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Original Article

CD80 expression and infiltrating regulatory T cells in idiopathic nephrotic syndrome of childhood

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Abstract ***Background*:** CD80 (also known as B7-1) is a co-stimulatory molecule that is expressed in biopsies and also excreted in urine in patients with minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS). CD80 is inhibited by the cytotoxic T-lymphocyte-associated-antigen 4 (CTLA4), which is mainly expressed on regulatory T cells (Tregs). Ineffective circulating Treg response is involved in the pathogenesis of nephrotic syndrome. In this study, we evaluated CD80 expression and infiltrating Tregs in children with MCD and FSGS.

***Methods*:** Evaluation of CD80 expression and semi-quantitative evaluation of Tregs (FOXP3-positive CD4 T cells) were carried out in 31 kidney biopsies (12 MCD, 19 FSGS) with immunofluorescence and immunohistochemistry staining.

***Results*:** All MCD sections were stained negative; whereas six out of 19 FSGS sections (all from steroid-resistant (SR) patients), including one from a Wilms' tumor 1 (WT1) mutation-positive FSGS patient, stained positive for anti-CD80 goat antibody, and negative for anti-CD80 rabbit antibody. FSGS biopsy specimens had significantly higher FOXP3-positive cells/mm^2 compared with MCD and control samples ($P < 0.001$). Biopsy samples from SR-FSGS patients ($n = 12$) with positive CD80 staining ($n = 6$) had significantly less Tregs (FOXP3-positive CD4 T cells) compared with CD80 (−) biopsies ($n = 6$; $P = 0.004$).

***Conclusion*:** CD80 expression was not detected in the majority of the archival biopsy sections and the results were not consistent across the different antibodies. In the SR-FSGS sections, however, CD80-positive biopsies had decreased FOXP3-positive CD4 T cells, suggesting that a decreased anti-inflammatory milieu may be the cause of increased CD80 expression.

Key words CD80, childhood, FOXP3, nephrotic syndrome, regulatory T cell.

Idiopathic nephrotic syndrome (INS), which is characterized by nephrotic-range proteinuria, hypoalbuminemia and edema, occurs in mainly two diseases in childhood: minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS). The pathogenesis of these two entities is unclear, and controversy exists on whether these two pathological diagnoses represent different stages of one common pathophysiological process or whether they are two different disease entities.[1] Historically, MCD has been considered as immune dysregulation syndrome of T cells, leading to podocyte foot process effacement, without podocyte death, and the disease attacks respond to steroids perfectly.[2] More than 80–90% of children with MCD, however, have one or more relapses, as well as long-term side-effects of steroid treatment.[2] FSGS has been attributed to a circulating proteinuric factor and genetic or metabolic disturbances, leading to podocyte injury, podocyte detachment and death.[3] Genetic testing can diagnose nearly one-third of steroid-resistant (SR) FSGS cases, in which patients have a very low chance of benefit from immunosuppressive drugs and a high risk of end-stage renal disease.[4] MCD and FSGS have similar clinical features at admission, and the treatment depends mainly on steroids, which have serious side-effects in children.[5] There is a need for biomarkers that can predict steroid dependence, disease resistance, and recurrence after transplantation, and also for more effective treatment modalities.[6]

A co-stimulatory molecule, CD80 (also known as B7-1) has recently been reported to be constitutionally expressed in biopsies from patients with proteinuric kidney diseases, including MCD, FSGS and membranous glomerulopathy, and that inhibition of CD80 by CTLA4-Ig (abatacept) represented a new treatment for SR and post-transplant FSGS.[7–9] CD80 is expressed on antigen-presenting cells and plays an important role in T-cell activation and is inhibited by the cytotoxic T-lymphocyte-associated-antigen 4 (CTLA4), which is mainly expressed on regulatory T cells (Tregs).[10] FOXP3 is the critical transcription factor

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for Treg development and function.[11] CD80 is also expressed on podocytes upon lipopolysaccharide stimulation, and its cytoplasmic tail blocks β1-integrin activation by competing with talin for β1-integrin binding.[8,9] According to a number of studies, during MCD relapse, an ineffective circulating Treg response or dysfunction of autoregulatory mechanisms, resulting in the inability of podocytes to express CTLA-4, is hypothesized to cause increased CD80 expression and podocyte motility.[12–15] The exact molecular mechanisms of the CD80–CTLA-4 interaction and clinical response to abatacept, however, remain the subject of debate.[16–18] Only a few studies have focused on infiltrating immune cells in INS.[19] The aim of the present study was to evaluate CD80 expression in kidney biopsy specimens from children with MCD/FSGS and clarify the role of infiltrating Tregs.

Methods

Patients

For immunohistochemistry and immunofluorescence analysis, we retrospectively evaluated all children with a clinical diagnosis of INS who were admitted to and followed at Hacettepe University Paediatric Nephrology Department. After closely assessing each patient's medical history and pathology, patients <1 year of age, diagnosed with secondary FSGS, non-nephrotic range proteinuria, mesangioproliferative glomerulonephritis and other glomerular diseases were excluded. All other available biopsy samples taken between 2014 and 2016 were analyzed in 2016. Twenty kidney tissue samples from the uninvolved portion of the nephrectomy specimens of Wilms' tumor patients were used as controls for immunohistochemistry and immunofluorescence staining.

Approval was obtained from the institutional ethics committee of Hacettepe University (GO 141210-22), and all patients or parents provided signed informed consent.

Immunofluorescence staining

Archival snap-frozen kidney biopsy tissues stored at −80°C for a median of 1.2 years (range, 0.1–1.8 years) were cut into 4 μm sections and air-dried for 10 min at room temperature. The sections were washed with phosphate-buffered saline (PBS) three times for 5 min and then fixed in 95% ethanol for 10 min. Subsequently, the sections were washed in PBS twice for 5 min each, incubated with a goat anti-human B7-1/CD80 antibody (R&D Systems, Minneapolis, MN, USA) at 1:100 dilution for 60–90 min at room temperature and then washed with PBS, followed by incubation with donkey anti-goat Alexa Fluor488 secondary antibody (Invitrogen, Carlsbad, CA, USA) at 1/100 dilution for 60–90 min at room temperature, as described by Yu *et al.*[9] A negative control slide (secondary antibody only) was run for each case, and a positive control slide from tonsil tissue for CD80 staining was run in each batch. Sequential sections were stained with an anti-human goat polyclonal antibody against a peptide mapped near the C-terminus of podocin (dilution 1/10; Santa Cruz Biotechnology, Dallas, TX, USA) and with a conjugated donkey anti-goat antibody (dilution 1/10; Santa Cruz Biotechnology) as a secondary antibody, as described by Agrawal *et al.*[20] Microimages were taken using a Zeiss microscope 4 axiocam HRC (Carl Zeiss AG, Oberkochen, Germany). Positivity for CD80 was defined as diffuse, linear podocyte staining in the CD80-stained section exceeding the score of the secondary antibody-stained control slide.

Immunohistochemistry staining

Immunohistochemical evaluation of the patient and control biopsy samples was performed using a Leica Bond-Max autostainer (Leica Biosystems, Buffalo Grove, IL, USA) on formalin-fixed paraffin-embedded and fresh frozen tissue samples. Sections of 3–4 μm were placed on poly-L-lysine-coated adhesive microscope slides. After dewaxing and rehydration or air-drying at room temperature, they were treated with Ethylenediaminetetraacetic acid (EDTA) and formalin and incubated with FOXP3 mouse monoclonal anti-human antibody (1:100 dilution, clone 236A/E7; Abcam, Cambridge, UK) and CD4 mouse monoclonal anti-human antibody (1:250 dilution; Abcam). For CD80 and podocin immunohistochemistry staining, anti-CD80 rabbit monoclonal anti-human antibody in a 1:150 dilution (clone EPR1157;[2] Abcam) and anti-NPHS2 (podocin) rabbit polyclonal antibody in a 1:75 dilution (Abcam) were serially stained. The sections were developed using the Bond Polymer Refine Detection system (Leica Biosystems) and then counterstained in hematoxylin, washed and dehydrated. Coverslips were then applied. Normal tonsil sections were used as positive controls for FOXP3 immunostaining. One positive control slide was stained with each batch. The primary antibody was substituted with PBS for negative control staining.

Semi-quantitative evaluation of immunostaining

Cells with clear lymphocyte morphology and distinct nuclear staining for FOXP3 but unstained cytoplasm (interpreted as Tregs) were scored as positive by a pathologist (D.O.), and the number of cells positive for FOXP3 was quantified, divided by the area (cells/mm^2). The pathologist (D.O.) was blinded to the patient clinical and laboratory parameters. The median area of the renal biopsy specimen was 6 mm^2, and the median number of glomeruli per biopsy specimen was 35 (range, 25–105 glomeruli).

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows 22.0 (IBM, Armonk, NY, USA). Descriptive analyses are presented as mean ± SD for normally distributed variables and as median (IQR) for non-normally distributed variables. Mann–Whitney *U*-test was used to compare laboratory results, and the chi-squared or Fisher's exact test (when chi-squared test assumptions did not hold due to low expected counts) was

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used to compare the immunostaining intensity of the biopsy samples. Correlation of biopsy findings with laboratory data was done with Spearman's test. $P < 0.05$ was considered statistically significant.

Results

Patients

In total, biopsy specimens were evaluated for 31 patient. Mean patient age at the time of biopsy was 10.1 ± 4.1 years. The main indication for biopsy was diagnosis of NS at ≥8 years of age or steroid dependency despite an adequate dose of calcineurin inhibitor. Twelve patients were diagnosed with MCD, six of whom were having their first NS attack at >8 years of age, were proteinuric, and were not using any drugs. The remaining patients with MCD were steroid dependent and were in remission with cyclosporine and low-dose steroids. Of 19 patients with FSGS, 12 patients had SR-FSGS and all were screened for podocin and WT1 mutations. Only one patient was found to have a WT1 exon:9: c.1228 + 4C > T mutation and included in the study. Clinical, pathological and laboratory findings at the time of biopsy are listed in Table 1.

CD80 immunofluorescence and immunohistochemistry staining

Twelve MCD and 19 FSGS sections were serially stained with podocin and goat anti-human CD80 antibody. All MCD sections stained negative with the goat anti-human CD80 antibody (Fig. 1c) (not exceeding the primary antibody negative slide Fig. 1a). Only 6/19 FSGS sections (31.6%) stained positive for CD80 antibody (Fig. 1d). These six sections were all from the 12 proteinuric SR-FSGS patients (Fig. 2. All sections from the same patients were also found to be negative for anti-CD80 rabbit monoclonal anti-human antibody (Fig. 3). Also, glomerular CD80 staining was noted in biopsies of a WT1 mutation-positive SR-FSGS patient taken at two different times.

FOXP3 immunostaining

Thirty-one available kidney biopsy samples were stained with FOXP3 and CD4. Glomerular, tubular and interstitial cells showed faint nuclear FOXP3 staining. Dense nuclear staining of FOXP3 and CD4 positivity confirmed FOXP3-positive T cells and was interpreted as Tregs (Fig. 4). FOXP3-positive CD4 T cells were observed in all biopsy specimens, predominantly in the interstitial area. FOXP3-positive cell count per mm^2 in FSGS biopsy specimens was significantly higher than that in MCD and healthy control sections ($P < 0.001$). The MCD sections, however, had a similar count of FOXP3-positive CD4 T cells per mm^2 in the interstitial area compared with control sections ($P = 0.843$). In the FSGS patients, the number of interstitial FOXP3-positive cells was not significantly correlated with serum albumin, estimated glomerular filtration rate or urine protein/creatinine ratio but had a strong, significant positive correlation with percentage of sclerotic glomeruli ($r = 0.830$, $P = 0.004$). In SRNS patients ($n = 12$), CD80 (+) biopsies ($n = 6$) had significantly fewer Tregs (FOXP3-positive CD4 T cells) compared with CD80 (−) biopsies ($n = 6$; $P = 0.004$) although there was no significant difference in other laboratory parameters or drug dosages (Table 2).

Table 1 Clinical, pathological and laboratory features of patients

	MCD ($n = 12$)		FSGS ($n = 19$)		
	First attack NS ($n = 6$)†	SDNS ($n = 6$)†	SDNS relapse ($n = 3$)†	SDNS remission ($n = 4$)†	Steroid-resistant NS ($n = 12$)†
Age at biopsy (year)	12 (10–13)	13 (9–14)	13 (8–15)	13 (11–15)	7 (4–14)
Early morning urine P/Cr (mg/mg Cr)	6.10 (4.50–8.90)	0.36 (0.15–0.40)	8.40 (7.54– 10.00)	0.36 (0.29–0.41)	5.60 (2.75–9.00)
Serum albumin (g/dL)	2.18 (2.02–2.30)	3.90 (3.60–4.23)	2.35 (1.89–2.40)	3.85 (3.75–4.05)	2.18 (1.82–3.05)
eGFR (mL/min)	145 (140–145)	122 (118–124)	154 (113–162)	112 (106–120)	145 (127–163)
Treatment at the time of biopsy					
Prednisolone	0	6 (100)	3 (100)	4 (100)	5 (41.7)
Cyclosporine	0	6 (100)	2 (66.7)	4 (100)	4 (33.3)
Prednisolone dose (mg/kg/day)	0	0.45 (0.34–0.47)	2.00 (0.95–15.00)	0.70 (0.47–1.00)	0.20 (0–.50)
Cyclosporine dose (mg/kg/day)	0	1.85 (1.20–2.30)	0.00 (0.00–3.40)	1.55 (0.50–2.65)	1.50 (0–2.45)
Sclerotic glomeruli (%)	0	0	1.5 (1.3–2.3)	1.9 (1.2–3.1)	31.5 (22.5–43.0)
FOXP3+ CD4+ cell count (cell/mm^2)	0.105 (0–0.305)	0.092 (0–0.203)	0.308 (0.248–0.308)	1.077 (0.556–1.214)	3.393 (1.672–5.788)
CD80 positivity	0 (0)	0 (0)	0 (0)	0 (0)	6 (50)

†Continous variables are showed as median (IQR) and categorical variables were shown as n (%). eGFR, estimated glomerular filtration rate (Schwartz formula); FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; NS, nephrotic syndrome; P/Cr, protein/creatinine; SDNS, steroid-dependent nephrotic syndrome.

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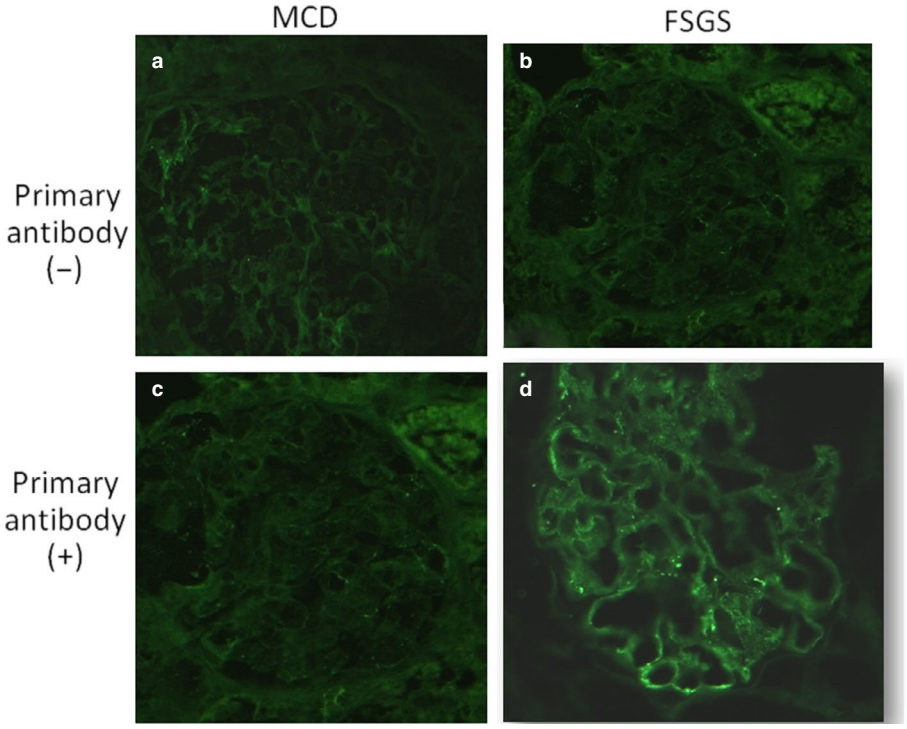

Fig. 1 (a,c) Negative immunofluorescence (IF) staining of minimal change disease (MCD) kidney section stained (a) only with secondary antibody (donkey anti-goat Alexa Fluor488 secondary antibody (Invitrogen) and (c) stained with primary antibody (goat anti-human B7-1/CD80 antibody, RD systems) and donkey anti-goat secondary antibody. (b) Negative IF staining of a focal segmental glomerulosclerosis (FSGS) kidney section stained only with secondary antibody. (d) Global 2+ diffuse, linear podocyte CD80 IF staining in an FSGS kidney section stained with primary antibody (goat anti-human B7-1/CD80 antibody, RD systems) and donkey anti-goat secondary antibody. (a–d) Original magnification × 200.

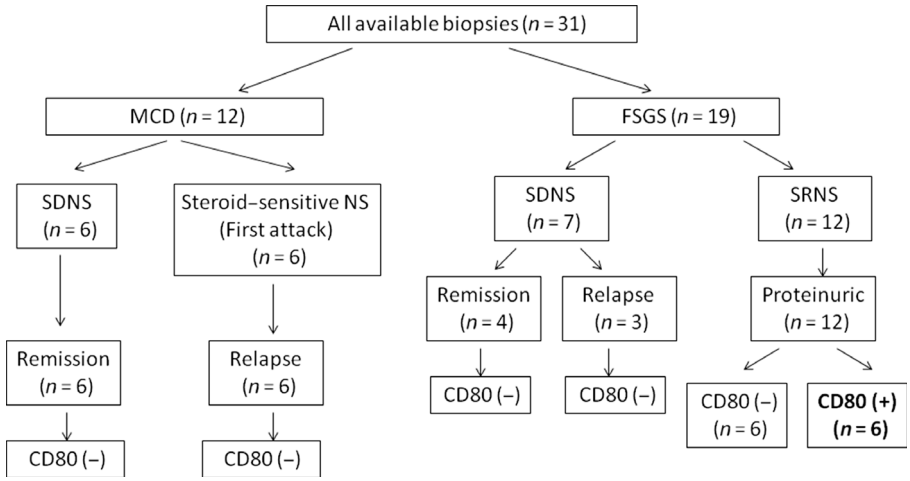

Fig. 2 Status of CD80 expression on goat anti-CD80 antibody staining. FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; NS, nephrotic syndrome; SD, steroid dependent; SR, steroid resistant.

Discussion

To our knowledge, this is one of the few studies evaluating CD80 expression in kidney biopsies of childhood NS patients, given that most previous studies involved mainly adult patients.[7,9,21] Garin *et al*. examined CD80 expression in a limited number of biopsies and noted CD80 expression specifically in MCD relapse sections.[7] Yu *et al*., however, noted CD80 expression in biopsies not only of MCD but also of FSGS patients, especially in cases of membranous glomerulopathy and of recurrent FSGS after transplantation.[9] They also suggested that abatacept (CTLA4-Ig, CD80 inhibitor),

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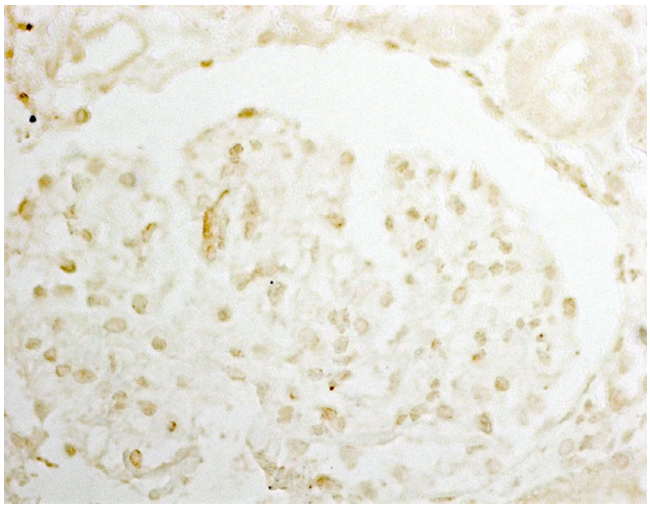

Fig. 3 Negative CD80 immunostaining in a focal segmental glomerulosclerosis specimen with anti-CD80 rabbit monoclonal anti-human antibody (Abcam; original magnification × 200).

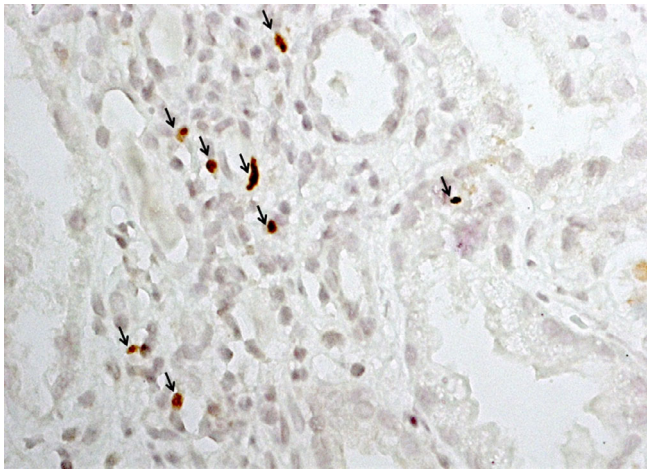

Fig. 4 Interstitial inflammatory cells showing strong nuclear FOXP3 immunopositivity (arrows; original magnification × 200).

induced remission in CD80-positive patients who had not responded to various immunosuppressive therapies before,[9] but their findings were not confirmed by groups who studied similar patient characteristics. Larsen *et al*. studied 60 patients with NS and did not detect CD80 expression on immunoperoxidase staining in paraffin-embedded tissue or on immunofluorescence staining in fresh frozen tissue.[21] Additional comprehensive studies also failed to detect CD80 expression in experimental murine models and biopsies from patients with proteinuric kidney disease.[16,22–24]

In contrast, increased urinary CD80 levels in MCD patients as compared with that in FSGS patients and healthy controls, has been reported.[7,25–27] Based on these studies, MCD was considered as a CD80pathy, and persistent CD80 expression was thought to be the major driving factor in MCD pathogenesis.[28,29] In addition, it was found that urinary CD80 could serve as a biomarker that could discriminate MCD from other histopathologies.[7,25–27] Recently, however, urinary CD80 was found to be elevated in patients with inherited NS and positively correlated with urinary protein level.[30] We did not detect CD80 expression in any of the biopsy samples of MCD patients using two different anti-CD80 antibodies, even in those who were not on immunosuppressive treatment. We also did not detect a linear staining pattern of CD80 in the majority of the FSGS sections (63.2%) on goat anti-CD80 antibody using similar immunostaining steps to those in previous studies.[9,21] Furthermore, we did not detect CD80 in any of the sections using an anti-CD80 rabbit monoclonal anti-human antibody, which questions the reliability of anti-CD80 antibodies in detecting CD80 expression. In the literature, there are several comments on this inconsistency. First, Benigni *et al*. suggested that CD80 staining may be an artifact of secondary antibody immunoglobulin G staining.[31] In order to exclude this possibility, we run a control slide not stained with the primary antibody, only with secondary antibody along with both primary and secondary antibody stained slide for each case. CD80 positivity was evaluated after comparing these two slides (Fig. 1). Second, the vintage effect mentioned by Mundel[6] could be a cause. We used kidney biopsies that had been snap-frozen and stored at −80°C for a median of 1.2 years (range, 0.1–1.8 years), but of the biopsy samples that tested positive for CD80, the storage duration ranged from 0.5 to 1.1 years, which was not different to that of the other sections. Third, the use of different antibodies for detection of CD80 in urine and biopsy samples could also be a cause, given that the enzyme-linked immunosorbent assay kits that quantify CD80 in urine detect soluble CD80 (23 kDa), whereas anti-CD80 antibodies used for CD80 detection in kidney biopsies target the extracellular domain of CD80. Further prospective studies are needed to correlate CD80 expression and excretion in INS.

We also noted positive CD80 expression in an SR-FSGS patient with *WT1* mutation. Positive CD80 expression in a patient with homozygous podocin mutation[32] and in an α3 integrin$^{-/-}$ mouse model has also been reported.[8] WT1 binds to *cis* regulatory regions of major cell-adhesion molecules of podocytes, including integrin and laminin,[33] which may explain the increased CD80 expression in the present patient. Also, urinary CD80 excretion was found to be elevated in patients with inherited NS, including a patient with *WT1* mutation.[30] It was suggested that CD80 is not only a marker of immunologic stimulus, but that some genetic disorders may also lead to increased CD80 expression, which may lead to podocyte activation in a vicious cycle.[34] Integrin was shown to play an important role not only for podocytes but also for Tregs, facilitating Treg cell contact-mediated suppression,[35] which may explain the decreased number of FOXP3-positive CD4 T cells in the FSGS patient with the *WT1* mutation.

According to Shalhoub's hypothesis, MCD is a disorder of cell-mediated immunity, particularly of T cells.[36] Many studies on the function and number of T-cell subsets in MCD have demonstrated increased pro-inflammatory T cells, especially the T-helper (Th)17 subset, in addition to decreased number and dysfunction of anti-inflammatory regulatory T cells,

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Table 2 SR-FSGS patient characteristics vs CD80 biopsy status (n = 12)

	C80 negative (n = 6)Median (IQR)	CD80 positive (n = 6)Median (IQR)	P-value
Sclerotic glomeruli (%)	34 (25–48)	27 (20–42)	0.470
Spot urine P/Cr (mg/mg)	3.1 (1.5–5.0)	7.5 (6.2–11.0)	0.078
Serum albumin (g/dL)	2.58 (1.94–3.20)	2.09 (1.69–2.70)	0.522
Serum creatinine (mg/dL)	0.22 (0.10–.69)	0.47 (0.41–0.60)	0.261
eGFR (mL/min)	149 (117–200)	145 (137–157)	0.749
Steroid dose at biopsy (mg/kg/day)	0.30 (0.10–0.50)	0.00 (0.00–0.50)	0.359
Cyclosporine dose at biopsy (mg/kg/day)	2.20 (1.00–2.50)	0.50 (0.00–2.00)	0.287
FOXP3+ CD4+ T cell count/mm^2	5.62 (3.75–32.49)	1.69 (1.49–2.20)	0.004

eGFR, estimated glomerular filtration rate (Schwartz formula); FSGS, focal segmental glomerulosclerosis; P/Cr, protein/creatinine; SR, steroid resistant.

particularly FOXP3-positive T cells in peripheral blood.[12] It was suggested that an imbalance in secreted cytokines by pro-inflammatory and anti-inflammatory cells may be a potential cause of increased podocyte activation, elevated CD80 expression and podocyte injury.[12,27] Supporting these findings, we found decreased FOXP3-positive CD4 T cells in CD80-positive biopsies compared with CD80(−) biopsies in SR-FSGS patients (n = 12). Given that we had a small number of patients and the majority of the patients were receiving immunosuppressive treatment, we could not reach a clear conclusion. We also found an increased number of infiltrating interstitial FOXP3-positive CD4 T cells in FSGS biopsies compared with healthy controls and MCD biopsies. FOXP3-positive CD4 T cells were positively correlated with sclerotic glomeruli percentage, suggesting that Tregs may be a marker of uncontrolled inflammation. Recently, in a diabetic nephropathy murine model, although CD80 expression did not increase compared with controls, it was found that abatacept, CTLA4-Ig, decreased albuminuria and infiltrating CD3+ T cell number.[37]

In conclusion, we did not detect CD80 expression in the majority of the present archival biopsy sections, and the results were not consistent across different antibodies. We did not detect CD80 expression in MCD biopsies even in patients who were not on immunosuppressive treatment. Only six of 19 FSGS biopsy sections (one of which was from a WT1 mutation-positive patient) were positive for CD80 expression with anti-CD80 goat anti-human antibody. The CD80 positive biopsies, however, had a decreased number of FOXP3-positive CD4 T cells, suggested that a decreased anti-inflammatory milieu may be a cause of increased CD80 expression.

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Disclosure

The authors declare no conflict of interest.

Author contributions

F.K.E., R.T., D.O. conceived of and designed the study. M.I., F.O., B.G., A.D., F.K.E., R.T., D.O. carried out data acquisition; D.O., R.T., F.K.E. analyzed and interpreted the data; R.T., F.K.E. carried out statistical analysis; R.T., D.O. provided technical support; supervision or mentorship. F.K.E. and R.T. wrote the manuscript. All authors read and approved the final version of the manuscript.

References

1 Maas RJ, Deegens JK, Smeets B, Moeller MJ, Wetzels JF. Minimal change disease and idiopathic FSGS: Manifestations of the same disease. *Nat. Rev. Nephrol.* 2016; **12**: 768–76.
2 Vivarelli M, Massella L, Ruggiero B, Emma F. Minimal change disease. *Clin. J. Am. Soc. Nephrol.* 2017; **12**: 332–45.
3 Barisoni L, Schnaper HW, Kopp JB. A proposed taxonomy for the podocytopathies: A reassessment of the primary nephrotic diseases. *Clin. J. Am. Soc. Nephrol.* 2007; **2**: 529–42.
4 Sadowski CE, Lovric S, Ashraf S *et al*. A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. *J. Am. Soc. Nephrol.* 2015; **26**: 1279–89.
5 Sethna CB, Merchant K, Reyes A. Cardiovascular disease risk in children with kidney disease. *Semin. Nephrol.* 2018; **38**: 298–313.
6 Mundel P. Podocyte-targeted treatment for proteinuric kidney disease. *Semin. Nephrol.* 2016; **36**: 459–62.
7 Garin EH, Mu W, Arthur JM *et al*. Urinary CD80 is elevated in minimal change disease but not in focal segmental glomerulosclerosis. *Kidney Int.* 2010; **78**: 296–302.
8 Reiser J, Mundel P. Danger signaling by glomerular podocytes defines a novel function of inducible B7-1 in the pathogenesis of nephrotic syndrome. *J. Am. Soc. Nephrol.* 2004; **15**: 2246–8.
9 Yu CC, Fornoni A, Weins A *et al*. Abatacept in B7-1-positive proteinuric kidney disease. *N. Engl. J. Med.* 2013; **369**: 2416–23.
10 Krummey SM, Ford ML. Braking bad: Novel mechanisms of CTLA-4 inhibition of T cell responses. *Am. J. Transplant.* 2014; **14**: 2685–90.
11 Hu M, Wang YM, Wang Y *et al*. Regulatory T cells in kidney disease and transplantation. *Kidney Int.* 2016; **90**: 502–14.
12 Bertelli R, Bonanni A, Di Donato A, Cioni M, Ravani P, Ghiggeri GM. Regulatory T cells and minimal change nephropathy: In the midst of a complex network. *Clin. Exp. Immunol.* 2016; **183**: 166–74.

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13 Cara-Fuentes G, Wasserfall CH, Wang H, Johnson RJ, Garin EH. Minimal change disease: A dysregulation of the podocyte CD80-CTLA-4 axis? *Pediatr. Nephrol.* 2014; **29**: 2333–40.
14 Le Berre L, Bruneau S, Naulet J *et al*. Induction of T regulatory cells attenuates idiopathic nephrotic syndrome. *J. Am. Soc. Nephrol.* 2009; **20**: 57–67.
15 Shimada M, Araya C, Rivard C, Ishimoto T, Johnson RJ, Garin EH. Minimal change disease: A "two-hit" podocyte immune disorder? *Pediatr. Nephrol.* 2011; **26**: 645–9.
16 Delville M, Baye E, Durrbach A *et al*. B7-1 blockade does not improve post-transplant nephrotic syndrome caused by recurrent FSGS. *J. Am. Soc. Nephrol.* 2016; **27**: 2520–7.
17 Garin EH, Reiser J, Cara-Fuentes G *et al*. Case series: CTLA4-IgG1 therapy in minimal change disease and focal segmental glomerulosclerosis. *Pediatr. Nephrol.* 2015; **30**: 469–77.
18 Salant DJ. Podocyte expression of B7-1/CD80: Is it a reliable biomarker for the treatment of proteinuric kidney diseases with abatacept? *J. Am. Soc. Nephrol.* 2016; **27**: 963–5.
19 Benz K, Buttner M, Dittrich K, Campean V, Dotsch J, Amann K. Characterisation of renal immune cell infiltrates in children with nephrotic syndrome. *Pediatr. Nephrol.* 2010; **25**: 1291–8.
20 Agrawal V, Prasad N, Jain M, Pandey R. Reduced podocin expression in minimal change disease and focal segmental glomerulosclerosis is related to the level of proteinuria. *Clin. Exp. Nephrol.* 2013; **17**: 811–8.
21 Larsen CP, Messias NC, Walker PD. B7-1 immunostaining in proteinuric kidney disease. *Am. J. Kidney Dis.* 2014; **64**: 1001–3.
22 Baye E, Gallazzini M, Delville M, Legendre C, Terzi F, Canaud G. The costimulatory receptor B7-1 is not induced in injured podocytes. *Kidney Int.* 2016; **90**: 1037–44.
23 Novelli R, Gagliardini E, Ruggiero B, Benigni A, Remuzzi G. Any value of podocyte B7-1 as a biomarker in human MCD and FSGS? *Am. J. Physiol. Renal Physiol.* 2016; **310**: F335–41.
24 Novelli R, Gagliardini E, Ruggiero B, Benigni A, Remuzzi G. Another piece of the puzzle of podocyte B7-1 expression: Lupus nephritis. *Nephron* 2016; **133**: 129–38.
25 Garin EH, Diaz LN, Mu W *et al*. Urinary CD80 excretion increases in idiopathic minimal-change disease. *J. Am. Soc. Nephrol.* 2009; **20**: 260–6.
26 Ling C, Liu X, Shen Y *et al*. Urinary CD80 levels as a diagnostic biomarker of minimal change disease. *Pediatr. Nephrol.* 2015; **30**: 309–16.
27 Mishra OP, Kumar R, Narayan G *et al*. Toll-like receptor 3 (TLR-3), TLR-4 and CD80 expression in peripheral blood mononuclear cells and urinary CD80 levels in children with idiopathic nephrotic syndrome. *Pediatr. Nephrol.* 2017; **32**: 1355–61.
28 Cara-Fuentes G, Lanaspa MA, Garcia GE, Banks M, Garin EH, Johnson RJ. Urinary CD80: A biomarker for a favorable response to corticosteroids in minimal change disease. *Pediatr. Nephrol.* 2018; **33**: 1101–3.
29 Ishimoto T, Shimada M, Araya CE, Huskey J, Garin EH, Johnson RJ. Minimal change disease: A CD80 podocytopathy? *Semin. Nephrol.* 2011; **31**: 320–5.
30 Minamikawa S, Nozu K, Maeta S *et al*. The utility of urinary CD80 as a diagnostic marker in patients with renal diseases. *Sci. Rep.* 2018; **8** (1): 17322.
31 Benigni A, Gagliardini E, Remuzzi G. Abatacept in B7-1-positive proteinuric kidney disease. *N. Engl. J. Med.* 2014; **370**: 1261–3.
32 Cara-Fuentes G, Araya C, Wei C *et al*. CD80, suPAR and nephrotic syndrome in a case of NPHS2 mutation. *Nefrologia* 2013; **33**: 727–31.
33 Dong L, Pietsch S, Englert C. Towards an understanding of kidney diseases associated with WT1 mutations. *Kidney Int.* 2015; **88**: 684–90.
34 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**: 1390–7.
35 Klann JE, Kim SH, Remedios KA. Integrin activation controls regulatory T cell-mediated peripheral tolerance. *J. Immunol.* 2018; **200**: 4012–23.
36 Shalhoub RJ. Pathogenesis of lipoid nephrosis: A disorder of T-cell function. *Lancet* 1974; **2** (7880): 556–60.
37 Herrera M, Söderberg M, Sabirsh A *et al*. Inhibition of T-cell activation by the CTLA4-Fc Abatacept is sufficient to ameliorate proteinuric kidney disease. *Am. J. Physiol. Renal Physiol.* 2017; **312**: F748–59.

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