reason to enhance predialysis alkalosis with a high dialysate bicarbonate concentration. We think this is especially true for patients with hypoalbuminemia, which facilitates alkalosis, or with hypotension, in which alkalosis-mediated vasodilatation may contribute to intradialytic hypotension.[3,4]

We believe that pre- and postdialysis serum bicarbonate concentrations should be in the lower and upper limits of the reference range. To eliminate unnecessary alkalinization, dialysate bicarbonate concentration should be tailored strictly to each patient by evaluating pre- and postdialysis acid-base status, especially if there is only a weak association between dialysate and predialysis serum bicarbonate concentrations (as evidenced by Tentori et al[4]). The interdialytic acidification can be reduced by oral intake of sodium bicarbonate[5] with respect to the patient's interdialytic weight gain and by dietary acid reduction.

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In Reply to 'Abnormal Serum Bicarbonate Concentration in Hemodialysis Patients' *and* 'A "Lingering Mystery" of Postdialysis Serum Bicarbonate Concentration'

Dr Marano[1] argues that we have sustained our patient's metabolic alkalosis by not reducing dialysate bicarbonate concentration and that it therefore is iatrogenic in origin. I disagree. The cause of the patient's high serum bicarbonate level is low endogenous acid production, with which Dr Havlin[2] agrees. However, Dr Havlin[2] states that we should eliminate unnecessary alkalinization by reducing dialysate bicarbonate concentration. There is no evidence that reducing dialysate bicarbonate concentration is helpful in this setting; the increase in mortality is due to factors unrelated to acid-base status. When I mention excessive alkali administration as a cause of metabolic alkalosis (Box 1 of our article[3]), I am referring to an *acute* increase in serum bicarbonate level. Our patient had a stable mildly elevated serum bicarbonate level, which does not require correction. There is no evidence in vivo that hypoalbuminemia facilitates metabolic alkalosis or that it mediates hypotension.

With regard to Dr Marano's[1] explanation of the "mystery" of nonequilibrium at the end of dialysis, removal of bicarbonate by carbon dioxide excretion by the lungs of course occurs, but only following bicarbonate neutralization, as indicated by Symreng et al.[4] Neutralization occurs as a result of hydrogen ions generated by nonbicarbonate buffers and increased organic acid production, though these reactions have been difficult to quantify. Dr Havlin[2] correctly indicates that in our patient, the Gibbs-Donnan effect accounts for the difference between serum plasma and dialysate bicarbonate concentrations at the end of dialysis. However, the Gibbs-Donnan effect does not account for this difference in the typical patient, for whom the disparity often is 7 mEq/L and occasionally is as high as 10 mEq/L.

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RESEARCH LETTERS

B7-1 Immunostaining in Proteinuric Kidney Disease

To the Editor:

B7-1–Positive proteinuric kidney disease is a recently described subcategory of primary podocytopathy in which immunostaining is reported to detect B7-1 in the podocyte cytoplasm.[1] Through inactivation of β1 integrin, podocytes upregulate B7-1 in many forms of experimental kidney diseases with proteinuria, including immune-mediated, drug-induced, genetic, and bacterial toxin–induced diseases.[1-3] A recent report observed podocyte B7-1 immunostaining in >50% of randomly selected kidney biopsy specimens from patients with proteinuric kidney disease.[1] This staining was thought to reflect disease-related B7-1 induction that might be a useful companion diagnostic for treatment of glomerulopathies with abatacept, a CTLA4 agonist that inhibits B7-1. This report generated significant excitement because it suggested an additional treatment for a disease that currently has limited therapeutic options. However, subsequent correspondence raised

Table 1. Clinical Characteristics of Cases Stained With B7-1, by Biopsy Diagnosis

Characteristic	Value
Primary FSGS, NOS	28 (47)
Age (y)	40.3 ± 21.9
Proteinuria (g/24 h)	6.7 ± 6.5
Native vs transplant biopsy	22:7
FSGS, tip variant	5 (8)
Age (y)	53.2 ± 6.4
Proteinuria (g/24 h)	7.7 ± 4.7
Native vs transplant biopsy	4:1
Collapsing glomerulopathy	5 (8)
Age (y)	49.6 ± 15.6
Proteinuria (g/24 h)	5.4 ± 3.5
Native vs transplant biopsy	5:0
Minimal change disease	19 (32)
Age (y)	35.3 ± 23.4
Proteinuria (g/24 h)	6.8 ± 3.6
Native vs transplant biopsy	19:0
Membranous glomerulopathy	3 (5)
Age (y)	46.0 ± 24.3
Proteinuria (g/24 h)	3.6 ± 0.4
Native vs transplant biopsy	3:0

Note: Continuous values given as mean ± SD, categorical values given as number (percentage).

Abbreviations: FSGS, focal segmental glomerulosclerosis; NOS, not otherwise specified.

doubts about the validity of the immunohistochemical procedures used to detect B7-1, as well as the frequency of response to this agent.[4,5] We sought to determine the prevalence of B7-1–positive proteinuric kidney disease in a large cohort using 2 different staining techniques.

Sixty biopsy specimens were stained for B7-1 during the study period. All patients had nephrotic syndrome at the time of the biopsy. Clinical and demographic details are shown in Table 1. All cases were subjected to an immunoperoxidase staining assay performed on paraffin-embedded tissue after retrieval and a previously described immunofluorescence assay on fresh tissue,[1] provided tissue was available for both assays (see Item S1).

For both staining techniques and in all cases, B7-1 was undetectable within podocytes. By immunofluorescence, membranous glomerulopathy cases showed strong granular capillary staining, but in each case, there was judged to be identically intense staining on the negative control (no primary antibody) slide, suggestive of staining by the secondary antibody. Similarly, many podocytopathy cases showed dusty granular podocyte staining that was identical to that seen on the negative control. These cases were not scored as positive because podocyte staining in the B7-1–stained section did not exceed that present in the negative control. In all cases, including those with secondary antibody staining by immunofluorescence, the immunoperoxidase stain did not detect B7-1–positive podocytes. Focal interstitial inflammatory cells stained positive for B7-1 in most cases and served as an internal positive control (Fig 1).

The report of B7-1–positive proteinuric kidney disease aroused hope for a new era of personalized medicine in nephrology. However, closer examination reveals several issues with the report by Yu et al[1] that must be addressed before patients are treated based on the results reported. First, the report is very small, with only 5 patients receiving treatment, all of whom showed positive staining for B7-1 within podocytes. It was presumed that the B7-1 staining indicated upregulation of this protein and an increased likelihood of treatment response to abatacept. However, no control group without staining was reported to have been treated, making it impossible to know whether B7-1–positive staining was a marker of disease response with abatacept. Even more disconcerting than the small sample size and lack of treatment controls is the lack of a negative control in the staining procedure, which almost certainly resulted in false-positive staining and reporting of B7-1 staining in podocytes that was the result of the secondary antibody.[4] Of note, Yu et al[1] reported strong staining in membranous glomerulopathy. Many cases in our study showed B7-1 staining by immunofluorescence, but it was identical to staining in the negative control and therefore was determined to be an artifact. In all our podocytopathy cases, the pattern of immunofluorescence staining for B7-1 and the negative control was consistent with the podocyte IgG staining, which commonly is regarded as nonspecific in these cases.[6] Our membranous cases showed a pattern of staining that also was identical to that typically seen by IgG. Using the immunoperoxidase technique, which does not have this secondary antibody artifact, no podocyte staining was detected.

Although results of our case series raise significant doubts about the utility of B7-1 staining as an indication that patients may respond to abatacept, this does not address the potential value of abatacept treatment of patients with steroid-resistant nephrotic syndrome. There are data to support upregulation of this gene in glomerulopathy[2] and a larger trial certainly is warranted given the response in the 5 patients described.[1] Future studies should focus on searching for additional biomarkers to guide therapeutic decision making.

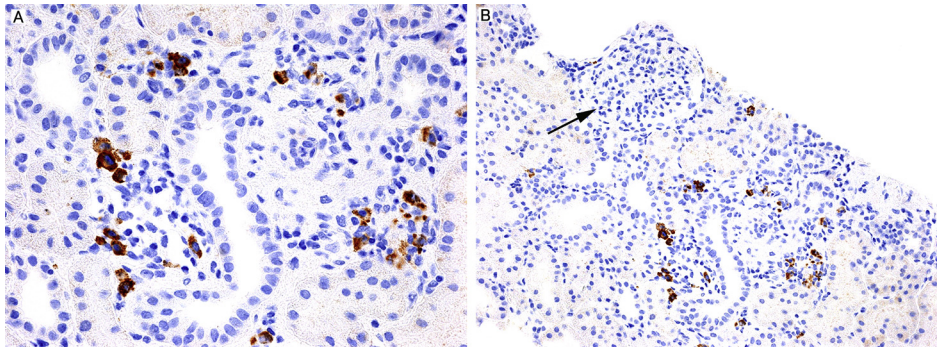

Figure 1. Immunohistochemical detection of B7-1 in a biopsy specimen from a patient with recurrent nephrotic syndrome after transplantation. (A) Interstitial activated B cells and monocytes show positive B7-1 staining. (B) Interstitial inflammatory cells show strong internal positive control staining for B7-1 while glomerulus (arrow) is completely negative.

In summary, we report the first large case series of B7-1 staining in kidney biopsies with a podocytopathy. We failed to identify any cases with positive B7-1 podocyte staining after evaluating 60 biopsy specimens using 2 different antibodies. One other recent report also supports the uncommon nature of this B7-1 staining in FSGS.[7] We conclude that B7-1 immunostaining is unlikely to serve as a useful diagnostic stain supporting the use of abatacept therapy.

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Contributions: Research idea and study design: CPL, NCM; data acquisition: CPL; data analysis/interpretation: CPL, NCM; supervision or mentorship: PDW. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. CPL takes responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained.

Supplementary Material

Item S1: Detailed methods.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2014.07.023) is available at www.ajkd.org

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Viable Podocyturia in Healthy Individuals: Implications for Podocytopathies

To the Editor:

The podocyte is a terminally differentiated cell of the renal glomerulus that, by wrapping around the basement membrane through its foot processes, controls the selective permeability of the glomerular filtration barrier.[1,2] In diabetic nephropathy and other chronic glomerular diseases, a gradual decrease in intraglomerular podocytes, paralleled by increased urinary excretion of these same cells (up to 60,000 daily) has been demonstrated,[2-5] suggesting a direct role of podocyturia in disease progression.[2,5] Slow "physiologic" podocyturia (~400 podocytes per day) recently has been found in healthy individuals.[5] Unexpectedly, in both health and disease, urinary podocytes show up as round viable cells able to proliferate once in culture,[5] possibly suggesting an underlying process of dedifferentiation.[6,7]

Whether urinary podocytes of healthy individuals consist of dedifferentiating cells and, if so, whether the dedifferentiation process precedes or follows detachment from the basement membrane presently is unknown. To clarify this, we collected sterile spot urine samples from 20 healthy individuals (mean age, 64.5 ± 4.1 [SD] years) and fragments of normal cortical tissue (derived from 15 adult kidneys removed for renal or urothelial carcinoma or from donor kidneys not suitable for transplantation) and searched for immature podocytes. Clinical characteristics of participants are provided in Table S1. The study was approved by the Ethics Committee of the San Raffaele Scientific Institute, and written informed consent was obtained from all participants.

Immature podocytes were defined as cells co-expressing the podocyte-specific antigens podocin, nephrin, and podocalyxin (Podxl)[1] and the embryonic transcription factors Nanog, Oct3/4, and Sox2 (with cytoplasmic localization) as markers of ongoing dedifferentiation.[8] The postmitotic podocyte marker synaptopodin also was evaluated.[9]

Analysis of RNA extracted from urine sediments showed expression of the podocyte and embryonic markers (Fig 1A). In experiments run in parallel, we identified (in cytospins of all 20 spot urine samples) viable podocytes, evident as round, mono- or binucleated, propidium iodide–negative, Podxl-positive cells (0.20 ± 0.02 viable podocytes per milligram of creatinine, equivalent to ~300 podocytes per day; Fig 1B). We detected Nanog in 96% of viable podocytes, in line with their being immature cells (Fig 1B). These urinary podocytes also coexpressed nephrin and podocin with Nanog (Fig 1C), as well as Oct3/4 and Sox2 (not shown). Synaptopodin was undetectable. When we seeded urinary sediments into culture medium, clusters of immature podocytes coexpressing Nanog and Podxl could be detected within a few days (Fig 1D).

In immunofluorescence studies of glomerular sections, we found cell clusters coexpressing Nanog with podocin (Fig 2A) and Podxl (Fig 2B). We also observed colocalization of Nanog with Oct3/4 (Fig 2C) and Sox2 (not shown). Consistent with these cells being immature, we detected no colocalization of Nanog and synaptopodin (Fig 2D). Clusters of immature cells were detected in ~60% of glomeruli considered, independent of donor age.

These study results show that viable podocyturia, as detectable in urine of healthy individuals, consists of dedifferentiating cells coexpressing mature podocyte antigens (with the exception of postmitotic synaptopodin) along with immaturity markers. The presence of immature podocytes inside the glomerulus alternatively could be interpreted as an ongoing differentiative process allowing a still unknown stem cell pool to replace dying or detaching podocytes. Although this hypothesis cannot be excluded, it nonetheless is not in line with the data in Fig 2, which show that no niches of undifferentiated stem cells expressing embryonic transcription factors