containing integrins in glomerular function? There has not been a detailed examination of kidneys in αv null mice or in conditional knockouts of the gene encoding αv integrin in any kidney lineages; therefore, it is possible some kidney defects have been missed. Conversely, much of the αv-containing integrin in the glomerulus is thought to be αvβ3, and β3 integrin null mice have relatively normal kidneys[11] that would argue against a developmental role for αvβ3 in kidneys. Borza *et al.* also demonstrated that α3β1 integrins bind the α3NC1 collagen domain and transdominantly inhibit adhesion mediated by αvβ3 integrin,[12] suggesting regulatory interplay between integrins on podocytes. In addition, some recent evidence posits an important role for αvβ3 integrin in kidney injury.[11] It is known that GPI-linked urokinase-type plasminogen activator receptors (uPAR) interact with several integrins that modulate their ligand-binding activities.[13] Wei *et al.*[11] showed that uPAR and αvβ3 integrin co-localize in podocyte foot processes and that uPAR-deficient mice are resistant to LPS-induced foot process effacement. αvβ3 integrin is among those that are modulated by uPAR, and activation of αvβ3 integrin in glomeruli (engaged by an activation-specific antibody) is decreased in uPAR-deficient mice.[13] Finally, expression of a constitutively active β3 integrin in podocytes results in proteinuria.

Together, the studies of Borza *et al.* and Wei *et al.* now indicate a role for αvβ3 integrin in glomerular disease and indicate a binding site for αvβ3 integrin in the KRGDS motif of human and primate α3 type IV collagen. Although the latter study indicates a role for αvβ3 in glomerular disease even in the absence of this motif in rodents, this does not preclude a role for the motif in modulating glomerular function in humans or from its having a role in human kidney disease. Further studies will need to examine the phosphorylation of this site in various models of glomerular disease and the effects of mutagenizing this site to prevent phosphorylation or integrin binding. Genetic studies in animals are difficult because this motif is not found in rodents. Perhaps immortalized human podocytes can be used as an *in vitro* model for additional studies.

DISCLOSURES

None.

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See related article, "Human Podocytes Adhere to the KRGDS Motif of the α3α4α5 Collagen IV Network," on pages 677–684.

Are Podocytes Passive or Provocative in Proteinuric Glomerular Pathology?

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Podocytes are highly specialized epithelial cells with unique structure and function that are essential to maintenance of the

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glomerular filtration barrier. Because of their pivotal role in glomerular filtration, their delicate fimbriated structure, and their limited ability for regeneration and repair, podocytes have been considered critical and vulnerable targets of glomerular injury. Disruption of the anatomic relationships between adjacent foot processes and between foot processes and the glomerular basement membrane (GBM) are among the earliest morphologic features of glomerular injury where proteinuria is a hallmark.

Podocytes are generally regarded as passive targets of both immune and nonimmune injury. In experimental models, they are highly susceptible to a variety of injurious agents, including complement, reactive oxygen species (ROS), and toxins such as puromycin aminonucleoside. Podocytes upregulate their expression of C5a receptors[1] and become the major target of complement-mediated immune injury in membranous nephropathy.[2] In Heymann nephritis, a rat model of human membranous nephritis, the combined insults of sublytic amounts of complement membrane attack complex and ROS induce podocyte injury and proteinuria.[3] Albuminuria in diabetic nephropathy results from nonimmune glomerular injury. The loss of $\alpha 3\beta 1$ integrin anchoring of foot processes to the GBM followed by podocyte detachment and shedding into the urine is now recognized as an early pathologic feature in experimental[4,5] and human diabetes.[4] In puromycin aminonucleoside–induced nephrosis, used as a model of idiopathic nephrotic syndrome, direct oxidative mechanisms induce podocyte foot process effacement[6] and apoptosis[7] leading to proteinuria.

Recent studies, including that by Banas *et al.*[8] in this issue of *JASN*, suggest podocytes may aggravate immune and nonimmune glomerular injury and contribute to their own demise through expression of receptors linked to pathways that induce proinflammatory molecules. Banas *et al.* studied immune complex–initiated injury in thymic stromal lymphopoietin transgenic mice that develop cryoglobulinemia and membranoproliferative glomerulonephritis.[8] Expression of mRNA encoding TLR-1, -2, and -4 are markedly upregulated early in the development of disease, and their expression is further increased when the severity of glomerulonephritis is augmented by deletion of an inhibitory Fc receptor (FcRIIb). TLR-4 expression is found on podocytes in nephritic glomeruli, and *in vitro* studies using immortalized mouse podocytes demonstrate induction of chemokine expression in response to lipid A, the specific TLR-4–binding component of LPS ligand. Interestingly, fibrinogen, an endogenous TLR-4 ligand, prominently deposited in glomeruli in this model and exhibits a similar ability to induce chemokine production by podocytes. These studies demonstrate the potential of podocytes to contribute actively to recruitment of inflammatory cells and glomerular injury by upregulating TLR-4 and production of proinflammatory chemokines in response to stimulation by either endogenous or exogenous TLR-4 ligands.

Although the studies by Banas *et al.* did not investigate the separate *in vivo* contributions to renal injury of TLR-4 expression by podocytes and leukocytes, a recent study by Brown *et al.*[9] addressed this issue using an acute anti-GBM antibody/lipid A–induced model of glomerular injury. Glomerular TLR-4 expression, rather than expression on leukocytes, is principally required for development of proteinuria in this model. TLR-4 expression on podocytes and mesangial cells and involvement of CXC chemokines in TLR-4–dependent injury was demonstrated, adding further weight to the argument that intrinsic renal cells, including podocytes, amplify glomerular injury through TLR-4–dependent pathways. Glomerular epithelial cells produce the proinflammatory cytokines TNF-α and IL-6 after LPS stimulation *in vitro*.[10] The ability of intrinsic renal cells to amplify inflammatory glomerular injury by their capacity to produce cytokines, particularly TNF-α, has been previously reported in murine anti-GBM nephritis,[11] and in human membranous nephritis, podocytes are a prominent source of TNF-α.[12]

Rapid induction of B7-1 (CD80) on podocytes and development of proteinuria in mice after administration of LPS and puromycin provides more evidence of the ability of podocytes to acquire a proinflammatory phenotype.[13] B7-1 is better known as a co-stimulatory molecule involved in antigen presentation to T cells, but its contribution to development of proteinuria in this study was independent of T cell participation. LPS-stimulated TLR-4 induces upregulation of B7-1 expression by podocytes *in vitro* and results in rearrangement of their actin cytoskeleton. In light of the work by Banas *et al.*,[8] induction of chemokine expression may have been expected, but this end point was not reported. Higher B7-1 expression on podocytes is also associated with more severe World Health Organization classes of glomerulonephritis in human lupus nephritis and more severe proteinuria and renal injury in murine lupus, suggesting involvement in inflammatory cell recruitment.

Podocytes amplify both immune and nonimmune glomerular injury by their capacity to produce and release ROS. Increased production of ROS by podocytes is also implicated in various experimental proteinuric diseases, including puromycin nephrosis,[14] Heymann nephritis,[15] Mpv17 gene–inactivated mice (a model of focal segmental sclerosis),[16] type 1 diabetes,[17] and human membranous nephropathy.[18] Podocytes express chemokine receptors and produce ROS in response to chemokine stimulation *in vitro*.[19] In turn, ROS stimulate podocyte production of the proinflammatory cytokine GM-CSF,[20] induce podocyte cell-cycle checkpoint proteins,[14] induce apoptosis in podocytes,[17] and directly injure the GBM.[3,15]

In diabetic nephropathy, evidence is emerging that acquisition of proinflammatory capacities by podocytes contributes to perpetuation of injury. Both hemodynamic and metabolic stimuli contribute to these changes in podocyte phenotype. For example, mechanical stretch can induce angiotensin II (AngII) expression in cultured podocytes.[21] An-

gII stimulates podocyte vascular endothelial growth factor production, which, in an autocrine manner together with TGF-β, stimulates production of α5(IV) collagen.[22] Targeted overexpression of AngII in podocytes leads to proteinuria and glomerulosclerosis in rats.[23] Exposure of cultured podocytes to high glucose concentrations increases ROS production and podocyte hypertrophy,[24] modulates production of matrix metalloproteases and α5(IV) collagen,[25] and stimulates 12-lipoxygenase and p38 mitogen-activated protein kinase signaling pathways.[26] The role of podocytes in diabetic nephropathy has been covered extensively in recent reviews.[27,28]

The study by Banas *et al.*[8] adds to accumulating evidence that podocytes provide can acquire functions that augment both immune and nonimmune glomerular injury in response to various pathogenic stimuli. This evidence challenges the view that podocytes are passive participants and merely a common final pathway or target of injury in proteinuric renal diseases.

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