SCIENCE BEHIND THE STUDY
Elizabeth G. Phimister, Ph.D., *Editor*

Kidney Disease and Antinephrin Antibodies

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For years, the pathogenesis of acute-onset nephrotic syndromes such as minimal change disease and focal segmental glomerulosclerosis (FSGS) has lacked delineation. However, a deficit in nephrin, a transmembrane surface and signaling protein of the podocyte,[1] is now in the spotlight as a driver of minimal change disease and a form of FSGS. (The podocyte is a specialized epithelial cell of the glomerulus.) Indeed, antibodies to nephrin that are injected into rats cause proteinuria,[2] and autoantibodies specific to nephrin in humans with minimal change disease were reported by Weins and colleagues in 2022.[3]

In this issue of the *Journal*, Hengel et al.[4] report that 46 of 105 patients (44%) with minimal change disease and 7 of 74 patients (9%) with primary FSGS had antinephrin autoantibodies; among patients with active disease who had not received immunosuppressive treatment, the percentages were 69% and 90%, respectively. The authors also describe how antinephrin autoantibodies may incite glomerulopathy. An understanding of the structure and function of the kidney and of its main task — the production of urine — is necessary to fully appreciate the implications of this study.

FILTRATION OF BLOOD AND FORMATION OF URINE

The blood in an average healthy adult is filtered through the approximately 2 million glomeruli contained within the kidneys — every 5 minutes! Thus, every 24 hours, an adult human produces the equivalent of eighteen 10-liter fish tanks of filtrate. Ultimately, most of this filtrate is resorbed downstream in the kidney tubules, resulting in the net production of a liter or two of urine, which, in healthy persons, is nearly free of protein and large molecules but contains smaller waste substances (Fig. 1). Impairment of the filtration process imposes a large burden: approximately 12% of persons with such impairment go on to have some form of kidney disease, and the majority of persons with kidney disease have dysfunctional glomeruli, characterized by reduced filtration of waste products from the bloodstream and increased loss of proteins that would otherwise maintain oncotic pressure within the circulation.

HOW DOES THE KIDNEY FILTER BLOOD?

Within each glomerulus, blood is filtered as it moves under pressure through specialized capillaries, the walls of which are made up of three layers: a fenestrated endothelial layer that retains red cells and molecules larger than approximately 6 to 8 nm, a basement membrane, and a layer of podocytes.[5] Each podocyte has many long tentacle-like "foot processes"; in the healthy kidney, the flat terminus (the sole plate) of each foot process is tightly sealed to the basement membrane, tethered to it by adhesion molecules called integrins. The proper functioning of this seal and that of the slit diaphragm, a specialized junction between neighboring podocyte foot processes that is made up of structural proteins (such as nephrin, which extends from the surface of the podocyte into the slit diaphragm), is critical to the blood filtration process. Indeed, pathogenic variants in more than 60 genes encoding podocyte proteins (several of which are components of the slit diaphragm) can cause kidney disease.[6] For example, variants in *NPHS1*, which encodes nephrin, cause a congenital form of nephrotic syndrome,[3] and variants in *TRPC6*, a transmembrane ion channel to which nephrin binds in *cis*, cause FSGS.[7]

WHAT CAUSES INJURY TO THE PODOCYTE?

Pathogenic genetic variants that cause changes to structural proteins represent one source of injury. However, the filtering apparatus of the glomerulus has mechanical and signaling properties

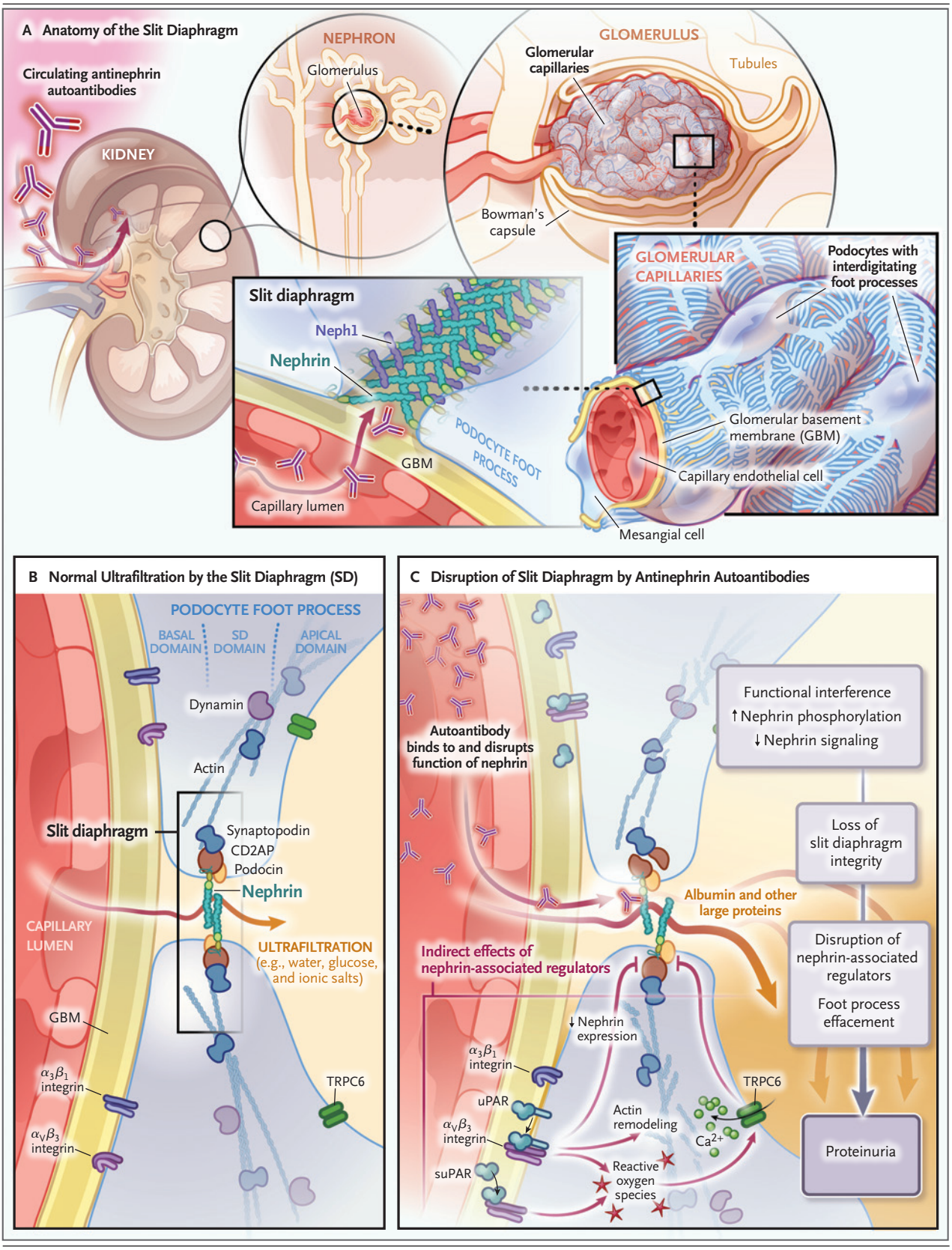

Figure 1 (facing page). The Kidney Ultrafiltration Barrier in Healthy Persons and Those with Nephrotic Syndrome.

Each kidney contains approximately 1 million nephrons (each nephron consists of a glomerulus and a tubule). The kidney filtration mechanism, which produces an ultrafiltrate of the blood, occurs throughout the glomerular capillaries and is made up of fenestrated endothelial cells lining the capillaries, a glomerular basement membrane (GBM), and, on the antiluminal side of the GBM, a layer of podocytes. Tightly bound connections between podocyte foot processes form a slit diaphragm (Panel A). This slit diaphragm, a coassembled network of proteins, is made up of the junction-forming proteins nephrin, Neph1, and podocin (Panel B). Nephrin possesses a signaling function that is regulated mainly through the phosphorylation of tyrosine residues in its intracellular domain. It interacts with podocin and the transient receptor potential channel TRPC6. Nephrin-binding autoantibodies or antibodies against unknown targets that functionally interfere with nephrin disrupt the glomerular filter without eliciting complement activation (Panel C). Nephrin can also become dysregulated indirectly through the activation of podocyte integrins by urokinase plasminogen activator receptor (uPAR) or by circulating soluble uPAR (suPAR). Such activation leads to actin remodeling, induction of TRPC6 expression, and nephrin down-regulation. The disruption of nephrin by autoantibodies or indirectly through the effects of uPAR or suPAR and TRPC6 undergird the effacement of podocyte foot processes and the dysregulation of kidney ultrafiltration, leading to proteinuria.[5-7] These events can occur in concert or separately from each other.

that are sensitive to circulating inflammatory proteins, toxins, and antibodies. Inflammatory insult may be caused by viral infection. In this instance, the integrin molecules on the sole plate of podocyte foot process may become activated by a messenger molecule of the innate immune system, the soluble urokinase plasminogen activator receptor (suPAR), thereby leading to changes in nephrin expression, a shortening of the slit diaphragm, and, eventually proteinuria.[8]

Changes in the slit diaphragm that are induced by antinephrin autoantibodies also compromise glomerular filtration. The fact that the levels of these antinephrin autoantibodies wax and wane in association with disease activity, particularly in patients with minimal change disease, implies that targeting these antibodies or the B cells that produce them might induce remission. Indeed, Hengel et al. report remission in two patients, one with minimal change disease and the other with FSGS, who received treatment with rituximab, which depletes B cells.

The finding of autoantibodies to different components of the glomerular filtration mechanism may explain other conditions, such as Goodpasture's syndrome (caused by autoantibodies to a type of collagen found in the glomerular basement membrane) and primary membranous nephropathy (caused by autoantibodies to proteins expressed by the podocyte). Exactly how autoantibodies interfere with components of the filtration apparatus has yet to elucidated.[6]

That said, Hengel et al. have provided some insight. They modeled podocytopathic kidney disease by immunizing mice with the ectodomain of mouse nephrin, which led to the rapid development of nephrotic syndrome with features of minimal change disease. Furthermore, the antibody led to a redistribution of nephrin from the cell surface into the cytoplasm of the podocyte. Hengel at al. also observed increased phosphorylation of nephrin at a specific amino acid of nephrin (tyrosine at position 1176); phosphorylation at this particular site is associated with endocytosis of nephrin and reorganization of the cytoskeleton. There were no signs of immune activation other than the antibody response.

What's Next?

The findings reported by Hengel et al. and others[9,10] point toward a clinical goal: the incorporation of routine and reliable quantitative assessment of autoantibodies as diagnostic tools. Hengel et al. developed a quantitative enzyme-linked immunosorbent assay (Fig. 2) to follow the waxing and waning of the autoantibody levels. These findings also incentivize research into how the effects of antinephrin autoantibodies might best be mitigated. Today, such mitigation can be broadly achieved through plasma exchange or the use of B-cell–depleting antibody-based drugs, such as rituximab. More specific therapies may be developed that reduce, block, or eliminate antinephrin autoantibodies.

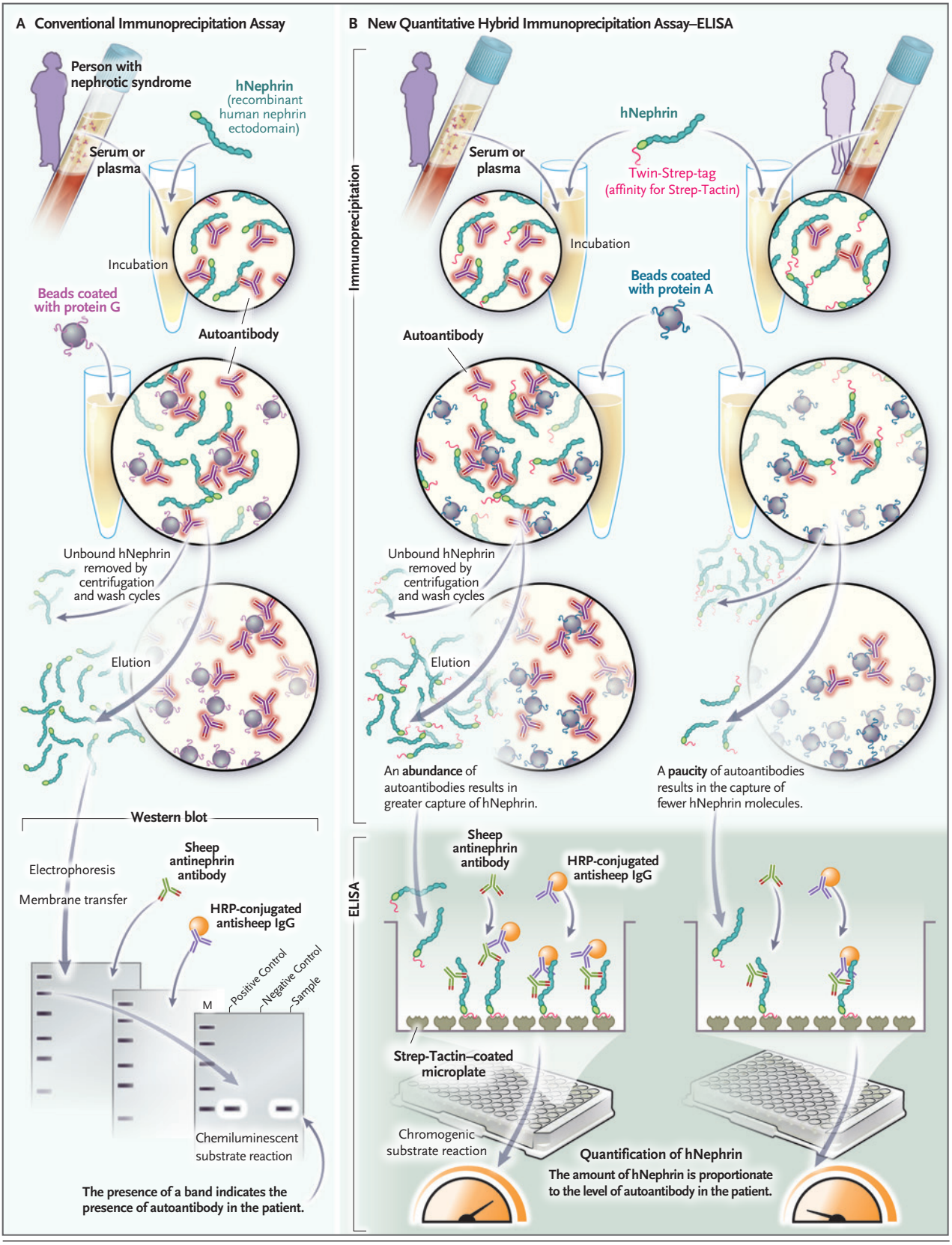

Figure 2 (facing page). Assays to Detect Nephrin Autoantibodies.

Hengel et al.[4] assayed antinephrin autoantibodies in the plasma or serum samples using two methods. Panel A depicts immunoprecipitation and elution of a labeled recombinant human nephrin ectodomain (hNephrin; amino acids A36 through L1052) followed by Western blotting. With this method, hNephrin is first incubated with human serum or plasma and beads coated with protein G. By binding to the Fc heavy chain of (and thus immobilizing) any autoantibody that may be present, the protein G–coated beads "pull out" (isolate) the autoantibodies that are, in turn, bound to the easy-to-quantify hNephrin. Excess hNephrin is washed away, and then the bound hNephrin is eluted from the bead–autoantibody conjugates, electrophoresed, blotted, and visualized, after blotting, with an antinephrin antibody. The results indicate the presence or absence of antinephrin autoantibody in the tested patient. Panel B shows the quantification of antinephrin antibodies through a two-step process involving an enzyme-linked immunosorbent assay (ELISA). In the first step, the patient's plasma or serum sample is incubated with hNephrin and protein A–coated beads. As with the protein G–mediated method of antibody assay, this step immunoprecipitates or "pulls down" any antinephrin antibodies, most of which are bound to hNephrin. Excess hNephrin is washed away, and then the immunoprecipitated hNephrin is eluted from the autoantibody–protein A conjugate. In the second step, hNephrin is quantified through a modified ELISA. The enhanced sensitivity and specificity of this assay make it suitable for the assessment of nephrotic syndrome subtypes, in which levels of autoantibodies increase with episodes of nephrotic syndrome and wane with remission. Twin-Strep-tag, a streptavidin short peptide affinity tag, is used together with Strep-Tactin, another streptavidin variant, to enrich recombinant nephrin so that it can be assessed quantitatively. HRP denotes horseradish peroxidase.

Ultimately, management guided by autoantibody assessment would require formal comparison with present clinical management.

Disclosure forms provided by the authors are available with the full text of this editorial at NEJM.org.

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