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The Glomerular Basement Membrane as a Barrier to Albumin

Jung Hee Suh and Jeffrey H. Miner

Renal Division, Department of Internal Medicine, Washington University School of Medicine, 660 South Euclid Ave., St. Louis, Missouri USA 63110

Abstract

The glomerular basement membrane (GBM) is the central, non-cellular layer of the glomerular filtration barrier that is situated between the two cellular components – fenestrated endothelial cells and interdigitated podocyte foot processes. The GBM is composed primarily of four extracellular matrix macromolecules – laminin-521, type IV collagen^{3 4 5}, the heparan sulfate proteoglycan agrin, and nidogen – that produce an interwoven meshwork thought to impart both size- and charge-selective properties. Although the composition and biochemical nature of the GBM have been known for a long time, the functional importance of the GBM vs. podocytes and endothelial cells for establishing the glomerular filtration barrier to albumin is still debated. Together with mouse genetics studies, the discoveries of four human mutations in GBM components in two inherited kidney disorders, Alport syndrome and Pierson syndrome, support essential roles for the GBM in glomerular permselectivity. Here we explain in detail the proposed mechanisms whereby the GBM can serve as the major albumin barrier and discuss possible approaches to circumvent GBM defects associated with loss of permselectivity.

Introduction

The glomerular basement membrane (GBM) is a thin (250 to 400 nm) meshwork of extracellular matrix proteins that is an integral part of the glomerular filtration barrier. Most of the GBM is situated between two cellular layers—glomerular endothelial cells and podocytes—in the peripheral capillary wall (Figure 1A); the remaining GBM segments lie between mesangial cells and podocytes at the bases of the capillary loops.¹ The GBM both provides structural support for the glomerular capillaries and harbors ligands for receptors on the surface of the adjacent endothelial cells, podocytes, and mesangial cells.^{2,3} Importantly, the GBM also contributes to glomerular permselectivity; as the second layer of the capillary wall that is encountered by filtrate, the GBM restricts the passage of plasma proteins across the glomerular filtration barrier. In support of this, among the nine major proteins found in the GBM, mutations in four of them are known to cause human kidney diseases^{4,5} (Alport syndrome and Pierson syndrome) that include proteinuria, the leakage of valuable plasma protein, most of which is albumin, into the urine.

While mutations affecting GBM components are important causes of kidney disease, environmental changes that affect the glomerulus can also lead to alterations in the composition and structure of the GBM. Diabetic nephropathy (DN) is one example in which the GBM is adversely affected by the microenvironment.⁶ DN is becoming more and more prevalent due to the worldwide increases in obesity and type II diabetes. About forty percent

Correspondence to: Jeffrey H. Miner, minerj@wustl.edu.

Competing interests

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of diabetics develop diabetic nephropathy, which then leads to more patients with chronic kidney disease in need of dialysis.⁷

Although it is clear that proteinuria and renal failure originate from both genetic and environmental factors, in all but a few cases it is very difficult to clearly define a genetic component. Much research has focused on the cellular components of the glomerulus—the podocytes, endothelial cells, and mesangial cells—because they can actively respond to genetic and environmental changes by producing gene products and cell signaling molecules. However, changes in these cells can also give rise to changes in the GBM, which can secondarily affect the properties and behavior of the neighboring cells through matrix-to-cell (outside-in) signaling events. Similarly, primary changes in the GBM may exert functionally important effects on the neighboring podocytes, endothelial, and mesangial cells and thereby impact glomerular filtration.

Whether and how the GBM contributes to the establishment and function of the glomerular filtration barrier to protein have been debated for several decades.⁸ Recent findings gleaned from genetic and physiological studies have provided a better view of how the GBM could function as a barrier. Further understanding the mechanisms in various disease models could help in the design of therapeutics that could prevent or reverse proteinuria by impacting GBM structure and function.

This review focuses mainly on the mechanisms of how the GBM functions to establish and maintain the glomerular filtration barrier. From genetic and biochemical studies in mice and humans, it is evident that the GBM is crucial to prevent the leakage of plasma proteins into the urine. We emphasize the critical role of the GBM as a permselective barrier that can be altered in different ways by genetic defects that cause kidney disease.

The components of GBM and their potential for contributing to the glomerular filtration barrier

In order to understand how the GBM might contribute to permselectivity, it is important to define its composition and to understand the properties of its major components. Like all basement membranes, the GBM is composed of laminin, type IV collagen, heparan sulfate proteoglycan, and nidogen.⁹ Components of the GBM are synthesized by both podocytes and endothelial cells,¹⁰ and during glomerulogenesis there is a fusion of separate podocyte- and endothelium-derived basement membranes to form the immature GBM. These features are together responsible at least in part for the GBM being thicker than most other basement membranes, as both cell layers synthesize ECM components and secrete them into the extracellular space between them. Furthermore, one can infer that changes in either podocytes or endothelial cells can result in altered GBM composition—and vice versa—possibly affecting glomerular filtration barrier function. In this context, podocytes, endothelial cells, and the GBM can be viewed as interconnected; this is evident not only through the obvious direct physical contacts within cell layers and between cells and the GBM, but also across the GBM through both cell-matrix-cell connections and cell-cell communication/signaling via diffusible factors. One excellent example of the latter is the VEGF signaling axis, in which podocyte-derived VEGF is crucial for endothelial cell homeostasis.¹¹

Historically, the GBM was considered by some to be a “crude prefilter”, while slit diaphragms between podocyte foot processes were thought to be responsible for the bulk of glomerular permselectivity.¹² However, as proposed by Farquhar and Palade, functional and physiological analyses of the glomerular filtration barrier using various tracers revealed the importance of the GBM as a size- and charge-selective filtration barrier.^{13–15} When neutral

tracers of various sizes were intravenously injected, they found that molecules larger than albumin were restricted to the inside of the glomerular capillary loops and were impaired from traversing the GBM. Likewise, when neutral, anionic and cationic tracers were infused, negatively charged molecules had the most difficulty crossing the GBM.¹⁵ From these studies, the function and necessity of the components of the GBM were inferred based on their biochemical properties. However, the most direct evidence as to whether each component of the GBM is essential for establishing the filtration barrier was provided by mouse genetic studies and discovery of human mutations that cause defects in glomerular permselectivity.

Laminins

Laminins are large heterotrimeric glycoproteins composed of three different homologous chains: α , β , and γ . In humans there are five α chains, four β chains, and three γ chains that assemble to form at least fifteen distinct laminin trimers¹⁶ that, depending on the α - β - γ chain composition, resemble cruciform, Y-shaped, or rod-shaped structures. Each chain has a laminincoiled-coil domain,¹⁷ and interactions among three coiled-coil domains and limited interchain covalent bonding contribute to the formation of the long arm of all laminin trimers.¹⁸ The remaining segment of each chain is called a short arm. The short arms of the cruciform trimers contain a laminin N-terminal (LN) domain, which plays an essential role in polymerization of trimers to form a network.^{19,20} Laminin α chains are unique because they also contain a C-terminal laminin globular (LG) domain, composed of five tandem sub-domains, which is situated distal to the long arm.²¹

LG domains link laminin trimers in basement membranes to neighboring cells by serving as ligands for two major cellular receptors, integrins and dystroglycan.^{22,23} Integrins are transmembrane heterodimers that are crucial in many different contexts for cell-to-extracellular matrix signaling and vice versa, as well as for direct cell-cell signaling.²⁴ Integrin $\alpha_3\beta_1$ is the predominant integrin normally present on the basal surface of podocytes, and deletion of $\alpha_3\beta_1$ results in severe kidney glomerular defects during development.²⁵ Integrin $\alpha_3\beta_1$ binds to laminin $\alpha_5\beta_1\gamma_1$ (LM-511) and $\alpha_5\beta_2\gamma_1$ (LM-521) through the α_5 chain's LG domain.²⁶ The binding of α_5 LG to integrin $\alpha_3\beta_1$ is essential for the formation of the typical glomerular capillary loop structure, likely because the mesangial cells that organize the capillaries also express integrin $\alpha_3\beta_1$.²⁷ Dystroglycan also binds to the LG domain of α chains,²⁸ but the deletion of dystroglycan from most kidney cells does not result in a kidney defect, suggesting that integrin $\alpha_3\beta_1$ is more responsible for extracellular matrix-to-cell adhesion and signaling in the kidney.²⁹

Secretory signal peptides are located at the N-terminus of each laminin chain, which targets them to the endoplasmic reticulum as they are synthesized. Laminin trimers assemble and become glycosylated in the endoplasmic reticulum and are processed further in the Golgi apparatus.³⁰ After secretion into the extracellular space, laminin trimers self-polymerize to form a laminin network by interactions among LN domains.²⁰ This polymerization step is a reversible and calcium-dependent process that requires a critical concentration of laminin trimers to form an initiating complex.¹⁹ This initial step is facilitated by the binding of laminin trimers to laminin receptors via their α chain LG domains,³¹ which increases the local concentration of laminin trimers and helps trigger laminin polymerization. Interactions that involve the other basement membrane molecules (type IV collagen, nidogen, and sulfated proteoglycan) then enables the assembly of a basement membrane. Interestingly, mice that lack α_1 and α_2 type IV collagen do assemble basement membranes during early embryonic development, but these basement membranes are unstable, leading to embryonic death by embryonic day 11.³² However, the deletion of laminin α_1 (a component of all early laminins) in mice caused much earlier lethality (embryonic day 5.5) and the total absence of basement membranes.³³ These data indicate that laminin is indispensable for the initial

formation of basement membranes, whereas type IV collagen is not. And as discussed below, the quantity of efficiently polymerizing laminin in the GBM is important for restricting the passage of plasma macromolecules across the glomerular filter.

Type IV Collagen

Type IV collagen is the most abundant protein found in basement membranes, comprising about 50% of total protein mass. There are six genetically distinct collagen IV chains, $\alpha 1$ through $\alpha 6$, that assemble to form three different heterotrimers referred to as protomers: $(\alpha 1)_2\alpha 2$, $(\alpha 3)_2\alpha 4$, and $(\alpha 5)_2\alpha 6$. Like all collagen chains, the collagen IV chains contain Gly-X-Y amino acid triplet repeats. However, unlike fibril-forming collagens of bone and cartilage, the Gly-X-Y repeat region of collagen IV displays multiple interruptions, imparting flexibility to the collagen IV protomer and to the network that it forms in basement membranes.

Each collagen IV chain has three domains: the N-terminal 7S domain, the collagenous domain containing the interrupted Gly-X-Y repeats (with X and Y usually being Lys or Pro), and the noncollagenous domain (NC1) at the C-terminus. Collagen IV protomers are assembled inside the ER and secreted into the extracellular space. There, they self-polymerize into a chicken wire-like network through hexameric and dodecameric interactions involving the NC1 and 7S domains, respectively, and become heavily cross-linked through disulfide bonds, sulfilimine bonds, and lysyl oxidase-mediated crosslinks.^{34,35}

In the GBM, there is a transition in the composition of collagen IV chains, from $(\alpha 1)_2\alpha 2$ in the immature GBM to $(\alpha 3)_2\alpha 4$ in the mature GBM.³⁶ This transition occurs coincidentally with the transition of laminin chains in the GBM. The molecular mechanisms controlling the switch of collagen IV and laminin chains in the GBM are unknown. However, the collagen IV transition might be required to accommodate the increased blood pressure in the adult, since $(\alpha 3)_2\alpha 4$ type IV collagen produces a more heavily cross-linked and more protease-resistant network compared to the $(\alpha 1)_2\alpha 2$ type IV collagen network.³⁷ The importance of the $(\alpha 3)_2\alpha 4$ type IV collagen network in the glomerular capillary wall is illustrated by the fact that its absence causes Alport syndrome, a glomerular disease discussed in detail below.

Nidogen

The nidogens, also known as entactins, are two homologous glycoproteins containing three globular-like (G) domains with two rod-like domains separating the G domains.³⁸ Nidogen-1 and nidogen-2, both found in the GBM, are encoded by two different genes. Nidogen-1 binds to both laminin $\alpha 1$ and type IV collagen, leading to the hypothesis that nidogen acts as a bridge linking the separate laminin and type IV collagen networks in basement membranes.³⁹ Deletion of either nidogen gene in mice does not cause any significant abnormalities, likely because of overlapping expression and redundant functions.^{40–43} Nidogen-1 and -2 double knockout mice, however, exhibit perinatal lethality due to lung and heart malformations, but many basement membranes form normally without nidogen, despite nidogen's ability to link laminin and type IV collagen networks.⁴⁴ The data suggest that nidogens provide extra stability to basement membranes under unusual stress, but they are not required for their initial formation. There is no definitive evidence that either nidogen alone contributes to the GBM's barrier properties, so they will not be discussed in that context.

Heparan Sulfate Proteoglycan

Heparan sulfate proteoglycans (HSPGs) have sulfated glycosaminoglycan side chains linked to a protein core. Although perlecan seems to be the prominent HSPG in most basement

membranes³⁹ and in the mesangial matrix, agrin is the major heparan sulfate proteoglycan in the GBM.⁴⁵ Agrin's sulfated glycosaminoglycan side chains give it a highly negative charge that is reflected by anionic sites within the GBM that can be detected with cationic probes such as polyethyleneimine and cationized ferritin.^{46,47} The N-terminal domain of agrin (NtA) binds avidly to the LM-521 long arm, and its C-terminus bears domains that can bind to cell surface receptors such as dystroglycan and integrins.⁴⁸ These properties of agrin suggest that it could have importance in mediating charge selectivity within the glomerular filtration barrier and in linking the GBM to the adjacent cells. In addition, heparan sulfate side chains are important in some contexts for binding and sequestering growth factors; one such growth factor secreted by podocytes that must cross the GBM and could benefit from the presence of such side chains is VEGF.^{11,49} However, podocyte-specific mutation of agrin in mice did not lead to any structural or functional defects in the glomerulus, although it did greatly reduce the density of negatively charged sites within the GBM and caused a dramatic reduction in the level of full length agrin protein in the GBM. These results suggest that agrin and the negative charge of the GBM do not play critical roles in the filtration barrier.⁴⁶ Furthermore, deletion of both agrin and the heparan sulfate side chains on perlecan also had little if any effect on permselectivity,⁵⁰ despite the fact that a previous report had revealed a potential role for perlecan in filtration in the context of protein overload.⁵¹ But because no critical role for agrin in the GBM, either as a matrix protein or as an anionic macromolecule, has been proven, agrin will not be discussed further in relation to the glomerular filtration barrier.

Diseases associated with malfunction of glomerular basement membrane

As mentioned above, there are two known and relatively well understood genetic kidney diseases that target components of the GBM: Pierson syndrome and Alport syndrome. Although both of these diseases involve primary defects in GBM components, they have very different clinical presentations; these differences provide some clues as to the specific roles for laminin and type IV collagen networks in influencing glomerular permselectivity.

Pierson syndrome

In the early 1960s, Pierson et al. described two patients from one family with a microcoria-congenital nephrosis syndrome⁵². Both patients presented with severe congenital nephrotic syndrome and early onset end-stage renal failure, and they died 2 weeks after birth. Their eyes' iris dilator muscles showed aplasia and/or atrophy with an abnormal lens. In 2004, Zenker and colleagues reported a congenital nephrotic syndrome with clinical features that included microcoria and mesangial sclerosis, similar to the features described by Pierson, thus the moniker Pierson syndrome.⁵³ From homozygosity mapping of five affected families, autosomal recessive mutations in *LAMB2*, the gene encoding laminin 2, were identified.⁵³ Follow up of the original cases via screening of healthy living family members revealed two different mutations in *LAMB2*, suggesting that the original cases were compound heterozygotes.⁵⁴ These findings highlight the critical role of laminin 2 for normal kidney function, especially glomerular filtration barrier function.

The recognition of Pierson syndrome as a specific disease entity by discovery of *LAMB2* mutations spurred more reports of Pierson syndrome cases.^{5,55} With increasing numbers of clinical and mutation reports, it has become clear that the onset and severity of Pierson syndrome varies greatly among patients in terms of progression of nephrotic syndrome and extra-renal manifestations, such as muscular hypotonia and neurodevelopmental deficits, resulting in a Pierson syndrome "spectrum" with genotype-phenotype correlations. The level of mutated laminin 2 expression and/or function seems critical for determining the severity of Pierson syndrome.^{56,57} Truncating mutations, which are dispersed throughout the *LAMB2* gene, generally show more severe phenotypes than missense mutations do, as

truncated proteins often lack the coiled-coil domain that is critical for the assembly of LM-521 trimers.⁵ In contrast, pathogenic missense mutations in LAMB2 are found primarily in the LN domain, likely resulting in laminin polymerization and/or secretion defects.^{5,58,59} Thus, for patients in the Pierson syndrome spectrum, the mutations in laminin 2 result in a total absence or reduced level of LM-521 in the GBM or in the formation and secretion of dysfunctional LM-521 trimers.

The straightforward interpretation for the mechanism of proteinuria in Pierson syndrome is that the lack of LM-521 causes a defect in the GBM's ability to attenuate the passage of albumin from plasma to Bowman's space. But given the evidence for the involvement of both podocytes and endothelial cells in regulating permselectivity, an alternative hypothesis is that defects in the GBM prevent proper cell/matrix interactions and signaling to cells, resulting in cellular defects leading to proteinuria. Rigorously investigating these hypotheses requires experiments that cannot be performed in humans. Fortunately, mice with a null mutation in *Lamb2* have been an excellent model to study Pierson syndrome, as they recapitulate the congenital nephrotic syndrome and die at about three weeks of age with severe proteinuria and neuromuscular defects.^{60,61} They also show accumulation of ectopic laminin chains in the GBM, including 1, 2, 3, 1, 3, and 2 (Figure 1B).⁶² This deposition of ectopic laminins into the GBM may be a compensatory response to the loss of laminin 2/LM-521, but it is not sufficient to establish a fully functional glomerular filtration barrier.⁶² Interestingly, the glomerular ultrastructure of proteinuric one week old *Lamb2* null mice reveals intact podocyte foot processes (Figure 1B) and slit diaphragms, suggesting a role for the GBM as an independent and indispensable filtration barrier to plasma proteins.⁶² Importantly, these data show that proper GBM laminin composition is not required for what appears to be the elaboration by podocytes of proper foot processes with slit diaphragms in functioning glomeruli, at least at early stages. In mice, and presumably in humans also, foot process effacement follows (Figure 1B), and perhaps is even caused by, the increasing proteinuria that eventually reaches nephrotic range.

In further support of a direct role for the GBM in mediating permselectivity, the use of ferritin as an electron dense tracer to monitor the permeability of the GBM to plasma macromolecules showed that the lack of LM-521 in *Lamb2*^{-/-} mice was associated with an increase in permeability relative to control littermates; this increase was apparent before widespread foot process effacement and loss of slit pores.⁶² And similar to previously reported results using albumin and ferritin tracing in situ,^{13,14,63} there was no accumulation of ferritin below the slit diaphragms, suggesting that they do not act as restrictive pores in the physiological state.⁶² These results are, however, in conflict with structural studies of the slit diaphragm that show pore sizes similar to or smaller than the size of albumin.⁶⁴

Alport syndrome

Alport syndrome is a hereditary glomerular, auditory, and ocular disease caused by mutations in the *COL4A3*, *COL4A4*, or *COL4A5* genes that encode the type IV collagen 3, 4, and 5 chains, respectively.^{4,65} Because the GBM's collagen IV network consists primarily of cross-linked 3 4 5(IV) protomers made by podocytes,⁶⁶ the lack of any one of the three chains (for example, due to a null mutation) prevents the protomer from forming. Moreover, missense mutations—particularly those that cause glycine substitutions in the collagenous domain—can lead to defects in protomer structure and in the collagen IV network and therefore also cause Alport syndrome.

In the absence of the 3 4 5(IV) network, there is a compensatory increase in the 1 1 2(IV) network, which is normally a minor component in the subendothelial aspect of the mature human GBM and barely detectable in normal mouse GBM. This compensation allows the GBM to form and function properly for several years, but eventually there is

hematuria that accompanies a characteristic splitting and thickening of the GBM (Figure 1C). This is followed by the onset of proteinuria that increases and signifies declining GFR. The majority of patients, most of whom are males carrying X-linked *COL4A5* mutations, eventually require renal replacement therapy. In contrast, only a minority of female carriers of X-linked Alport syndrome progress to ESRD. The rarer autosomal forms of Alport syndrome affect males and females equally.

That proteinuria appears late in the disease process suggests that collagen IV is not as important as laminin for maintaining glomerular permselectivity. However, studies of a mouse model of Alport syndrome show that Alport GBM is more permeable to ferritin than is normal mouse GBM, indicating that collagen IV is important for the filtration barrier.⁶⁷ The areas of increased permeability also showed ectopic deposition of laminin chains (laminin 1 plus increased levels of 5),⁶⁷ suggesting the possibility that secondary changes in the laminin network, together with the collagen IV network defect, might be responsible for increased permeability in Alport GBM. That proteinuria appears late in the course of the disease may be because any early increase in the level of filtered albumin may be masked by increased albumin uptake by proximal tubular cells.

Treatment of Alport mice and humans with ACE inhibitors has been shown to slow the onset of proteinuria and the decline in GFR.^{68,69} A reduction in blood pressure should put less stress on the defective, imperfectly cross-linked GBM, on the adjacent podocyte, and on the entire glomerulus, which exhibit increased deformability.⁷⁰ Moreover, biomechanical strain has been proposed to change gene expression in ways that exacerbate glomerular disease in Alport syndrome.⁷¹

Concluding thoughts

What are the mechanisms whereby laminin and collagen IV might be directly involved in glomerular permselectivity? According to concepts proffered by Smithies,⁷² the GBM behaves like a concentrated gel with size-selective properties into which macromolecules such as albumin permeate primarily by diffusion. As the major components of the GBM, the laminin-521 and collagen IV networks, in concert with the other GBM components, likely impart the GBM with its characteristic porosity. By analogy to the polyacrylamide gel, which also has size-selective properties, it is easy to understand how 1) reducing laminin or type IV collagen concentration might be similar to reducing the percentage of acrylamide; or 2) how changing laminin or type IV collagen isoforms might be similar to changing the ratio of acrylamide to bis-acrylamide. In either of these cases, the result could be increased permeability via increased pore sizes. Consistent with this, our hypothesis that *Lamb2*^{-/-} mice develop nephrotic syndrome due to a paucity of laminin in the GBM, and therefore a defective laminin network in the GBM, was supported by our data from transgenic mice with podocyte-specific overexpression of laminin 1/LM-511. On the *Lamb2*^{-/-} background, the forced secretion of high levels of LM-511 from podocytes into the GBM prevented the nephrotic syndrome that would have otherwise developed, and the mice lived a long life.⁷³ These data also suggest that in the GBM the quantity of laminin is important, as LM-511 could efficiently substitute for LM-521 if expressed at a high enough level. An exciting conclusion from this study is that upregulation of the unaffected *LAMB1* gene in the podocytes of patients with Pierson syndrome should be an effective therapy. Moreover, our recent study showed that secretion of a secretion-defective mutant laminin 2 (C321R-LAMB2) could be improved in vitro by treatment with a chemical chaperone,⁷⁴ which promotes proper protein folding. This result suggests a possible drug therapy for Pierson syndrome patients who have missense *LAMB2* mutations that impair protein folding and/or secretion. Finally, additional studies in mice suggest that the three C-terminal segments of the LG domain of laminin 5 are critical for a proper filtration barrier⁷⁵ for reasons not yet

understood. Future studies concentrating on the mechanism for this could reveal novel approaches for tightening the glomerular barrier to albumin and reducing albuminuria.

Given the well-demonstrated importance of multiple podocyte proteins for proper foot process architecture and for permselectivity (see accompanying article by Brinkkötter et al.), it is important to determine how this can be reconciled with the concept that the GBM is a major contributor to the physical filtration barrier. We propose, based on cell biological concepts put forth previously,⁷⁶ that the podocyte cytoskeleton and slit diaphragms are so critical for normal filtration because they are connected (either directly or indirectly) to cell surface receptors—primarily integrins—that link to the GBM via LM-521. In turn, these receptors are critical for organizing the arrangement of the GBM's components and can thereby regulate its architecture, its porosity, and thus its permselectivity.

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Key points

- The glomerular basement membrane is the extracellular matrix component of the glomerular filtration barrier. It is flanked by the podocyte and glomerular endothelial cell layers.
- The major GBM components are laminin-521, collagen 3 4 5(IV), nidogen, and the heparan sulfate proteoglycan agrin
- Mutations in *COL4* genes that result in absence of the collagen 3 4 5(IV) network cause Alport syndrome, a hereditary nephritis accompanied by hearing defects
- Mutations in laminin 2 (*LAMB2*) cause Pierson syndrome, a congenital nephrotic syndrome with eye and neurologic abnormalities
- Studies using mouse models of Pierson and Alport syndromes have shown the defective GBM to be more permeable to macromolecules, suggesting a role in permselectivity

Figure 1. The components of glomerular basement membrane (GBM) and known alterations in Pierson and Alport syndromes

(A) The normal GBM is composed of laminin-521 (521), type IV collagen $\alpha3\alpha4\alpha5$, nidogen and heparan sulfate proteoglycan (HSPG; primarily agrin). Podocytes and endothelial cells each contribute at least a subset of these components to the GBM (arrows; see text for details). Most of the plasma albumin (black dots) is restricted to the capillary lumen. FP, foot processes. (B and C) Mutations in two GBM components result in albuminuria both in human patients and in mouse models. (B) The GBM of *Lamb2* knockout mice lacks laminin-521 and instead contains ectopic laminins (shown in dark gray text), such as laminin-111, -211, -332, and -511. However, the laminin network made of these ectopic laminins is defective, leading to increased passage of plasma protein across the barrier. At 1 week, *Lamb2* mutant mice have proteinuria due to the defective GBM, but without podocyte foot process effacement. At 2 weeks, podocyte abnormalities can be detected, followed by increasing proteinuria and widespread effacement. These results indicate that proteinuria precedes podocyte abnormalities in *Lamb2* mutant mice, highlighting the importance of the GBM as a barrier to plasma protein. (C) The GBM of *Col4a3* mutant mice lacks the collagen $\alpha3\alpha4\alpha5$ (IV) network. Although there is increased deposition of collagen $\alpha1\alpha1\alpha2$ (IV) as a compensatory mechanism, the resulting GBM becomes split and thickened and accumulates multiple ectopic laminins (shown in dark gray text). Eventually there is increased loss of plasma protein across the filtration barrier and proteinuria.