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Danger Signaling by Glomerular Podocytes Defines a Novel Function of Inducible B7-1 in the Pathogenesis of Nephrotic Syndrome

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Podocyte foot processes (FP) and the interposed slit diaphragms (SD) form the final barrier to protein loss, explaining why podocyte injury is typically associated with marked proteinuria (1). The highly dynamic FP actin cytoskeleton is linked to the SD and proteins that regulate podocyte actin dynamics therefore are of critical importance for structural maintenance and sustained function of the glomerular filter. We recently made the novel finding that under pathologic conditions, with FP effacement and proteinuria, podocytes upregulate B7-1 (2). B7-1 (also termed CD80) is a transmembrane protein expressed on the surface of B cells and other antigen-presenting cells (APC). On B cells and other APC, B7-1 provides a co-stimulatory signal for T cells through binding to its receptors CD28 and CTLA-4 (3–5). The immune function of B7-1 has been well described (3–5). To explore the function of B7-1 in podocytes, we developed a novel, unique murine model of LPS-induced, B7-1–dependent transient nephrotic syndrome that shares several key features of human minimal-change disease (MCD) (2). On the basis of our observations, we propose that LPS induces transient B7-1–dependent nephrotic syndrome through the reorganization of the podocyte FP actin cytoskeleton and disruption of the SD (2). Our findings suggest a novel function for B7-1 in danger signaling by podocytes (2).

Podocytes Are Important for Glomerular Homeostasis

Renal podocytes are highly differentiated cells with a complex cellular morphology. They are located inside the kidney glomerulus, a twisted corpuscle of capillaries through which the blood is filtered hydrostatically through a high-volume/high-discrimination filter (1,6). Podocyte FP are anchored to the glomerular basement membrane (GBM) *via* $\alpha 3\beta 1$ integrin (7) and α-/β-dystroglycans (8,9). Neighboring FP are connected by a specialized cell–cell junction, the glomerular SD, which represents the main size selective filter barrier in the kidney (6,10,11). The SD is thought to be a modified adherens junction (12) and is composed of a growing number of proteins, including P-cadherin (12), nephrin (13–15), FAT (16,17), podocin (18), and Neph1 (19–21). Podocytes are injured in many forms of human and experimental glomerular disease, including MCD, focal segmental glomerulosclerosis, membranous glomerulopathy, diabetes, and lupus nephritis (6,22). Independent of the underlying disease, the early events are characterized by molecular alterations of the SD without visible morphologic changes or, more obvious, by a reorganization of the FP structure with apical displacement of the SD (6,23,24). From a clinical standpoint, it is important to recognize that early structural changes in podocyte morphology, such as FP effacement, have to be reversed within a certain period of time to prevent development of severe and progressive glomerular damage (23,25).

Role of B7-1 Beyond T Cell Co-stimulation

The role of B7-1 in T cell co-stimulation is well established (3–5). Much less is known about B7-1–mediated signaling in B cells and other APC. Two regions in the cytoplasmic tail of B7-1 are thought to be important for B7-1 membrane distribution and T cell co-stimulation in APC (26,27). Another report showed B7-1 mediated outside-in signaling in B cells through tyrosine kinases (28). Cross-linking of B7-1 on B lymphoma Raji cells induced tyrosine phosphorylation of 160-, 120-, 55-, 46-, and 44-kD proteins, which was inhibited by genistein, a tyrosine kinase inhibitor. B7-1–mediated signaling blocked DNA synthesis and induced cell spreading in a fibroblast-like manner, which was blocked by genistein (28). These results suggest that B7-1 is involved in transmembrane outside-in signaling in B cells, and its biologic effects seem to be mediated by tyrosine kinases (28). Further support for a role of B7-1 in outside-in signaling comes from a recent report showing that CTLA-4-Ig–mediated ligation of B7-1 regulates tryptophan catabolism in dendritic cells (29).

Novel Function for B7-1 in Danger Signaling by Podocytes

Podocytes of $\alpha 3^{-/-}$ mice are unable to maintain normal podocyte structure, including the elaboration of mature FP

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along the GBM (30). To identify genes that are critically involved in the development of FP effacement and proteinuria, we performed a genomic screen comparing wild-type and $\alpha 3^{-/-}$ podocytes. With this approach, we uncovered an unanticipated novel role for B7-1 in podocytes as inducible modifier of glomerular permselectivity (2). B7-1 in podocytes was found in genetic, drug-induced, immune-mediated, and bacterial toxin–induced experimental kidney diseases with nephrotic syndrome (2). The clinical significance of our results is underscored by the observation that podocyte expression of B7-1 correlated with the severity of human lupus nephritis. LPS signaling through TLR-4 reorganized the podocyte actin cytoskeleton *in vitro*, and activation of B7-1 in cultured podocytes led to reorganization of vital slit diaphragm proteins (2). *In vivo*, LPS rapidly upregulated B7-1 in podocytes of wild-type and SCID mice, leading to nephrotic-range proteinuria. Mice lacking B7-1 were protected from LPS-induced nephrotic syndrome. Taken together, these data established a causal link between podocyte B7-1 expression and urinary protein loss that is independent of lymphocyte infiltration or activation (2). It has not escaped our attention that LPS-induced nephrotic syndrome shares several key features of human MCD in that the FP effacement and proteinuria are transient and present without signs of glomerular inflammation.

Separating the Good and the Bad in Nephrotic Syndrome

The upregulation of B7-1 in podocytes suits well to provide a unifying molecular explanation for the pathogenesis of proteinuria in nephrotic syndrome. However, it is difficult to believe that the sole (and harmful) function of podocyte B7-1 should be to disassemble the glomerular filter. Hence, it is intriguing to speculate that transient B7-1–dependent proteinuria may be a physiologic response that is desirable under certain conditions, *e.g.*, in patients with Gram-negative infection. The disruption of the glomerular filter by TLR-induced B7-1 may help the innate immune system in clearing the circulation from harmful agents by dumping them into the urine. In this scenario, the *transient* urinary protein loss would be the price that we pay for the rapid decay of the harmful agent. Consistent with this idea, the development of transient proteinuria has been found during the course of gram-negative sepsis (31). Moreover, a prospective study showed that in postoperative septic patients, microalbuminuria is an early indicator of increased glomerular permeability but not in nonseptic patients (32). Clearly, this is highly speculative, and future studies will be required to explore this hypothesis in detail. In MCD, the onset of nephrotic syndrome is often preceded by an infection or allergic reaction (33). Some children present with fever and bacteremia. In a number of these patients, the peritoneal cavity is the site of the infection, and *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli* are commonly isolated (33). A urinary tract infection is occasionally present, and a history of upper respiratory tract infection immediately preceding the first clinical signs of nephrotic syndrome may exist and stimulate the activation of podocyte danger signaling pathways, thereby leading to B7-1–induced nephrotic syndrome. This raises the intriguing possibility that prolonged nephrotic syndrome in MCD may result from the persistence of a normally beneficial response caused by genetic defects in the B7-1 pathway or by the persistence of other unphysiologic activators of podocyte B7-1. The sustained B7-1 activation in podocytes, in turn, may result in the continuation or recurrence of nephrotic syndrome in this setting.

The activation of the innate immune system by LPS (2) (and potentially other pathogen-associated particles) leads to the upregulation of podocyte B7-1. In light of these results, it is tempting to speculate that podocyte B7-1 may not only act to reorganize the slit diaphragm but also participate in the activation of circulating T cells. Under normal conditions, T cells are unable to enter the glomerulus, but in advanced stages of nephrotic syndrome, it may be facilitated for T cells to enter Bowman's space and directly interact with podocytes. A podocyte–T cell interaction may result in stimulation and activation of T cells. In inflammatory glomerulonephritis, T cells can be found in the glomerulus in Bowman's space, and B7-1 as well as B7-2 regulate crescentic glomerulonephritis (34). Therefore, podocyte B7-1 may also modulate T cell–mediated cytotoxicity in immune and nonimmune kidney diseases with proteinuria. The expression of B7-1 by podocytes in glomerulonephritic situations may serve to recruit T cells to sites of GBM damage and breakdown and promote further inflammation.

It has been known for a long time that T cell dysfunction is thought to occur in MCD, and T cell activation was proposed to play a pathogenic role in this disease (35,36). In the context of our results that LPS-induced nephrotic syndrome can occur in SCID mice (2), the role of T cells in MCD may be reinterpreted in the following way. Rather than causing disease, the activation of T cells may reflect the activation of the immune system, including the upregulation of podocyte B7-1 in response to any of the above discussed stress (*e.g.*, infection, allergens). Clearly, this concept is highly speculative and converse to the classical idea that T cell activation precedes glomerular dysfunction, and future studies will be necessary to confirm or refute this hypothesis.

Conclusion

Genomic profiling identified B7-1 expression in podocytes under conditions of FP effacement and proteinuria (2). The biologic work-up of podocyte B7-1 expression unraveled a novel function of B7-1 as an inducible modifier of glomerular permselectivity (2). The induction of B7-1 in podocytes by LPS through TLR-4 signaling suggests that the podocyte is a novel component of the innate immune system that is equipped with a danger signaling machinery (37). In summary, upregulation of B7-1 in podocytes may contribute to the pathogenesis of proteinuria by disrupting the glomerular filter and provides a novel molecular target to tackle proteinuric kidney diseases (2).

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