Induction of B7-1 in podocytes is associated with nephrotic syndrome

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Kidney podocytes and their slit diaphragms form the final barrier to urinary protein loss. This explains why podocyte injury is typically associated with nephrotic syndrome. The present study uncovered an unanticipated novel role for costimulatory molecule B7-1 in podocytes as an inducible modifier of glomerular permeselectivity. B7-1 in podocytes was found in genetic, drug-induced, immune-mediated, and bacterial toxin–induced experimental kidney diseases with nephrotic syndrome. 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. In vivo, exposure to low-dose LPS rapidly upregulates B7-1 in podocytes of WT and SCID mice, leading to nephrotic-range proteinuria. Mice lacking B7-1 are protected from LPS-induced nephrotic syndrome, suggesting a link between podocyte B7-1 expression and proteinuria. LPS signaling through toll-like receptor-4 reorganized the podocyte actin cytoskeleton in vitro, and activation of B7-1 in cultured podocytes led to reorganization of vital slit diaphragm proteins. 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. Our findings suggest a novel function for B7-1 in danger signaling by nonimmune cells.

Introduction

The kidney glomerulus is a highly specialized structure that ensures selective ultrafiltration of plasma so that essential proteins are retained in the blood. The common denominator in a variety of kidney diseases, such as diabetes mellitus, focal segmental glomerulosclerosis, and systemic lupus erythematosus, is glomerular dysfunction (nephrotic syndrome), involving a massive loss of protein in the urine (proteinuria) (1). Kidney or systemic diseases that result in nephrotic syndrome are important causes of morbidity and mortality affecting both adults and children. Glomerular podocytes serve as the final barrier to urinary protein loss by the formation and maintenance of podocyte foot processes (FPs) and the interposed slit diaphragm (SD) (1). All forms of nephrotic syndrome are characterized by abnormalities in podocytes, including retraction (effacement) of podocyte FPs and/or molecular reorganization of the SD. FP effacement is a dynamic process that correlates closely with the development of proteinuria both in human disease and in experimental models. FP effacement requires a precise interplay of multiple cellular functions, including structural alterations of the cytoskeleton, movement of FPs over the basement membrane, and reconstruction of the SD (1). From a clinical perspective, it is important to recognize that early structural changes in podocytes such as substructural alterations in SD composition or FP effacement have to be reversed within a certain period of time to prevent development of severe and progressive glomerular damage (2). Recent years have been extremely fruitful in providing an extensive amount of information on the molecular composition of the SD and its anchoring structures, which form multiple interactions and provide signaling platforms. The discovery of several novel podocyte proteins and their mutation analysis, including nephrin (3), CD2AP (4), α-actinin-4 (5), podocin (6), and neph1 (7), have shed light on the pathogenesis of proteinuria and emphasized the critical role of the podocyte and the SD in maintaining the function of the glomerular filtration barrier.

Podocyte FPs form a scaffolding around the capillary loops that are anchored to the glomerular basement membrane (GBM) via α3β1 integrin (8) and α-/β-dystroglycans (9, 10). α3β1 integrin represents a receptor for certain isoforms of laminin, including laminin-5 in the epidermal basement membrane and laminin-10/11 in the GBM (11). In podocytes, α3β1 integrin is expressed at the basal side of FPs. Podocytes of α3 integrin–deficient (α3−/−) mice bear a strong morphological resemblance to podocytes in congenital nephrotic syndrome and other pathological conditions with FP effacement (11, 12). The α3 integrin deletion therefore represents a genetic model.
system that allows the analysis of the molecular basis of FP assembly and dynamics.

B7-1, also termed CD80, is a transmembrane protein normally expressed on the surface of B cells and other APCs (13–15). On APCs, B7-1 acts as a costimulatory molecule through binding to its cognate receptors CD28 and CTLA-4 on T cells (13–15). Little is known about B7-1 expression by non–bone marrow–derived cells. In human keratinocytes, B7-1 gene expression is upregulated by allergens and irritants (16). In P815 mastocytoma tumor cells, B7-1 gene expression is induced by oxidative stress (17), which is reminiscent of the induction of B7-1 in podocytes by puromycin, described below. In the kidney, B7-1 can be induced in glomerular endothelial cells by warm ischemia/reperfusion injury in rats (18).

The innate immune system senses pathogen-associated molecular motifs through toll-like receptors (TLRs), thereby initiating an immunological response (19). In APCs, LPS is the strongest endogenous inducer of B7-1 expression through TLR-4 signaling (20). Here we show that danger signaling occurs also in non–bone marrow–derived glomerular podocytes and underlies a dramatic physiological response. Podocytes sense LPS by TLR-4, which triggers the reorganization of the kidney-filtration apparatus, leading to podocyte FP effacement and proteinuria. This response requires the induction of costimulatory molecule B7-1, since proteinuria was abrogated in B7-1–/– mice. We discuss a novel function for B7-1 in the danger signaling of nonimmune cells.

Methods

Cell culture. WT podocytes were cultured as described before (21). We generated conditionally immortalized α3–/– podocyte cell lines by intercrossing α3 integrin heterozygous mice (12) with the ImmortoMouse (Charles River Laboratories Inc., Wilmington, Massachusetts, USA), which carries a temperature-sensitive T antigen as transgene (22). Glomeruli were isolated from kidneys of E18 homozygous embryos, and eight α3–/– clonal podocyte cell lines were obtained by limiting dilution. To show that observed changes in podocyte gene expression and function were due to the absence of α3 integrin, three α3+/– clones were stably transfected with human α3 integrin cDNA as described before for α3+/– collecting duct epithelial cells (23).

Differential display PCR. Total RNA was isolated from differentiated WT and α3–/– podocytes that had been grown under nonpermissive conditions for 14 days using the RNAeasy kit (QIAGEN Inc., Valencia, California, USA). After DNase treatment, reverse transcription was carried out with three different anchored synthetic oligonucleotide primers from the RNAimage kit (GenHunter, Nashville, Tennessee, USA). Differential display PCR reactions were performed in duplicate using 96 different primer combinations exactly as described before (24). Differentially expressed PCR products were cloned into the pCR2 vector using the TA cloning kit (Invitrogen, Carlsbad, California, USA) and sequenced. Sequence analyses and database searches were done with the software program package HUSAR (Heidelberg Unix Sequence Analysis Resources; German Cancer Research Center, Heidelberg, Germany). The following primers specific for B7-1, yielding a 172-bp product, were used: sense, 5′-TTGATGCCAGAAACGTT-3′; antisense, 5′-AGCCCGATCCACG-TGATTA-3′.

Antibodies. The following protein-specific primary antibodies were used: anti–B7-1 (16-10A1; Pharmingen, San Diego, California, USA), anti–B7-1 (R&D Systems Inc., Minneapolis, Minnesota, USA), and mouse monoclonal anti-synaptopodin (25). Rabbit was a gift from L. Holzman (University of Michigan, Ann Arbor, Michigan, USA); rabbit polyclonal anti-nephrin CD2AP was a gift from A. Shaw (Washington University, St. Louis, Missouri, USA); polyclonal anti-ZO-1 was from Zymed Laboratories Inc. (South San Francisco, California, USA).

Immunocytochemistry. Human and murine kidneys were harvested and snap-frozen according to standard protocols and fixed after sectioning in ice-cold acetone for 10 minutes. For immunofluorescent labeling, sections were washed once with PBS and incubated with blocking solution (2% FCS, 2% BSA, 0.2% fish gelatin) for 30 minutes at room temperature before further incubation with one of the primary antibodies for 1 hour at room temperature. For double labeling, sections were washed three times with PBS for 5 minutes, and the second primary antibody was applied for 60 minutes. Antigen-antibody complexes were visualized with secondary antibodies (Rockland Inc., Gilbertsville, Pennsylvania, USA) conjugated with fluorochrome (Cy2 or Cy3). Pictures were captured with a Spot cooled CCD camera (Diagnostic Instruments, Sterling Heights, Michigan, USA) and processed with Adobe Photoshop 5.0 software (Adobe Systems Inc., San Jose, California, USA). Confocal microscopy and processing of micrographs were performed as reported before (25).

LPS treatment of cultured podocytes. Differentiated podocytes were exposed to different doses of LPS (1–50 μg/ml) for 3.5 to 72 hours. RT-PCR for B7-1 was done as described above, and changes in the organization of the actin cytoskeleton were assessed by phallolidin staining as described before (26). Each set of experiments was repeated at least five times.

Nephrin–/– mice. The generation and functional characterization of nephrin–/– mice has been reported before (27). For the present study, kidneys were harvested from 1-day-old nephrin–/– and WT littermate animals, snap-frozen in liquid nitrogen, and processed for immunohistochemistry as described above.

LPS-induced FP effacement and proteinuria. All animal studies were approved by the Albert Einstein College of Medicine Animal Institute Committee. B7-1–/– mice were generated and backcrossed onto a BALB/c background (>12 generations) as previously described (28). SCID mice (29) on a BALB/c background as well as WT 129 and C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, Maine, USA). The base-line urine protein excretion of 4- to 6-week-old female 129 (∼10), C57BL/6 (∼25), BALB/c (∼13), B7-1–/– (∼5), and SCID (∼6) mice was analyzed by urine dipstick (Albustix; Bayer, Tarrytown, New York, USA) before animals were injected intraperitoneally with either 200 μg LPS (1 mg/ml in sterile LPS-free PBS) in a total volume of 200 μl, or equal volumes of sterile LPS-free PBS (PBS: BALB/c WT, n = 5; B7-1–/–, n = 3; SCID, n = 3). Twenty-four hours after injection, urinary protein excretion was measured again, and kidneys were harvested and processed for H&E and immunofluorescence staining. FP effacement was assessed by transmission electron microscopy according to our published protocols (30). In a subset of C57BL mice (n = 5), urine was collected before and 24 hours after LPS injection and analyzed by dipstick. From the same urines we determined the protein/creatinine ratio with Albuwell M and Creatinine Companion protein/creatinine ELISA kits (Exocell Inc., Philadelphia, Pennsylvania, USA), respectively. All measurements were performed in duplicate. The following results were obtained: protein/creatinine.
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Figure 1

B7-1 expression in podocytes. (A) Differential display PCR of WT (+/+ and α3–/–) podocytes. The arrow indicates the position of B7-1. (B) Induction of B7-1 mRNA expression in α3–/– podocytes. RT-PCR from three independent samples (a–c) on serial dilution (1:5 to 1:25) of cDNA standardized for β-actin. Con, H2O control. (C) Rescue (Res) of α3–/– podocytes by stable transfection with human α3 integrin restored B7-1 expression to WT levels. RT-PCR for GAPDH shows equal RNA concentrations. (D) Dose-dependent increase of B7-1 mRNA in WT podocytes in response to puromycin (0.1, 1, 5, 10, and 50 μg/ml). (E) Time course of B7-1 and α3 integrin mRNA expression in puromycin aminonucleoside–treated (PAN-treated) WT podocytes. As early as 6 and 24 hours, B7-1 is induced by PAN, whereas α3 integrin levels remain unchanged. (F) B7-1 is not expressed in E18 WT kidneys (left panel) but is expressed in podocytes of α3–/– kidneys as shown by double labeling with synaptopodin (right panels).

Results

B7-1 is induced in podocytes in genetic and drug-induced models of FP effacement. Podocytes in kidneys of α3–/– mice are unable to maintain normal podocyte structure, including the elaboration of mature FPs along the GBM (12). Therefore α3–/– mice provide an excellent genetic model to study podocyte FP effacement. α3–/– mice die during the perinatal period with severe kidney and lung defects, precluding extensive study of the role of this cell matrix receptor in podocytes in vivo. To delineate the molecular mechanisms underlying FP effacement in α3–/– mice, we took advantage of our previously established cell culture system for WT podocytes (21) and generated α3–/– conditionally immortalized podocyte cell lines. In an effort to identify genes critically involved in the development of FP effacement and proteinuria, we performed differential display PCR of total RNA harvested from cultured WT and α3–/– podocytes. Three PCR products (A31, A32) were differentially expressed between WT and α3–/– podocytes. Two clones, A31 (Figure 1A) and A32, were expressed by α3–/– cells and one, A33, only by WT cells (data not shown). We confirmed the differential expression of all three clones by quantitative RT-PCR (24) (Figure 1B and data not shown). The expression of A31 was 14-fold higher in α3–/– than in WT podocytes (Figure 1B) and therefore was evaluated in detail. The 400-bp product of A31 was identical with a mouse myotube (Figure 1A) and A33, only by WT cells (data not shown). These results showed that changes in urinary protein detected with both tests were comparable.

Microbead experiments. The coating of microbeads was adapted from the method of Sedwick and coworkers (31). Sulfate-coated blue fluorescent microbeads (6.2 μm in diameter; Interfacial Dynamics Corp., Portland, Oregon, USA) were coated with polyclonal anti–B7-1 (16-10A1) antibody or polyclonal antidendrin (negative control). In addition, murine CTLA-4-Ig dendrin (negative control). In addition, murine CTLA-4-Ig was used. Twenty microliters of beads per 5 μg protein were used for surface coating. Podocytes growing in 24-well plates were incubated overnight with the coated beads, followed by immunocytochemistry.

Figure 1

A

B

C

D

E

F

ratio: control, 26 ± 12.78; 24 hours’ LPS, 205 ± 150.01, P < 0.01; dipstick: control (trace = 0), 0.0 ± 0.21; 24 hours’ LPS, 2.0 ± 0.71, P < 0.001. These results showed that changes in urinary protein were

Figure 1

A

B

C

D

E

F

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Podocyte B7-1 expressed in human and murine lupus nephritis correlates with disease stage and severity of proteinuria. To determine the significance of glomerular B7-1 expression in human glomerulonephritis, we analyzed B7-1 expression in human kidney biopsies from different stages of lupus nephritis. Glomerular B7-1 expression correlated with the disease stage in that it increased from WHO class II to V and was strongest in WHO class V kidney biopsies (Figure 2, D and E). As in the murine condition (Figure 2C), glomerular B7-1 colocalized with the podocyte marker synaptopodin (25) (Figure 2E). Taken together, these data underscore the clinical relevance of B7-1 expression in podocytes. They suggest that B7-1 in podocytes contributes to the pathogenesis of proteinuria.

Induction of B7-1 in podocytes of nephrin−/− mice. The nephrin gene NPHS1 is the target gene of congenital nephrotic syndrome of Finnish type, an autosomal-recessive disorder, characterized by massive proteinuria in utero and nephrosis at birth (3). Inactivation of NPHS1 in mice results in massive proteinuria, edema, and perinatal lethality (27, 38). To test whether the induction of B7-1 in podocytes under nephrotic conditions is specific for lupus nephritis, we analyzed the expression of B7-1 in nephrin−/− mice (27). B7-1 colocalized with synaptopodin in podocytes of nephrin−/− mice (Figure 3A). In contrast, no podocyte B7-1 expression was found in WT littersmates (Figure 3B). These data imply that podocyte B7-1 expression is not specific for lupus nephritis but is also found in noninflammatory conditions with proteinuria and is not dependent on immune complexes.

B7-1 expression in podocytes in vitro is induced by LPS through TLR-4 signaling and leads to the reorganization of the actin cytoskeleton. B7-1 expression in APCs can be induced by LPS through TLR-4/CD14-mediated signaling (20). Interestingly, podocytes constitutively express TLR-4 and its coreceptor CD14 and hence respond to LPS (Figure 4A). Therefore, we explored the effects of LPS on podocytes. Exposure to LPS specifically upregulated B7-1 (Figure 4B) and induced the disruption of stress fibers (Figure 4C), creating a cortical ring of F-actin and a submembranous space lacking actin fibers (Figure 4D). This cytopathic effect is reminiscent of the actin changes that are observed in FP effacement in vivo (1) and suggests that LPS may contribute to the pathogenesis of FP effacement and proteinuria through the reorganization of the podocyte actin cytoskeleton.

LPS upregulates B7-1 in podocytes in vivo and leads to nephrotic syndrome in WT but not in B7-1−/− mice. To test this hypothesis further, we injected a single low dose of LPS intraperitoneally into adult female WT BALB/c mice and analyzed the effects on kidney histology, podocyte B7-1 expression, and urinary protein excretion. Histologically, kidneys of LPS-injected mice were

**Figure 2**

B7-1 expression in podocytes in murine and human lupus nephritis correlates with the severity of proteinuria. (A) B7-1 is not expressed in normal mouse kidney (left). In the lupus kidney, strong glomerular (G) B7-1 expression is observed (middle). In addition, infiltrating interstitial B cells stain positive (arrows). At higher magnification (right), B7-1 expression is found in podocytes (arrows) in the glomerulus. (B) Double labeling with synaptopodin confirmed that intraglomerular B7-1 expression is restricted to podocytes in 4+ proteinuric (4+ prot) nephritis. (C) B7-1 is absent in an age-matched lupus mouse with trace proteinuria (trace prot). (D and E) In human lupus nephritis, glomerular B7-1 expression increases from WHO class II to V and colocalizes with synaptopodin in podocytes (E).
not distinguishable from PBS-injected controls (Figure 5A). In particular, no signs of inflammation were found. LPS induced upregulation of B7-1 in podocytes (Figure 5B) and FP effacement within 24 hours (Figure 5D, left panel). In contrast, PBS injection did not induce podocyte B7-1 expression (Figure 5C) or FP effacement (Figure 5D, right panel). Most significantly, LPS injection caused severe proteinuria (n = 13; base line, 0.8 ± 0.4, vs. LPS, 2.7 ± 0.9; P < 0.001, two-sided t test) within 24 hours (Figure 5G). In contrast, no proteinuria was found in PBS-injected controls (data not shown). We next established that LPS-induced nephrotic syndrome is independent of the mouse strain, since we found it also in WT 129 (n = 9; base line, 0.7 ± 0.5, vs. LPS, 2.1 ± 0.78; P < 0.001, t test) and C57BL/6 mice (n = 23; base line, 0.5 ± 0.29, vs. LPS, 1.9 ± 0.56; P < 0.0001, t test) (Figure 5G). The LPS-induced proteinuria was transient and returned to base-line levels after 72 hours (data not shown). To establish a causal relationship between podocyte B7-1 upregulation and development of proteinuria, the experiments were repeated with B7-1−/− mice (28). No proteinuria was observed in LPS-injected B7-1−/− mice (n = 5; base line, 1.0 ± 0, vs. LPS, 0.6 ± 0.55; P not significant, t test) (Figure 5G). The differences in proteinuria between WT and B7-1−/− mice were statistically significant (P < 0.001, t test). These data suggest that B7-1 induction in podocytes is necessary for the development of LPS-induced proteinuria.

**Discussion**

This study is the first to demonstrate that B7-1 is expressed in podocytes under a variety of stress conditions. Moreover, the results of this study establish the clinical significance of B7-1 upregulation in podocytes contributing to the pathogenesis of proteinuria. To test whether the ligation of B7-1 transduces intracellular signals in podocytes, thereby modulating the composition of the SD, we used a microbead approach to cross-link B7-1. With this approach, three key proteins of the SD, nephrin (3), CD2AP (4), and ZO-1 (39), were analyzed in α3−/− podocytes by immunocytochemistry after binding of microbeads coated with CTLA-4-Ig or anti–B7-1 antibody (Figure 6). The detailed microscopic analysis revealed that nephrin was redistributed from cell-cell contacts (Figure 6A) and accumulated around CTLA-4-Ig–coated (Figure 6B) or anti–B7-1–coated microbeads (Figure 6C). The association of SD proteins with anti–B7-1–coated microbeads was confirmed by confocal microscopy, which showed a ring of nephrin lining the microbeads (Figure 6D).

Similar results were obtained for CD2AP, which was redistributed from the cell membrane and the actin filaments (Figure 6E) to microbeads (Figure 6F-H). ZO-1 relocated from cell-cell junctions (Figure 6I) to the microbeads (Figure 6, J-L). To show that the redistribution of SD proteins was specifically mediated by B7-1, several control experiments were performed. First, microbeads were coated with a mutated variant of murine CTLA-4-Ig that can no longer bind to Fc receptor. Second, microbeads were coated with an irrelevant antibody with an intact Fc fragment. The microbeads coated with the irrelevant antibody (dendrin) could to some extent adhere to podocytes but did not cause the rearrangement of ZO-1 (Figure 6M). Third, uncoated microbeads were used. No binding was observed with uncoated microbeads or beads coated with the irrelevant antibody (data not shown). Moreover, B7-1 clustering beads had no effect on cultured WT podocytes, which do not express B7-1 (data not shown). In summary, the results of the bead experiments are specific and conclusive. They suggest that B7-1 may contribute to the pathogenesis of proteinuria by disrupting the SD complex.

**Figure 3**

Induction of B7-1 in podocytes of nephrin−/− mice. (A) B7-1 is induced in podocytes of nephrin−/− mice as shown by double labeling with synaptopodin. (B) B7-1 is absent from podocytes of WT littermate controls. These data establish that the expression of B7-1 in podocytes in vivo can occur independently of immune complexes or inflammatory cells.

**Figure 4**

LPS-induced upregulation of B7-1 and actin cytoskeleton reorganization in podocytes in vitro. (A) Cultured podocytes express the LPS receptors CD14 and TLR-4 as shown by RT-PCR. (B) LPS treatment upregulates B7-1 mRNA expression and reorganizes the podocyte cytoskeleton from a stress fiber pattern (C), creating a cortical ring of F-actin (D; arrowheads) and a submembranous space devoid of actin fibers (asterisks).

**Figure 5**

Cultured podocytes from WT mice (A and C) and B7-1−/− mice (B and D) were treated with LPS or PBS for 24 hours. (A) and (B) show localization of B7-1 with nephrin. (C) and (D) show localization of actin with nephrin. The results of this study establish the clinical significance of B7-1 upregulation in podocytes contributing to the pathogenesis of proteinuria. To test whether the ligation of B7-1 transduces intracellular signals in podocytes, thereby modulating the composition of the SD, we used a microbead approach to cross-link B7-1. With this approach, three key proteins of the SD, nephrin (3), CD2AP (4), and ZO-1 (39), were analyzed in α3−/− podocytes by immunocytochemistry after binding of microbeads coated with CTLA-4-Ig or anti–B7-1 antibody (Figure 6). The detailed microscopic analysis revealed that nephrin was redistributed from cell-cell contacts (Figure 6A) and accumulated around CTLA-4-Ig–coated (Figure 6B) or anti–B7-1–coated microbeads (Figure 6C). The association of SD proteins with anti–B7-1–coated microbeads was confirmed by confocal microscopy, which showed a ring of nephrin lining the microbeads (Figure 6D).

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podocyte B7-1 expression and elucidate its role in the pathogenesis of nephrotic syndrome. We specifically demonstrate that podocyte B7-1 reorganizes the actin cytoskeleton of podocytes and modulates SD organization. The effects are independent of T cells and B cells and represent a novel unique function of B7-1. This study also is the first to show that LPS injection in mice induces B7-1-dependent nephrotic syndrome with FP effacement and urinary protein loss.

So far, B7-1 had been assumed to be predominantly expressed on B cells and other professional APCs. The traditional role of B7-1 is T cell costimulation (13–15). Along with the interaction of the T cell receptor with antigen-MHC complexes, the interaction of B7 with its receptors CD28 and CTLA-4 on T cells plays a pivotal role in T cell–mediated immunity (13–15). In T cells, binding of B7-1 to its receptors on the T cell surface triggers migration of protein kinases, actin-binding proteins, and other accessory molecules to the T cell–APC interface (40, 41). This formation of the immunological synapse causes a rearrangement of the T cell actin cytoskeleton (40, 42–44), activates protein tyrosine kinases that couple to PI3K, and controls the activity of Ras and Rho-family GTPases in T cells (45).

The role of B7-1 in T cell costimulation is well established and defines a dialogue between a B7-1–expressing APC and a T cell to which the costimulatory signal is conveyed (13–15). In contrast, B7-1–mediated outside-in signaling in B7-1–expressing APCs is still obscure. With respect to outside-in signaling through B7-1 on B7-1–expressing cells, two studies have been published. One study showed that cross-linking of B7-1 on B lymphoma Raji cells induces tyrosine phosphorylation of intracellular proteins, indicating a role of B7-1 in outside-in signaling (46). Further support for a role of B7-1 in outside-in signaling comes from a recent report showing that CTLA-4Ig–mediated ligation of B7-1 regulates tryptophan catabolism in dendritic cells (47). These changes are reminiscent of the changes seen in SD protein redistribution after ligation/activation of B7-1 in podocytes (Figure 6).

In human keratinocytes, B7-1 gene expression is induced by allergens and irritants (16). In P815 mastocytoma tumor cells, B7-1 gene expression is induced by oxidative stress (17). In APCs, MyD88-independent LPS/TLR-4 signaling is the strongest activator of B7-1 expression (20, 48). Similarly, B7-1 induction in podocytes appears to be downstream of various signaling pathways, including TLR signaling (Figure 5). Generally, somatic cells grown in culture are, for the most part, quite indifferent to LPS (20), and earlier studies had shown that macrophages are the primary target cells of LPS in septic shock (20). Here we demonstrate that podocytes constitutively express the LPS receptor TLR-4 and its coreceptor CD14 and respond to LPS exposure with upregulation of B7-1. This is another unanticipated finding of the present study.

The LPS experiments (Figures 4 and 5) allowed us to explore several important questions. First, they established that the induction of podocyte B7-1 in conditions with FP effacement and proteinuria was causative and not the consequence of the nephrotic condition, since B7-1–/– mice were protected from LPS-induced proteinuria. Second, one might argue that the effects of LPS are predominantly mediated through the action of T cells. Our data refute this hypothesis, because (a) SCID mice lacking T and B cells showed the same degree of FP effacement and proteinuria as WT mice; (b) exposure of cultured podocytes in the absence of any other cells was sufficient to upregulate B7-1 and reorganize the podocyte actin cytoskeleton; and (c) the cell-free microbead-mediated cross-linking of B7-1 on α3+/– podocytes was still obscure.
sufficient to redistribute vital SD proteins. Collectively, these data suggest that B7-1 expression by podocytes represents a pathogenic mechanism for disruption of the glomerular filtration apparatus that is independent of T cell activation.

An intriguing question remains, why do podocytes upregulate B7-1? In the grass shrimp, Palaemonetes pugio, podocytes are involved in the ultrafiltration of hemolymph where micro- and macromolecular substances are cleared (49). In the pre-molt stage of this shrimp, podocyte assemblages extend into the efferent hemolymph channels and are in direct contact with immune cells, and they may serve as a component of the LPS-sensing (20) innate immune response. The upregulation of podocyte B7-1 by LPS may be a phylogenetically preserved mechanism, which can be reactivated under pathological conditions, e.g., in patients with Gram-negative sepsis. Consistent with this idea, the development of transient proteinuria has been found during the course of Gram-negative sepsis (50). Moreover, a prospective study showed that in postoperative SCID mice control for T or B cell–mediated effects, it is still possible that dendritic cells or other APCs might contribute to the observed effects. Future studies with a podocyte-specific B7-1 knockout should allow us to address this question.

We propose that transient B7-1–induced nephrotic syndrome may be a physiological response. Clearly, future studies will be required to explore this hypothesis in detail, but it could potentially explain how a normally beneficial danger-sensing mechanism can have deleterious effects in certain genetic backgrounds. Understanding the role of B7-1 in podocytes should allow new avenues toward the development of novel, selective podocyte-protective therapies that modulate danger signaling in podocytes and tackle proteinuria. It will be interesting to see whether B7-1–dependent pathways are also operative in chronic kidney disease.

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Figure 6
Redistribution of SD proteins to sites of B7-1 engagement. (A) In untreated α3+/+ podocytes, nephrin is expressed at cell-cell contacts. (B and C) Incubation of podocytes with microbeads coated with CTLA-lg (B) or anti–B7-1 antibody (C) redistributes nephrin around microbeads (arrows). (D) The association of nephrin with microbeads was confirmed by confocal microscopy. (E–L) Similar redistribution patterns were seen for CD2AP (E–H) and ZO-1 (I–L). Arrows in F and J: accumulation of CD2AP (F) and ZO-1 (J) around microbeads. Asterisk in K: microbead. (M) In contrast, microbeads coated with an irrelevant antibody could to some extent adhere to podocytes (arrowhead) but did not affect the localization of ZO-1 at cell-cell contacts (arrows).

Figure 7
Pathways leading to B7-1–mediated proteinuria. Various stimuli may lead to podocyte B7-1 induction. These include genetic stimuli (e.g., deletion of α3 integrin or nephrin), toxic stimuli (PAN-induced reactive oxygen species [ROS]), or direct stimulation of the TLR-4/CD14 receptor on the podocyte. B7-1 then induces FP effacement and disruption of the SD complex, thereby modifying glomerular permselectivity.
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