s.e.m.)) was not different than the observed excretion in the FSGS patients who were younger than 21 years (35 ± 6 ng/g creatinine (mean ± s.e.m.)) and, in contrast to what would have been

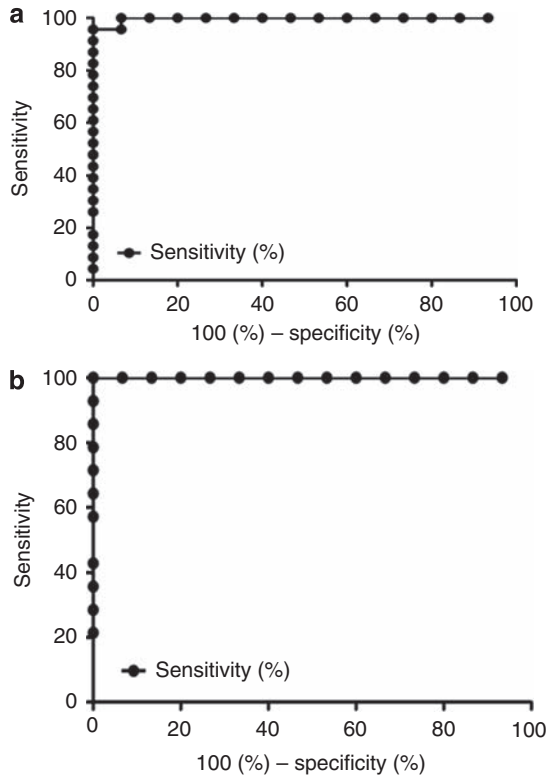

Figure 2 | Receiver operating characteristic curves for differentiating MCD and FSGS. ROC analysis of urinary CD80 levels comparing MCD patients in relapse and FSGS (**a**) and MCD patients in relapse and remission (**b**). MCD, minimal change disease.

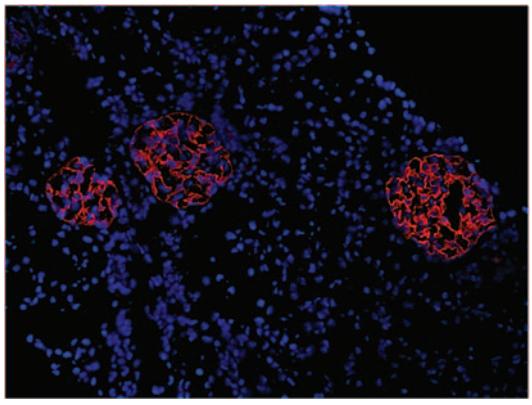

Figure 3 | Minimal change disease in relapse. CD80 is present in glomeruli (red stain) but not in tubules.

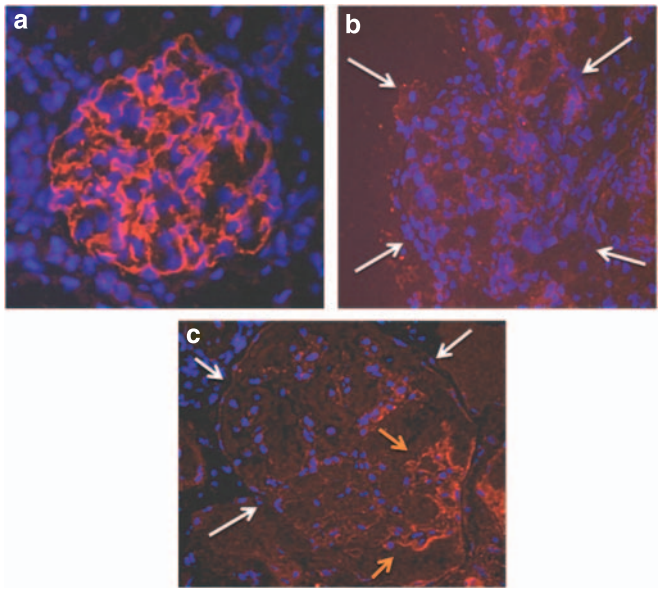

Figure 4 | CD80 in glomerulus of minimal change disease and focal segmental glomerulosclerosis patients. (**a**) CD80 is expressed (red stain) in glomerulus from an MCD patient in relapse. (**b**) CD80 stain is absent in glomerulus of minimal change disease patient in remission and (**c**) minimal segmental stain for CD80 in patient with focal segmental glomerulosclerosis. Urinary CD80 excretion in this patient was 75 ng/g creatinine.

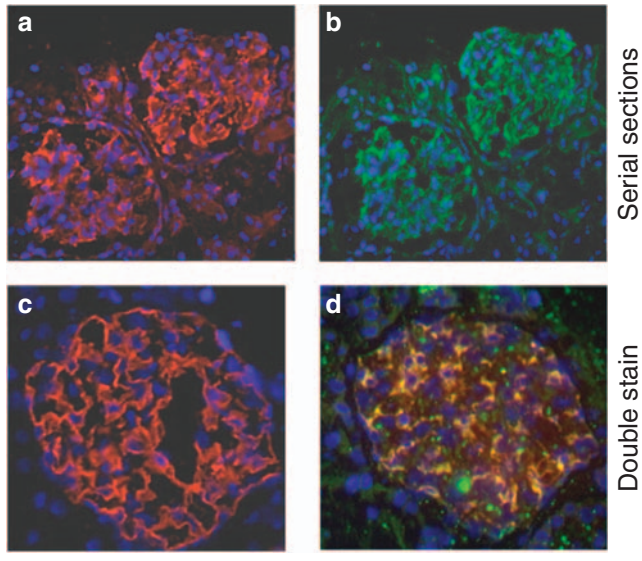

Figure 5 | Co-localization of CD80 and podocin in glomerular capillary walls. (**a** and **c**) CD80 is expressed (red stain) in glomeruli of two MCD patients in relapse. (**b**) Podocin is expressed (green stain) in glomeruli of MCD patients in relapse. (**d**) CD80 and podocin co-localize at the glomerular capillary wall.

expected, the CD80 urinary excretion in those greater than 21 years tended to be higher (68 ± 12 ng/g creatinine (mean ± s.e.m.)) and not lower than those with FSGS who were less than 21 years ($P = 0.16$). Furthermore, the remarkable separation of urinary CD80 excretion, coupled with the differences in immunostaining for CD80 of renal biopsies, suggests that the differences are likely due to the disease itself rather than to the age of the subject. Clearly additional studies by other groups are needed to confirm these findings.

In conclusion, urinary CD80 excretion is increased in MCD subjects in relapse but not in FSGS patients and, therefore, may be useful to distinguish MCD from FSGS. Although more renal

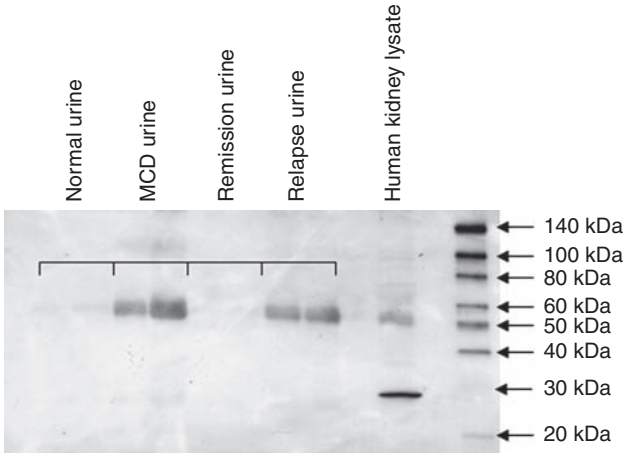

Figure 6 | Western blot of CD80 protein in normal urine from patients with MCD, in remission and relapse. Urine CD80 was immunoprecipitated to eliminate the interference from albumin present in some urines and is present as a ~53 kDa protein. For comparison, CD80 from human kidney lysate shows both a 53 and 26 kDa protein. MCD, minimal change disease.

biopsy samples need to be tested, our studies would support the hypothesis that podocyte CD80 expression in the pathogenesis of proteinuria in subjects with MCD.

MATERIALS AND METHODS

Patients

Seventeen patients with biopsy proven MCD (ages 1–19 years) were studied. Ten of the 17 patients were also studied in our previous publication.[8] However, data of these 10 patients in this report originated from different urine samples collected on subsequent relapses or on follow up evaluations while in remission. No urine samples from the previous study were rerun. Thirteen were studied both in relapse and after remission was achieved. Two were studied only during remission and two only during relapse. All MCD patients were followed at the University of Florida. Twenty-two patients with biopsy proven FSGS (ages 14–81 years) were included in the study (Tables 1–3). Seventeen of these patients presented with active nephrotic syndrome and five with proteinuria. Three patients with FSGS were seen at the University of Florida, Gainesville, FL, USA and 19 at the Medical University of South Carolina.

Definitions

MCD was defined based on renal biopsy findings according to established criteria by the International Study for Kidney Diseases in Children.[23] FSGS was also defined by renal biopsy findings showing focal and segmental consolidation of the glomerular tuft with increased extracellular matrix obliterating the capillary lumen with or without synechiae and hyalinosis and with either negative immunofluorescence or only segmental IgM or C3 staining.

Relapse was defined as proteinuria (>3.0 urinary protein (mg)/creatinine (mg) ratio or 3+ or greater by using the tetrabromophenol-citrate buffer colorimetric qualitative dipstick test) and a serum albumin <3.5 g/dl. Remission was defined as a urinary protein/creatinine ratio <0.2 (or less than 0.5 for children under the age of 5 years) and serum albumin >3.5 g/dl). Immunosuppressive therapy at the time of the study is shown in Tables 1–3.

The study was approved by the Institutional Review Board of the University of Florida and the Medical University of South Carolina. Informed consent was obtained from each patient.

Urinary CD80 measurements

Urinary CD80 was measured using a commercially available ELISA kit (Bender MedSystems, Burlingame, CA, USA) and results adjusted for urinary creatinine excretion. Urinary creatinine and protein and serum albumin were measured by an autoanalyzer.

Western blotting and protein extraction

CD80 molecular size was determined by western blot analysis. Owing to the high level of albumin in some urines, which can obscure CD80 detection, the protein was analyzed by antibody precipitation followed by western blot. An amount of patient urine equivalent to 37 μg creatinine was added to a microcentrifuge tube and 1 × PBS added to a final volume of 1 ml (total protein levels varied by 268–2394 μg). The diluted urine was first precleared of rabbit antibody by addition of 20 μl of Protein A agarose slurry (Roche, Indianapolis, IN, USA) and incubation at 4 °C with gentle mixing for 2 h. The sample was centrifuged at 6000 *g* at 4 °C for 2 min and the supernatant transferred to a new tube. A rabbit anti-human CD80 antibody (1 μl, no. NB110-55564, Novus, Littleton, CO, USA) was added and the sample incubated with mixing at 4 °C overnight. A 20 μl aliquot of Protein A agarose was added to bind the antibody and the sample incubated with mixing for 2 h at 4 °C. The sample was then centrifuged at 13,000 *g* for 5 min at 4 °C and the supernatant carefully removed. An equal volume (~30 μl) of 2 × loading buffer was added to the agarose pellet and the sample boiled for 5 min, centrifuged at RT at 13,000 *g* for 5 min and 30 μl of sample subjected to separation in a 4–20% gradient PAGE. The gel was transferred to PVDF membrane, blocked and incubated with Goat anti-human CD80 antibody (2 μl, no. AF140, R&D Systems, Minneapolis, MN, USA) overnight. The membrane was washed and incubated with a mouse anti-goat HRP-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 h. The blot was visualized using ImmunoStar HRP substrate (BioRad, Hercules, CA, USA).

Immunohistochemistry

Snap frozen fresh tissues in liquid nitrogen were embedded in OCT Compound and stored at −80 °C. Tissue sections were cut (4- to 8-μm thick) on a Lecia cryostat and mounted on superfrost plus (Fisher Scientific) slides. Slides were stored at −80 °C until use. Before staining, slides were warmed at room temperature for 30 min, fixed in ice-cold acetone for 5 min and air dried for 30 min. Subsequently, sections were rinsed in 1 × PBS-0.1%Tween 20 twice for 2 min at room temperature. Sections were incubated in normal donkey serum blocking solution, and then incubated for 1 h at room temperature using anti-human polyclonal CD80 goat antibody (R&D Systems) and rinsed twice with PBS-Tween 20 for 3 min each time. Antigen–antibody complexes were visualized with donkey anti-goat IgG-conjugated with red fluorescent 594 dye secondary antibodies (Alexa Fluor, catalog no. A-11058, Invitrogen, Carlsbad, CA, USA). Sections were incubated with the secondary antibody for 30 min at room temperature and rinsed with PBS-Tween 20 three times for 2 min. A cover slip with anti-fade fluorescent mounting medium containing DAPI was placed and sealed with nail polish. Slides were examined by immunofluorescence microscopy and analyzed by Zeiss Image Software. Frozen tissue sections were also double immunostained for podocin (Santa Cruz Biologicals) with

modifications of a previously published double immunofluorescence staining protocol.[24]

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

Support for this project was provided by generous monies from Gatorade and also from a startup package for RJJ at the University of Colorado.

REFERENCES

1. Nephrotic syndrome in children: prediction of histopathology from clinical and laboratory characteristics at time of diagnosis. A report of the International Study of Kidney Disease in Children. *Kidney Int* 1978; **13**: 159–165.
2. The primary nephrotic syndrome in children. Identification of patients with minimal change nephrotic syndrome from initial response to prednisone. A report of the International Study of Kidney Disease in Children. *J Pediatr* 1981; **98**: 561–564.
3. Meyrier A, Niaudet P. Minimal changes and focal segmental glomerulosclerosis. In: Davison AM, Cameron JS, Grunfeld JP, *et al.* (eds). *Textbook of Clinical Nephrology*. Oxford University Press: Oxford, 2005, pp 439–469.
4. Appel GB, Pollack MR, D'Agati V. Focal segmental glomerulosclerosis genetic and spontaneous causes. In: Feehally J, Floege J, Johnson RJ (eds). *Comprehensive Clinical Nephrology*. Mosby Elsevier: Philadelphia, 2007, pp 217–230.
5. Shalhoub RJ. Pathogenesis of lipoid nephrosis: a disorder of T-cell function. *Lancet* 1974; **2**: 556–560.
6. Meyrier AY. Treatment of focal segmental glomerulosclerosis with immunophilin modulation: when did we stop thinking about pathogenesis? *Kidney Int* 2009; **76**: 487–491.
7. Savin VJ, Sharma R, Sharma M *et al.* Circulating factor associated with increased glomerular permeability to albumin in recurrent focal segmental glomerulosclerosis. *New Engl J Med* 1996; **334**: 878–883.
8. Garin EH, Diaz LN, Mu W *et al.* Urinary CD80 excretion increases in idiopathic minimal-change disease. *J Am Soc Nephrol* 2009; **20**: 260–266.
9. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol* 2005; **23**: 515–548.
10. Wong CK, Lit LC, Tam LS *et al.* Aberrant production of soluble costimulatory molecules CTLA-4, CD28, CD80 and CD86 in patients with systemic lupus erythematosus. *Rheumatology (Oxford)* 2005; **44**: 989–994.
11. Kakoulidou M, Giscombe R, Zhao X *et al.* Human soluble CD80 is generated by alternative splicing, and recombinant soluble CD80 binds to CD28 and CD152 influencing T-cell activation. *Scand J Immunol* 2007; **66**: 529–537.
12. Reiser J, von Gersdorff G, Loos M *et al.* Induction of B7-1 in podocytes is associated with nephrotic syndrome. *J Clin Invest* 2004; **113**: 1390–1397.
13. Eto N, Wada T, Inagi R *et al.* Podocyte protection by darbepoetin: preservation of the cytoskeleton and nephrin expression. *Kidney Int* 2007; **72**: 455–463.
14. Tain YL, Chen TY, Yang KD. Implications of serum TNF-beta and IL-13 in the treatment response of childhood nephrotic syndrome. *Cytokine* 2003; **21**: 155–159.
15. Yap HK, Cheung W, Murugasu B *et al.* Th1 and Th2 cytokine mRNA profiles in childhood nephrotic syndrome: evidence for increased IL-13 mRNA expression in relapse. *J Am Soc Nephrol* 1999; **10**: 529–537.
16. Lai KW, Wei CL, Tan LK *et al.* Overexpression of interleukin-13 induces minimal-change-like nephropathy in rats. *J Am Soc Nephrol* 2007; **18**: 1476–1485.
17. Taylor PA, Lees CJ, Fournier S *et al.* B7 expression on T cells down-regulates immune responses through CTLA-4 ligation via T-T interactions [corrections]. *J Immunol* 2004; **172**: 34–39.
18. Araya C, Diaz L, Wasserfall C *et al.* T regulatory cell function in idiopathic minimal lesion nephrotic syndrome. *Pediatr Nephrol* 2009; **24**: 1691–1698.
19. Magee CC, Ansari MJ, Milford EL. Clinical management. In: Brenner BM (ed). *Brenner and Rector's The Kidney*, 8th edn. Saunders Elsevier: Philadelphia, 2008, pp 2138–2167.
20. Abbate M, Zoja C, Remuzzi G. How does proteinuria cause progressive renal damage? *J Am Soc Nephrol* 2006; **17**: 2974–2984.
21. Zandi-Nejad K, Eddy AA, Glassock RJ *et al.* Why is proteinuria an ominous biomarker of progressive kidney disease? *Kidney Int Suppl* 2004; **92**: S76–S89.
22. Kriz W. The pathogenesis of 'classic' focal segmental glomerulosclerosis-lessons from rat models. *Nephrol Dial Transplant* 2003; **18**(Suppl 6): vi39–vi44.
23. Churg J, Habib R, White RH. Pathology of the nephrotic syndrome in children: a report for the International Study of Kidney Disease in Children. *Lancet* 1970; **760**: 1299–1302.
24. Hugo C, Pichler R, Gordon K *et al.* The cytoskeletal linking proteins, moesin and radixin, are upregulated by platelet-derived growth factor, but not basic fibroblast growth factor in experimental mesangial proliferative glomerulonephritis. *J Clin Invest* 1996; **97**: 2499–2508.