Glomerular epithelial cells, or podocytes, are an essential part of the glomerular filtration barrier that prevents passage of proteins of the size of albumin or larger from the circulation into glomerular ultrafiltrate. Podocytes are specialized cells that consist of the cell body and primary, secondary, and tertiary processes. The cytoskeleton of the cell body and major processes consists primarily of microtubules and vimentin intermediate filaments, whereas the major cytoskeletal component of the tertiary processes, or foot processes, is actin microfilaments. Actin cytoskeleton maintains the architecture of the foot processes and responds to signals from outside of the podocyte to modify its structural and functional characteristics.

Neighboring podocytes are interconnected with a specialized cell adhesion structure, the slit diaphragm, that shares features reminiscent of both adherens and tight junctions. In 1998, upon identification of nephrin, the first integral protein of the slit diaphragm, characterization of the slit diaphragm proteins and their functions became a subject of intensive research. Thereafter, several other immunoglobulin superfamily members sharing homology with nephrin, as well as members of the cadherin superfamily, among other proteins, have been localized to the slit diaphragm. Importantly, many of these proteins are essential for maintaining the normal podocyte morphology and function, as lack of or mutations in, for example, nephrin, its binding partners NEPH1, podocin, and CD2AP, or the cadherin superfamily member FAT1 lead to podocyte foot process effacement (flattening) (for references, see Johnstone and Holzman). Foot process effacement is associated with detachment of podocytes from the glomerular basement membrane, loss of slit diaphragms and their replacement by tight junctions, and development of proteinuria.

Foot process effacement is a dynamic and reversible process and requires active interplay between the slit diaphragm proteins and their extracellular ligands as well as their cytosolic effectors that connect the protein complexes to signaling networks and actin cytoskeleton. Even though the regulation of actin polymerization/depolymerization is well characterized, little has been known about the ligand-induced signaling events that regulate actin dynamics in podocytes. Several recent publications, including the one by Uchida et al. in this issue of *Kidney International*, elegantly demonstrate the involvement of the slit diaphragm protein complexes in ‘outside-in’ signaling pathways that participate in the regulation of the actin cytoskeleton of the podocytes in health and disease.

The cytosolic domain of nephrin contains several putative tyrosine phosphorylation sites that have emerged as important regulatory sites of nephrin function. Uchida et al. characterize two new phospho-nephrin-specific antibodies that recognize rat nephrin, which is tyrosine phosphorylated on either residue Y1204 or Y1228, and show that nephrin is phosphorylated on these residues in normal rat glomeruli *in vivo*. Further, nephrin phosphorylation on these residues is decreased upon puromycin aminonucleoside (PAN)-induced podocyte damage and proteinuria. Already 1 day after PAN injection, and before the development of overt proteinuria, the level of phosphorylated nephrin was significantly decreased; it was hardly detectable after 7 and 14 days and returned back to the normal level 28 days after PAN injection. The reduction reflects a decrease in nephrin phosphorylation, as the reduction of total nephrin was less than that of its phosphorylated form. Supporting these findings, nephrin was shown to be phosphorylated in normal rat glomeruli, and this phosphorylation was decreased in PAN nephrosis. It has also been shown that nephrin tyrosine phosphorylation is minimal in normal rats but increases 3 days after induction of PAN nephrosis. In addition, in protamine sulphate-induced foot process effacement in mice and passive Heymann nephritis in rats, nephrin phosphorylation increases. The above discrepancies may be explained by differences in the animal models and experimental protocols and antibodies used, but they may also reflect the dynamic nature of nephrin phosphorylation under changing circumstances. Nonetheless, the data indicate an important role for nephrin phosphorylation status in glomerular disorders and call for careful time sequence studies on glomeruli *in vivo*, especially during disease progression and recovery, and for the specific targeting of each phosphorylation site in nephrin.

Several of the tyrosine residues in the cytosolic domain of nephrin are

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phosphorylated by Fyn and other Src family kinases,6,9,10 producing potential binding sites for SH2 domain-containing proteins. Accordingly, the SH2 and SH3 domain-containing adaptor protein Nck has been identified as a binding partner of nephrin.4,8 The binding of Nck to nephrin is tyrosine phosphorylation dependent and is mediated via the SH2 domain of Nck. The SH3 domains of Nck in turn recruit proteins of the actin polymerization machinery, including N-WASp and Arp2/3, to the complex (Figure 1). In line with this, engagement of nephrin extracellular domain (by antibody-induced clustering) leads to nephrin tyrosine phosphorylation, recruitment of Nck to the complex, and induction of localized actin polymerization, indicating a role for nephrin and Nck in the regulation of actin polymerization. The essential role of Nck in podocytes is further substantiated by the finding that lack of both Nck isoforms (Nck1/2) in podocytes leads to defects in foot process formation and to proteinuria.5

Whereas the above studies used cultured cells to study the mechanisms by which nephrin regulates actin skeleton, Uchida et al.3 took one step further to analyze actin dynamics in glomeruli in vivo in health and disease. They found that upon decreased nephrin phosphorylation on Y1204 and Y1228, the amount of filamentous actin (F-actin) transiently decreased and globular actin (G-actin) increased in PAN nephrosis. Further, the decrease in F-actin appeared most prominent in podocytes. Interestingly, Li et al. show that these two residues, Y1204 and Y1228 of rat nephrin (conserved in rat, mouse, and human), both contribute significantly to the binding of nephrin with Nck, and that Y1228 may be the preferred binding site in vivo.6 Additionally, nephrin–Nck interaction was reduced on day 7 of PAN nephrosis.6 Collectively, these findings suggest that reduced phosphorylation of nephrin on Y1228 may result in decreased binding of nephrin to Nck and alterations in actin polymerization in podocytes.

In addition to Nck, phosphoinositide 3-kinase (PI3K) provides an alternative pathway for nephrin-mediated regulation of the actin cytoskeleton (Figure 1). Tyrosine phosphorylation of nephrin increases its interaction with PI3K and induces activation of Akt and Rac1, both known regulators of the actin cytoskeleton. This leads to a decrease in stress fibers and an increase in membrane ruffles and lamellipodia in cultured podocytes.5 The mechanisms leading to decreased stress fibers involve inactivation of RhoA, a small GTPase, and activation of coflin, an actin filament-severing protein. In PAN nephrosis, both nephrin–PI3K interaction and Akt activation decreased, supporting a role also for this actin regulatory pathway in modulating podocyte architecture and function.

Without any doubt, the regulation of podocyte function(s) is complex and requires cooperative mechanisms. This is well illustrated by Garg et al., who found that the nephrin homolog NEPH1 is phosphorylated by Fyn, leading to recruitment of Grb2 and localized actin polymerization at the plasma membrane (Figure 1).7 Furthermore, nephrin and NEPH1 together were more potent in inducing actin polymerization than either of them alone, indicating a cooperative function for nephrin and NEPH1. In addition to immunoglobulin superfamily members, cadherin superfamily members also are components of the slit diaphragm and associate with the actin cytoskeleton and/or proteins regulating actin (Figure 1) (for references, see Johnstone and Holzman8).
It should be noted that not all of the interactions between the slit diaphragm proteins and the cytoskeleton are regulated by phosphorylation. For example, nephrin and NEPH1 associate with CD2AP and ZO1, respectively, both of which are proteins associated with actin (Figure 1) (for references, see Johnstone and Holzman), and phosphorylation-dependent regulatory mechanisms have not been identified for these interactions thus far. Thus the nephrin–NEPH1 protein complex has multiple ways to associate with and regulate the actin cytoskeleton. Further studies using cell culture and especially animal models are necessary to define the cooperative functions of the slit diaphragm protein complexes.

Finally, the key task is to define the molecular mechanisms regulating podocyte actin dynamics during development of podocyte injury and proteinuria in various human glomerular diseases. Uchida et al. analyzed by immunofluorescence microscopy seven minimal-change nephrosis patients for the presence of tyrosine-phosphorylated nephrin in podocytes and found that the level of nephrin Y1228 phosphorylation was significantly lower than in control patients. Although the patient number was quite small, the result, together with studies on animal models and cultured cells, supports a role for the nephrin protein complex not only as a structural component of the slit diaphragm structure, but also as an active signaling scaffold modulating the structural and functional characteristics of podocytes. A further challenge will be to define the molecular mechanisms and signals from within the podocyte that regulate ligand engagement and functional behavior of the slit diaphragm proteins.

ACKNOWLEDGMENTS

The Academy of Finland is acknowledged for financial support.

REFERENCES


The classic clinical triad of rash, fever, and eosinophilia in a patient with acute renal failure, especially if non-oliguric, would prompt a search for urinary eosinophils and a discontinuation of methicillin or any other potential offending medication. Recent studies document that the full hypersensitivity triad is not often present, and suspicion of AIN must be present with any of these features in a patient with renal failure on suspect medications.1–4 When nonsteroidal anti-inflammatory drugs (NSAIDs) were reported to have unique clinical features, such as onset of the nephrotic syndrome, in association with acute renal failure and AIN, this was rapidly absorbed by clinicians. Thus, clinical criteria for medication-induced AIN have been established for years. Likewise, nephropathologists have become adept at diagnosing AIN and even predicting medications as the etiology of the AIN by noting not only ‘tubulitis,’ but eosinophilic infiltrates and at times granulomatous changes when the offending agent was stopped.

Although the spectrum of acute interstitial nephritis (AIN) encompasses many entities, including sarcoidosis, tubulointerstitial nephritis and uveitis syndrome, lupus, and other autoimmune interstitial nephritides, medication-related AIN remains the most common and clinically relevant form in native kidneys.1–3 Although numerous medications have been incriminated, methicillin and other β-lactam antibiotics were the prototype offending agents for many years. Studies documented an epidemiologic relationship between the renal lesion and the penicillin, there were cases with recurrence on rechallenge, and remissions of the clinical disease occurred when the offending agent was stopped.

Acute interstitial nephritis (AIN) is an uncommon form of acute renal failure that is usually medication related. Although the clinical features and renal histopathology are well recognized, therapy beyond discontinuing the offending drug has been a challenge. The use of corticosteroids, although supported by numerous small retrospective studies and anecdotal case reports, has been controversial. The study by González et al., although it has limitations, provides solid support for the early use of corticosteroids in the treatment of drug-related AIN.

The treatment of acute interstitial nephritis: More data at last

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