Podocyte Expression of B7-1/CD80: Is it a Reliable Biomarker for the Treatment of Proteinuric Kidney Diseases with Abatacept?

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doi: 10.1681/ASN.2015080947

The story of B7-1 (also known as CD80) and its possible association with nephrotic syndrome began with a chance observation that the gene for B7-1 is overexpressed in podocytes from mice deficient in the α3 integrin.1 The α3β1 integrin is an important adhesion receptor in podocytes and the deficient mice have abnormal podocytes and are proteinuric.2 The finding of B7-1 in podocytes was somewhat surprising because it is best known as a costimulatory receptor on antigen presenting cells, where it engages its activating (CD28) and inactivating (CTLA4) counter-receptors on lymphocytes. In a thorough and logical series of studies, Reiser et al. showed that B7-1 is induced in podocytes and leads to cytoskeletal alterations in vitro and proteinuria in vivo in a variety of genetic, toxic, and inflammatory conditions, including activation of innate immune signaling via TLR-4 by bacterial endotoxin (LPS).1 Most compelling were the observations that B7-1-deficient mice did not develop proteinuria after LPS injection, whereas mice with severe combined immunodeficiency (SCID) became proteinuric and had increased B7-1 immunostaining on podocytes, which documented that the effects of LPS were mediated by B7-1 and suggested that the effects were not dependent on an intact systemic immune system. Thus B7-1 was identified as a danger signal on injured podocytes, setting the stage for translation to proteinuric human diseases.3

Based on this preclinical data, B7-1 expression on podocytes was proposed as both a biomarker that can identify proteinuric patients that might benefit from treatment with CTLA4-Ig, and as a therapeutic target. Interestingly, Garin et al. had previously shown that CD80 (B7-1) is expressed by the podocytes but not the tubules of patients with minimal change disease in association with increased urinary excretion of CD80,4,5 although urinary CD80 correlated with minimal change disease and not FSGS.6 Nonetheless, these observations raised the exciting possibility that an agent such as abatacept, that was approved by the US Food and Drug Administration and clinically tested with a good safety profile, could be repurposed to treat proteinuric kidney diseases mediated by podocyte B7-1 overexpression, including progressive and recurrent FSGS and intractable diabetic nephropathy, for which there are no effective measures to halt a disease in which renin-angiotensin-aldosterone system inhibitors merely slow progression.

The first such study was reported by Yu et al. in 2013 and included five patients with nephrotic syndrome who had FSGS (four with rituximab-resistant recurrent FSGS after transplantation, requiring plasmapheresis, and one with steroid-resistant primary FSGS).7 All demonstrated podocyte B7-1 immunostaining on kidney biopsy and all experienced a partial or complete remission of proteinuria after treatment with abatacept. The study by Yu et al. also offered further insights into a mechanism whereby abatacept might exert its beneficial effect. Increased expression of B7-1 was shown to destabilize B1-integrin by competing for its association with talin, a focal adhesion protein. This converted cultured podocytes from a quiescent to a more motile phenotype, the in vitro equivalent of foot process effacement. Abatacept blocked the interaction of B7-1 with talin, thereby stabilizing B1-integrin activity and preventing podocyte motility.7 To paraphrase the accompanying editorial, these findings were considered a breakthrough that might offer customized treatment for patients with otherwise refractory forms of proteinuric kidney disease, and corroboration was eagerly awaited.8

Support for the concept that podocyte B7-1 expression might be a more general feature of proteinuric kidney diseases came in the form of a study of diabetic nephropathy reported in the Journal of the American Society of Nephrology by Fiorina et al. in 2014.9 This was considered especially important because diabetic nephropathy represents the largest unmet need for an effective treatment in nephrology, as it accounts for almost half of all patients with ESRD in the United States.10 As in the original mouse studies, the first clue came from a gene expression study that showed association of two single nucleotide polymorphisms in the B7-1 gene in patients with type II diabetes and nephropathy, B7-1 was found to be induced in kidney biopsy specimens from patients with type II diabetes and nephropathy, and specific podocyte B7-1 immunostaining was detected in almost 50% of biopsies. Moreover, the intensity of B7-1 staining corresponded to the
severity of glomerular pathology. In addition, high baseline circulating levels of the soluble form of the B7-1 ligand, sCD28, forecast the risk of ESRD with an odds ratio of 2.8 (95% CI, 1.0 to 7.8). The findings were further supported by experimental studies showing CTLA4-Ig–inhibitable, PI3-kinase–dependent B7-1 expression and cell injury in podocytes cultured in the presence of high glucose concentrations. CTLA4-Ig also protected podocytes and inhibited the development of proteinuria in two murine models of diabetic nephropathy (25-week-old db/db mice and C57BL/6 mice 12- and 16-weeks after administration of streptozotocin). Thus it was concluded that B7-1 expression identifies a pathway of hyperglycemia-induced podocyte injury that might be interrupted with CTLA4-Ig to prevent or treat diabetic nephropathy.

So what is the problem? If we are to depend on podocyte B7-1 expression on podocytes to identify patients that are potential candidates for CTLA4-Ig treatment, we need to know that it is a reliable and reproducible biomarker. Unfortunately, it turns out that the immunohistochemical techniques to detect B7-1 on podocytes in human kidney biopsies are very finicky. Even expert nephropathologists have had trouble corroborating the observations in relatively large numbers of cases, and others have raised questions about the specificity for primary FSGS. Therefore, before embarking on clinical trials of CTLA4-Ig in diabetic nephropathy based on podocyte B7-1 expression, we would need confirmation that B7-1 immunostaining reliably identifies candidates for such treatment.

In this issue of the Journal of the American Society of Nephrology, Gagliardini et al. report that they were unable to confirm that B7-1 is induced in the podocytes of humans and experimental animals with diabetic nephropathy. The authors examined frozen kidney biopsy specimens of 31 patients with diabetic nephropathy by indirect immunofluorescence with a polyclonal goat anti-B7-1 antibody, and a subgroup of 13 cases by immunoperoxidase staining of paraffin-embedded tissues with a monoclonal anti-B7-1 antibody used in this study was the same as that used in the study by Fiorina et al. and reported in the supplementary material. As previously reported in FSGS, immunoperoxidase staining with monoclonal anti-human B7-1 antibody also failed to detect B7-1 on podocytes of the diabetic nephropathy kidneys, despite the detection of a signal in interstitial inflammatory cells and tubular epithelial cells. Gagliardini et al. also examined the kidneys of two murine models of diabetic nephropathy (BTBR ob/ob mice at 20–22 weeks of age and C57BL/6 mice 16–20 weeks after administration of streptozotocin) and failed to detect B7-1 podocyte staining with the same primary anti-mouse B7-1 antibody used by Fiorina et al. Thus, the authors issue a cautionary note about the reliability of podocyte B7-1 as a biomarker and potential therapeutic target in diabetic nephropathy, and argue that non-specific staining might have influenced previous findings.

If immunohistochemistry is unreliable, what is the evidence that B7-1 is expressed by podocytes in vivo? It is clear that the gene and protein are expressed by cultured podocytes, but is that also the case in vivo? Fiorina et al. detected B7-1 mRNA by real-time PCR in diabetic kidneys, however the message might have arisen and been amplified from interstitial or tubular cells because whole kidney rather than isolated glomeruli or podocytes were analyzed. What about the original mouse studies by Reiser et al. in which B7-1 knockout mice were protected from LPS-induced injury? Here again, definitive proof is lacking because the mice were global knockouts not podocyte-specific B7-1 knockout mice, and the beneficial effects might have been from B7-1 deficiency in conventional immune cells. However, one might argue that immune-deficient SCID mice were not protected from the proteinuric effects of LPS so the effects were not dependent on an intact systemic immune system. However, the SCID mice were not crossed with B7-1 knockout mice, so we do not know for sure that the effects of LPS in the SCID mice were mediated by B7-1. Indeed, the only evidence in favor of the role of podocyte B7-1 in the SCID mice was the appearance of podocyte B7-1 immunostaining, which brings us back full circle to the reliability of such staining as a biomarker.

If podocyte B7-1 staining is an imperfect marker and uncertain target in proteinuric kidney diseases like FSGS and diabetic nephropathy, how do we explain the remarkable benefit of abatacept in the five cases of nephrotic syndrome reported by Yu et al.? I do not presume to have the answer, but can suggest a few possibilities. First, we know that one or more circulating factors may cause primary FSGS, especially in those cases that recur soon after kidney transplantation as in the report by Yu et al., and we are still uncertain where they come from. Therefore, it is conceivable that the effect of abatacept was to inhibit the production of such a permeability factor and that it had nothing to do with podocyte B7-1 expression. Perhaps abatacept does indeed inhibit a form of B7-1 on podocytes that is poorly identified by available antibodies and techniques. Independent confirmation by in situ hybridization and immunohistochemistry that the gene and protein are expressed by podocytes in vivo would provide impetus to improve detection methods. Lastly, an off-target ligand-independent effect of CTLA4-Ig cannot be completely excluded, although the only known ligand-independent isoform of CTLA4 acts intracellularly and would not be relevant to the action of CTLA4-Ig.

Given our desire to see breakthroughs for the treatment of intractable and progressive kidney diseases, we are quick to embrace new and exciting discoveries such as those reviewed here. While this is understandable, we owe it to our patients, the
public, and our own scientific discipline to insist on corroboration before hastening toward implementation. While the paper by Gagliardini et al. has not corroborated the value of podocyte B7-1 as a biomarker in diabetic nephropathy, at least with the best techniques available at present, we are still uncertain about the general efficacy of CTLA4-Ig in certain proteinuric kidney diseases such as recurrent FSGS. Hopefully, an answer to that will emerge through rigorously designed studies.

ACKNOWLEDGMENTS

The author appreciates the help of Dr. Laurence H. Beck Jr. in reviewing and commenting on the manuscript.

This work was supported by research grant DK090029 from the National Institute of Diabetes and Digestive and Kidney Diseases/National Institutes of Health.

DISCLOSURES


REFERENCES


See related article, “B7–1 Is Not Induced in Podocytes of Human and Experimental Diabetic Nephropathy,” on pages 999–1005.

Nephrin Trafficking beyond the Kidney—Role in Glucose–Stimulated Insulin Secretion in β Cells

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The Ig superfamily member nphrin is the key structural component of the slit diaphragm, which interconnects neighboring podocyte foot processes in kidney glomerulus. Mutations in the nphrin gene (Nephrotic Syndrome Gene 1 [NPHS1]) cause the congenital nephrotic syndrome of the Finnish type (NPHS1) characterized by severe proteinuria during the first weeks of life.1 The disease is most prominent in Finland, with an incidence of one in 8200 births, and >90% of the Finnish patients have two truncating mutations, Finn-major and Finn-minor,1 which lead to total lack of nphrin protein expression.2 NPHS1 caused by Finn-major or Finn-minor mutations is a progressive disease, with renal transplantation providing the only curative therapy. Worldwide, >200 NPHS1 mutations have

Published online ahead of print. Publication date available at www.jasn.org.

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