Editorial Focus:

B7-1 Biomarker Bites the Dust

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Focal segmental glomerulosclerosis (FSGS) and minimal change disease (MCD) are leading causes of primary nephrotic syndrome, yet their etiologies are unknown. Evidence supports the concept that a circulating “permeability factor” or toxin acts directly on the podocyte to mediate podocyte dysregulation, effacement and proteinuria. In FSGS, irreversible podocyte injury causes podocyte depletion and progressive glomerulosclerosis (4). Despite decades of research, both the identity and source of the permeability factor(s) remain elusive (7). Whereas MCD is highly responsive to glucocorticoid therapy, FSGS responds in only 40% of cases and is often resistant to alternate immunosuppressive agents. This contrasts with secondary forms of FSGS, for which it is often possible to treat the cause, such as HIV infection, pamidronate toxicity, or morbid obesity (4). Given the dismal prognosis of primary FSGS and its potential to recur in the allograft, there is pressing need for biomarkers that inform more targeted therapy.

Against this backdrop, the recent report of B7-1 as biomarker and potential therapeutic target in a subcategory of primary FSGS was met with enormous enthusiasm. In 2013, Yu et al. reported that B7-1 (CD80) was induced in podocytes in 13 of 21 randomly selected native kidney biopsies of patients with proteinuric kidney disease (10). The images (relegated to supplemental materials) showed weak and sparse B7-1 staining in glomeruli of primary FSGS and MCD, of equivocal specificity. Stronger glomerular B7-1 staining was found in such immune complex-mediated glomerular diseases as membranous nephropathy (MN), where the pattern suggested cross-reactivity of secondary antibody with human IgG in the deposits. The authors described 4 patients with recurrent FSGS after transplantation and one with primary FSGS in whom abatacept induced partial or complete remissions of proteinuria (10). Abatacept, which is CTLA4-Ig, directly inhibits T-cell costimulatory molecule B7-1.

B7-1 (CD80) is a molecule expressed on B cells that provides co-stimulation to T cells via interaction with CTLA4 and CD28. The story of B7-1 was first introduced to nephrologists in 2004, when Reiser et al. reported that LPS injection in mice causes podocyte B7-1 induction, transient proteinuria and effacement through TLR-4/CD14 signaling (9). Podocytes constitutively express both TLR-4 and its co-receptor CD14, suggesting they possess innate immune function. The podocyte response to LPS was dependent on B7-1 but independent of B and T cells. This work opened the field of podocyte signaling by danger-associated molecular patterns (DAMPs) as a potential means of protection from gram-negative sepsis by inducing transient proteinuria and urinary elimination of injurious factors. Mechanistically, in vitro data suggested that B7-1 mediates podocyte injury and proteinuria by disrupting the binding of talin to β1 integrin and that abatacept prevents this disruption (10).

As with any new biomarker, there is a necessary period of validation. Several rapid-fire letters to the editor described lack of reproducibility and nonspecificity of the
immunostaining results (3) and lack of efficacy of abatacept in recurrent FSGS, even in biopsies expressing B7-1 (1). Meanwhile, nephrologists implored renal pathologists to initiate B7-1 immunostaining of renal biopsy specimens to identify those patients most likely to benefit from abatacept. Many renal pathology laboratories tried for months to set up a well-validated staining protocol but failed despite following the published method (10) and exploring different reagents and conditions with appropriate controls. At a companion meeting of the Renal Pathology Society in 2015, even the pathologist who had performed the original immunostaining reported by Yu et al. (10) acknowledged difficulty interpreting the ambiguous staining results and the need for further biomarker validation.

The important paper by Novelli et al. (8), published in this issue alerts the nephrology community to the unreliability and apparent uselessness of B7-1 immunostaining as a biomarker assay in human podocytopathies. They performed immunostaining of both frozen and paraffin-embedded renal biopsies from patients with MCD and FSGS and were unable to detect specific B7-1 staining of podocytes. For the human frozen samples, they used the same primary (goat anti-human) B7-1 antibody and experimental procedure described in the publications that report B7-1 expression by podocytes (6, 10). To confirm their data, they analysed biopsies using another primary (mouse anti-human) B7-1 antibody and a previously described immunoperoxidase assay (5). Again, they did not detect any B7-1 podocyte signal. Importantly, both assays detected strong B7-1 staining of interstitial inflammatory cells as internal positive controls, confirming the reliability of the assays and sensitivity of the antibodies employed. Their positive staining of LPS-activated cultured human splenocytes demonstrated that the primary polyclonal goat antibody was indeed specific for human B7-1. They justified their choice of a different secondary antibody (human IgG-adsorbed rabbit anti-goat antibody) by demonstrating that the secondary donkey anti-goat antibody used by Yu et al. (10) gave nonspecific staining when used alone in negative controls and was cross-reacting with human IgG in MN biopsies (3).

Adding fuel to the fire, they then explored several mouse models of FSGS, including the LPS model and adriamycin nephropathy. They found no podocyte expression of B7-1 by immunostaining of tissue sections, despite the induction of proteinuria and the ability to stain B7-1 expressing interstitial dendritic cells as internal positive controls.

This study has advanced the B7-1 field in several important ways: It sampled a larger cohort of patients, including 15 MCD and 16 FSGS and 4 nephrectomy normal controls. It studied 2 animal models of podocyte injury. It used several different primary antibodies and immunostaining protocols in fresh and fixed tissue, including the original described by Yu et al (10). Positive and negative controls are well-validated. A limitation is that the only method of B7-1 detection employed was immunostaining and not potentially more sensitive molecular techniques.
Immunostaining is often tricky and depends not only on the quantity of antigen, stability of the target epitope, its conformation and accessibility to antibody binding, type and duration of fixation, differences in antibody sensitivity and specificity, and image exposure time and contrast. The authors demonstrated the validity of the techniques and reliability of the image acquisition by using internal controls and double labeling as reference signal. There was no B7-1 signal in podocalyxin-expressing podocytes from human biopsies with MCD and FSGS or the mouse models studied, but there was strong B7-1 signal in human interstitial CD20+ lymphocytes and in murine CD11c+ dendritic cells, indicating that their techniques were working.

So what is the current state-of-the-art on B7-1 podocyte expression? One can reasonably conclude that immunostaining for B7-1 in tissue sections is not suitable for biomarker guidance in human MCD or FSGS. What is more, the lack of detectable B7-1 staining in several experimental models, including LPS and adriamycin nephropathy, raises new doubt about B7-1 as a podocyte injury marker. Because the authors did not perform highly sensitive molecular analyses of expression levels of B7-1 mRNA or protein by Western blot in microdissected glomeruli, one cannot exclude the possibility that B7-1 expression by podocytes is present at very low levels, beneath the detection threshold of immunostaining techniques. Nonetheless, the data convincingly show no utility of B7-1 immunostaining in kidney biopsies as a means of triaging patients for abatacept therapy. The study does not address the efficacy of abatacept in this population, which is another point of contention (1, 6).

Lesson learned is that biomarker discovery requires independent validation in other patient cohorts, large cohorts, by different investigators, and with proper positive and negative controls. By way of contrast, since the initial discovery of PLA2R as target antigen in primary MN (2), renal pathology laboratories world-wide have adopted routine immunostaining for PLA2R, both in frozen and fixed tissue, and private and commercial laboratories offer serum assays for circulating anti-PLA2R antibody. That is a true success story. As for B7-1, it is advisable to abandon B7-1 immunostaining of kidney biopsies in clinical practice, pending new evidence to the contrary.


