SECTION 2

Principles of Renal Physiology

CHAPTER 7

The Physiology of the Glomerulus

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OBJECTIVES

The chapter will:

1. Explain the structure of the glomerulus.
2. Describe the many regulatory pathways that control blood flow and urine filtration and how some diseases and medications can affect these pathways.
3. Identify and describe initial symptoms of some common diseases that affect the glomerulus.

The study of the glomerulus is a study of vasculature. Glomerular flow regulation is key to kidney function and a window into the hemodynamics of the body. As the interface between the nephron and systemic bloodstream, glomerular injury often reflects broader systemic disease processes.

BASIC ANATOMY

Each kidney contains roughly 1 million glomeruli, and together these filter approximately 20% of cardiac output.[1] Virtually all renal blood flow will travel through the glomerular capillaries, and 20% of this plasma is filtered. Glomerular capillaries are surrounded by Bowman's capsule, which comprises an inner visceral epithelium that encases the capillaries and an outer parietal epithelium. Between these two membranes is a space analogous to the potential space of a serous membrane like the pleural space, which is named Bowman's space, and is where plasma filtrate begins its path down the nephron. Blood flows into the glomerular capillaries from the afferent arteriole and leaves via the efferent arteriole. The filtration fraction is the fraction of the plasma entering the glomerulus that is filtered into the renal tubule; it does not leave through the efferent arteriole. The glomerular filtration rate measures how much plasma actually is filtered. This normal filtered volume adds up to 144 L daily. A typical patient may make approximately 1 L of urine per day, so most of the 144 L of filtered blood will be reabsorbed along the nephron (Fig. 7.1).

Afferent and efferent arterioles have a complex anatomy that is specialized for managing renal blood flow in response to various conditions. Even the size of these vessels changes in different sections of the kidney. In the cortex, where the emphasis is on filtration and reabsorption, afferent arterioles have a larger diameter than efferent arterioles, whereas in the juxtamedullary glomeruli, where the emphasis is on maintaining a hypertonic gradient and concentrating urine, the afferent and efferent arterioles are much larger than in the cortex. More important, after blood leaves glomerular capillaries, it does not enter a venous system but instead flows into efferent arterioles and perfuses different parts of the kidney.

Some efferent arterioles have branches that form peritubular capillaries, and these capillaries take up water and solute that is reabsorbed by the tubule, as well as to provide oxygen and nutrients to the nephron. In a sense, peritubular capillaries perform a mirror function to that of the glomerular capillaries: glomerular capillaries deposit water and ions into the tubule, and the peritubular capillaries take up water and ions that were reabsorbed by the tubule. These different capillaries have comparable structure, including fenestrations. The peritubular capillaries branching from the efferent arteriole of one glomerulus actually may provide nutrients to and remove waste from a different glomerulus. Thus, if the capillaries of one glomerulus are damaged, then they no longer supply blood to filter, and this injury also may reduce total blood flow to peritubular capillaries of a different glomerulus. Other efferent arterioles perfuse the medulla, and this represents about 25% of renal blood flow. These efferent arterioles break up into descending vasa recta in the corticomedullary junction. Descending vasa recta eventually will feed into capillary beds in the medulla. Thus the blood flows through two capillary beds: first the glomerular capillaries and then the medullary capillaries. The medullary capillary beds then will join to form ascending vasa recta, and the ascending vasa recta travel back up to the corticomedullary junction and feed into arcuate veins. There are more ascending vasa recta

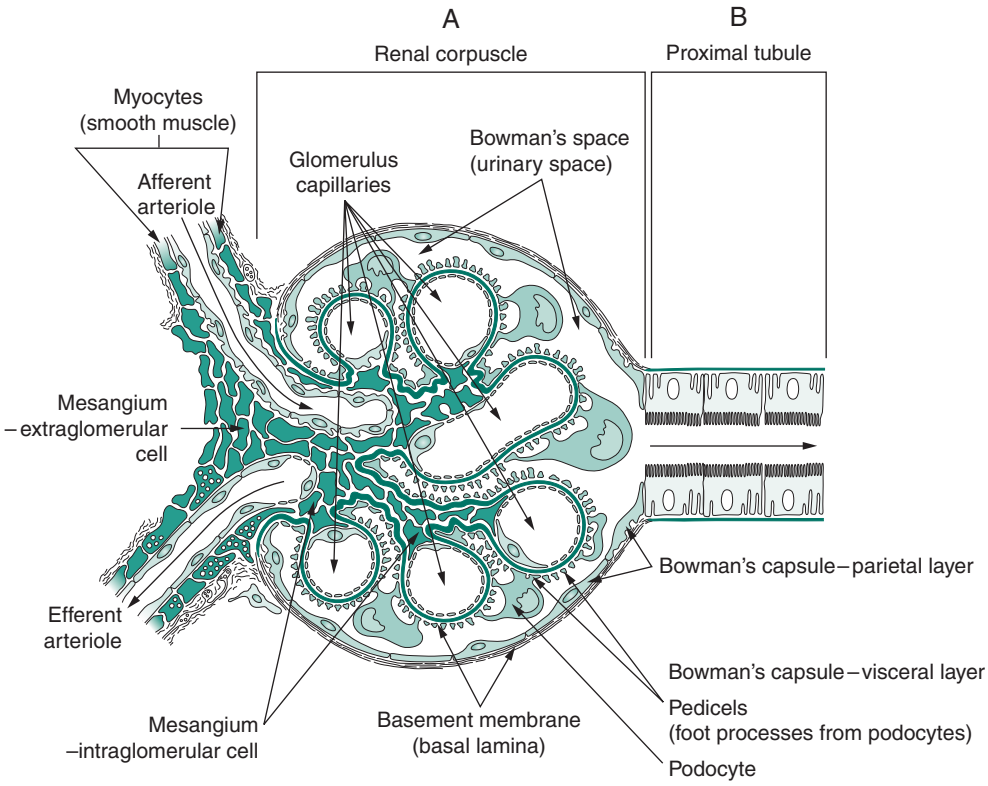

FIGURE 7.1 Glomerular structure.

than descending vasa recta, possibly to accommodate lower pressure after the blood has passed through a second capillary bed.

FLOW REGULATION

Blood flowing to the glomerulus is under higher pressure in the afferent arteriole than in the efferent arteriole, and the difference is the pressure driving filtration across the glomerular filtration barrier known as ΔP. There is higher pressure in glomerular capillaries (60 mm Hg) than in other capillary beds (20 mm Hg), which helps drive plasma across the filtration barrier into the urinary space. This pressure is regulated tightly, and the kidney continually constricts and dilates the afferent and efferent arterioles to respond to different systemic blood pressures and blood flow rates.

Role of Flow Regulation

Renal blood flow regulation is important for several reasons. Afferent arteriole vasoconstriction provides vascular resistance that protects against vascular injury from high blood pressure. In extremely high pressure, normal protective mechanisms are overwhelmed and the kidney is still exposed to elevated blood pressures. Glomerular injury ensues as part of the progression to renal failure.[2,3] In addition, early diabetes leads to loss of afferent arteriole pressure control, despite normal systemic blood pressure,[4,5] and this may play a part in the renal injury associated with diabetes. Finally, as demonstrated in experiments on rats, loss of one kidney to nephrectomy redirects more blood flow to the remaining kidney, potentially causing injury.[6]

Limiting glomerular blood pressure by constricting the afferent arteriole is also clinically protective during kidney injury. For example, ischemic injury can injure the proximal tubule, where approximately 70% of sodium is reabsorbed. When this happens, less sodium is reabsorbed in the proximal tubule, which can lead to massive diuresis, causing rapid volume depletion. Some sodium may be reabsorbed by the distal tubule, but that cannot compensate for losing the large reabsorption that would normally be done in the proximal tubule. For example, if a kidney filters 144 L daily, most of this volume is reabsorbed, because there is only 1 L of urine made daily. Up to 70% may be reabsorbed by the proximal tubule. However, if that 70% of filtered volume, which is 100 L, were not reabsorbed by the proximal tubule, then a significant percentage would be lost to urine, leading rapidly to volume depletion. To prevent such catastrophic volume loss, sensors in the macula densa detect this sodium wasting and provide a feedback that constricts the afferent arteriole limiting filtration and volume loss. The afferent and efferent arterioles also are important when blood pressure is low. Lower afferent arteriole resistance and higher efferent resistance allows the glomerulus to maintain normal filtration in spite of low blood pressure ensuring continued filtration efficiency. There are limits to this compensation, however, and renal blood flow drops off significantly when systolic blood pressure drops below 80.

Myogenic Response

The afferent and efferent arteriole vascular muscle constricts or dilates rapidly, within 0 to 8 seconds, in response to

pressure sensing. Stretch or distortion of the plasma membrane in the muscle cells causes the membrane to change conductance and depolarizes the membrane. This opens voltage-gated calcium channels and leads to increased intracellular calcium. The muscle cell has a positive feedback cascade: intracellular calcium causes further release of calcium from the sarcoplasmic reticulum, leading to actin/myosin interaction and muscle contraction. The result is to contract muscle cells in response to increased pressure, so it maintains vessel diameter in face of higher pressure. This is more prominent in the afferent arteriole than the efferent arteriole.

Tubuloglomerular Feedback

Tubule cells between the thick ascending limb and the distal convoluted tubule are in close proximity to the glomerular afferent arteriole. These macula densa cells release chemical signals and interact with specific cells, called juxtaglomerular cells, in the afferent arteriole just proximal to the glomerulus. Macula densa cells monitor intratubular salt concentrations to regulate renal blood flow via afferent arteriole constriction and dilation. The juxtaglomerular cells also contain renin granules, which can send out a wider signal to control vascular resistance through the renin-angiotensin-aldosterone pathways. The coordinated effects of the macula densa cells and the juxtaglomerular cells to control vascular tone and renal blood flow is called tubuloglomerular feedback.

Tubuloglomerular feedback works more slowly than the myogenic response. The feedback responds to the concentration of sodium chloride in the tubule that reaches the macula densa; increased renal blood flow carries more sodium chloride into the tubule and eventually to reach the macula densa. Macula densa cells contain NKCC2 ion channels on their apical plasma membrane, which are similar to the NKCC ion channels in the thick ascending limb. When concentrations of sodium and chloride arrive at the macula densa, these channels will take up more ions, which increases intracellular concentrations of the ions. The increased ion concentrations activate Na/K-ATPase transporters in the macula densa cells' basolateral membranes, and these transporters will in turn use adenosine triphosphate (ATP), producing adenosine diphosphate (ADP) and adenosine monophosphate (AMP).

The increased levels of ADP and AMP are converted to adenosine and then released from the cell. Released adenosine binds to A1 receptors on the juxtaglomerular vascular smooth muscle cells and stimulates calcium signaling in these cells. There is also separate evidence that AMP or ADP binds to P2× receptors on the vascular smooth muscle cells. In all cases, the adenosine signal leads to smooth muscle cell contraction and therefore vasoconstriction of the afferent arteriole. As a result, this is negative feedback: increased renal blood flow will lead to more sodium, potassium, and chloride in the tubule, which is detected by the macula densa cell that then causes the afferent arteriole to constrict to reduce blood flow.

Evidence suggests that other signals besides adenosine may be involved in tubuloglomerular feedback. For example, the angiotensin 1 receptor seems to be involved, because AT1-antagonists in normal mice reduce tubuloglomerular feedback. So, potentially, angiotensin-receptor blockers (such as Losartan or Valsartan) may diminish autoregulation by tubuloglomerular feedback and make patients more vulnerable to moderate blood pressure drops. There is also evidence that ACE inhibitors may blunt the feedback. Tubuloglomerular feedback can be affected by anything that affects these ion concentrations. If the proximal tubule did not reabsorb the ions, such as when there is ischemic injury to the proximal tubule, then this increases the concentrations reaching the macula densa and causes a negative feedback that constricts the afferent arteriole. Alternately, loop diuretics (such as furosemide) will inhibit the NKCC2 ion channel and therefore blunt the tubuloglomerular feedback.[7–9] Specifically, loop diuretics reduce autoregulation and preserve glomerular filtration rate, even when there is high flow inside the tubule. So, a loop diuretic will cause diuresis by blocking sodium, potassium, and chloride reabsorption in the thick ascending limb and maintain diuresis by blocking absorption of these same ions in the macula densa, thus blunting tubuloglomerular feedback.

Angiotensin II

Renin is released by the juxtaglomerular granular cells (Peti-Peterdi) and is a catalyst for conversion of angiotensinogen to angiotensin I, which is later enzymatically converted to angiotensin II by the angiotensin converting enzyme. Angiotensin II can have diverse effects throughout the body, but it also contributes to regulation of renal blood flow through its actions on the efferent arteriole.[10] Angiotensin II is a vasoconstrictor[11] mainly for efferent arterioles. It activates angiotensin II type I receptor (AT1), a G-protein receptor, which will activate intracellular Rho kinase in smooth muscle cells. Rho kinase then inactivates myosin light-chain phosphatase and increases smooth muscle myocyte contraction. As a result, Rho kinase causes contraction of smooth muscle cells around the afferent arteriole, as well as other vessels such as interlobular arteries.

Angiotensin II acts on AT1 receptors in afferent and efferent arterioles, but it will constrict the efferent arteriole 10 to 100 times more than the afferent arteriole.[12,13] As a result, it will tend to increase glomerular capillary pressure and lead to more plasma filtered. This explains part of the effects of the drug class of angiotensin-converting enzyme (ACE) inhibitors. These inhibit the angiotensin-converting enzyme lowering the level of angiotensin II, even when there are elevated levels of renin and angiotensin I. Lower angiotensin II reduces the contraction of the efferent arteriole, thus allowing more blood to flow out of the glomerulus and lowering glomerular filtration. This is why serum creatinine rises when they start an ACE inhibitor. If the decline is limited, it does not necessarily mean the kidney is injured; instead a small decline in the glomerular filtration rate (GFR) means less plasma is being filtered, but renal blood flow is either constant or increases. Of course, the effects of this shunting are limited, so a larger drop in GFR may signal actual renal damage. In addition, by relatively dilating the efferent arteriole and reducing intraglomerular pressure, an ACE inhibitor also will reduce proteinuria. Proteinuria is a signal of glomerular injury (discussed below) and can be inflammatory. This is partly why an ACE inhibitor is used as part of the treatment for nephrotic proteinuria. Angiotensin II, however, plays a complex role in vascular management, both inside the kidney and systemically. For example, angiotensin II also acts as a vasodilator. It activates angiotensin II type 2 receptor (AT2), causing endothelial cells to release vasodilatory paracrine agents.[14] Angiotensin II also activates EP4 receptors to cause synthesis of PGE2, PGI2, and NO in the afferent arterioles. These vasodilators will counteract the effect on Angiotensin

II vasoconstriction on afferent arterioles. This explains partly why angiotensin II affects the efferent arteriole more than the afferent; it causes only vasoconstriction in the efferent but a mix of vasoconstriction and vasodilation of the afferent. In the glomerulus, angiotensin II also activates mesangial cells to contract, thus causing capillary constriction.

Endothelial Factors

Several paracrine agents are released by endothelial cells that contribute to glomerular flow regulation. In many cases these are local signals released by endothelial cells in response to systemic signals such as angiotensin II or vasopressin. They are not so much a separate regulatory pathway but rather one step in a multi-step control pathway that will constrict or dilate the arterioles to control glomerular flow. For example, nitric oxide causes vasodilation and is released by endothelial cells in response to systemic signals such as bradykinin, thrombin, platelet-activating factor, endothelin, and calcitonin gene-related peptide. Endothelin is a vasoconstrictor that is released by endothelial cells in response to systemic signals such as transforming growth-factor beta, tumor necrosis factor-alpha, platelet-derived growth factor, angiotensin II, vasopressin, insulin, bradykinin, thromboxane, and thrombin. The diversity of these triggers suggests that several different processes, from inflammation to the clotting cascade, may promote vasoconstriction or vasodilation or both. Several agents, such as bradykinin and thrombin, also may cause vasodilation and vasoconstriction by triggering release of dilatory or constricting paracrine agents in different vessels. Vascular smooth muscle cells detect these signals through a variety of receptors, often G-protein receptors. Several G-protein receptors activate phosphokinase C inside the cell, which triggers an enzyme cascade that leads to increased intracellular calcium. Intracellular calcium binds calmodulin, and together they activate myosin light chain kinase, which causes myocyte contraction through repositioning of the actin and myosin filaments. Calcium enters the smooth muscle cell through several means, but an important channel is the "transient receptor potential cation channels," or TRPC. There are several types of TRPC, although TRPC3 and TRP6 are common in vascular smooth muscle in renal arterioles. Calcium also enters cells through Na/Ca exchange transporters. However, there are different types of G-protein receptors on vascular smooth muscle cells. For example, prostaglandin E2 (PGE2) and prostacyclin activate different G-protein receptors, which, in turn, activate adenylyl cyclase to synthesize cyclic adenosine monophosphate (cAMP). Elevated levels of cAMP lead to lowering calcium levels inside the cell, either by pumping calcium out of the cell or by sequestering it in the sarcoplasmic reticulum. The lower calcium levels lead to muscle relaxation and thus vasodilation. Other local paracrine signals act directly on the cell and do not activate a G-protein receptor. For example, nitric oxide from endothelial cells and atrial natriuretic peptide (ANP) directly activate guanylate cyclase to synthesize cGMP inside the cell. Intracellular cGMP, like cAMP discussed above, lowers calcium and thus induces vasodilation.

In all cases, these signals to constrict or dilate muscle cells work by changing levels of calcium inside the smooth muscle cell. Interestingly, calcium channel blockade can inhibit these autoregulatory mechanisms.[15] This raises the interesting possibility that a patient on a calcium channel blocker may be more sensitive to renal injury from hypotension, such as that experienced during surgery or sepsis.

Short- and Long-Term Regulation

Dilation and constriction of the afferent and efferent arterioles is a rapid response to changing blood flow, but this is a short-term response and may be overshadowed by a longer-term, more systemic response.

Short-term responses modulate blood pressure in the glomerular capillaries, but the long-term response manages sodium balance, which affects systemic blood pressure. For example, tubuloglomerular feedback may vasoconstrict the afferent arteriole in response to increased sodium and chloride levels in blood. This leads to temporarily reduced glomerular filtration rate. Over the longer term, however, high blood volume will cause a drop in angiotensin II, which will dilate the efferent arteriole. The lower angiotensin II, however, also leads to a lower level of aldosterone, so less sodium is reabsorbed, and this finally reverses the original elevation in vascular volume.

There is empiric evidence that increased perfusion pressure will lead to decreased sodium reabsorption in at least some nephrons,[16] an effect termed *pressure natriuresis.* This leads to a salt-wasting diuresis, which is a negative feedback: the kidney responds to higher systemic blood pressure by wasting salt and volume. However, this raises questions of how the kidney senses the higher pressure, and how does it force salt wasting? There are several theories to explain why increased interstitial pressure will lead to salt wasting. One theory proposes that increased hydrostatic pressure in the interstitium will lead to increased capillary pressure in the endothelium. The endothelial cells respond to this increased pressure by releasing NO, and NO will have a paracrine effect on the thick ascending limb and collecting duct, causing reduced sodium reabsorption. There are, however, competing theories on how this mechanism works. In all cases, though, salt wasting will cause a diuresis and lower blood volume. This will help maintain a blood pressure and steady glomerular pressure even when systemic blood pressure changes.

GLOMERULAR CAPILLARIES

After entering the glomerular capsule the afferent arteriole branches into five to seven primary capillary branches.[17] In humans (and rats but not mice) these capillaries form a network of branches and merges until they finally combine into the efferent arteriole. Glomerular capillaries are unique in several ways. They bridge between two arterioles, they are the only capillaries not surrounded by interstitial tissue, and they support high blood pressures to bring about filtration.

Vascular Endothelial Growth Factor

Endothelial and mesangial cells express receptors that respond to vascular endothelial growth factor (VEGF). Podocytes secrete VEGF and during embryonic organogenesis this is a chemoattractant to the angioblasts that form the glomerular endothelium. Deficiency of VEGF understandably prevents normal glomerular development. VEGF also has autocrine effects on podocyte cell survival and differentiation, and knockout mice with no VEGF also seems to have paracrine effects on mesangial cell migration and survival.[18]

A deficiency of VEGF also may be clinically significant in a mature kidney. For example, in a pregnant woman preeclampsia is a disease of proteinuria and hypertension.

Preeclampsia patients have elevated levels of a competitive inhibitor of VEGF (soluble fms-like tyrosine kinase, also called sFlt-1 or VEGF-R1).[19] Early in a normal pregnancy there are elevated levels of placental growth factor (PlGF, a member of the VEGF family), which likely contributes to placental development. Later in a normal pregnancy there is a rise in sFlt-1, which is an antagonist of VEGF and PlGF. Preeclampsia is associated with elevated levels of sFlt-1 too soon in the pregnancy, causing an early drop in PlGF and VEGF and direct glomerular injury. Microscopically, preeclampsia causes glomerular injury and swollen endothelial cells, leading to proteinuria. Experiments have injected sFlt-1 into pregnant rats and induced the same glomerular lesions of preeclampsia.[20] An excess of VEGF also is associated with disease. For example, people with diabetes have elevated levels of VEGF, and this leads to part of diabetic nephropathy.[21,22] Chronic hyperglycemia (as in diabetes) causes increased VEGF production by podocytes,[23] and podocyte abnormalities are an early manifestation of diabetic nephropathy.[24] Overexpression of VEGFA has been shown to cause collapse of the glomerular tuft and depletion of endothelial cells.[25]

Glomerular Capillary Diseases

Several diseases have particular effects on the glomerular capillary endothelium. Thrombotic microangiopathies present with anemia, thrombocytopenia, and renal injury. Thrombotic thrombocytopenic purpura (TTP) is a disease of ADAMTS13, an enzyme that cleaves von Willebrand factor (vWF) and so controls the clotting cascade. Without ADAMTS13, vWF multimers grow large enough to spontaneously trigger clotting. TTP often occurs as an acquired disease resulting from an autoantibody to ADAMTS13, but it also may occur as a congenital deficiency in the ADAMTS13 enzyme.

Hemolytic uremic syndrome is a different thrombotic microangiopathy caused by dysregulation of the alternate complement pathway that leads to excessive inflammation, endothelial cell injury, and apoptosis. This inflammatory overactivation may be triggered by Shiga toxin from O157:H57 *E. coli,* and in these cases usually is accompanied by diarrhea. Diarrhea also may be absent in other cases, however; this is called atypical hemolytic uremic syndrome. The cause of atypical hemolytic uremic syndrome is not clear but may be related to congenital or acquired deficiency of complement pathways regulation enzymes, such as factor H, or other regulating enzymes such as the D7E lipid enzyme. Atypical hemolytic uremic syndrome often has waxing/waning course with multiple relapses and often leads to ESRD. The difference between hemolytic uremic syndrome and thrombotic thrombocytopenic purpura is thrombotic thrombocytopenic purpura is defective ADAMTS13, and hemolytic uremic syndrome is direct endothelial injury through the complement cascade.

MECHANICAL FILTRATION

Plasma in the glomerular capillaries pass through the glomerular filtration barrier as a first step in urine formation. The glomerular filtration barrier filters molecules based on size and charge.[26] This filtration barrier is made of three types of filters: the endothelial fenestrations in glomerular capillaries, the glomerular basement membrane, and the visceral epithelial cell podocyte slit diaphragm. Injury at any of these layers can disrupt the filtration barrier and lead to proteins or blood cells entering Bowman's space as part of urine (Fig. 7.2).

Understanding the content of urine will tell us what is happening at this filtration boundary. For example, injury on the vascular side of the glomerular filtration barrier allows red blood cells to leave the capillary causing microscopic hematuria. These red blood cells usually do not pass freely but rather "squeeze" through gaps in the endothelium and emerge misshapen. This is why "dysmorphic" red blood cells are a significant finding. It suggests there are gaps in the endothelium and is more associated with nephritic than nephrotic diseases. Alternatively, injury on the epithelial side of the basement membrane allows large amounts of protein to pass, and a urinalysis shows significant proteinuria. This is usually a nephrotic disease.

Filtered Molecules

Normally, a healthy glomerular filtration barrier will freely pass water and small molecules, but filtration of larger

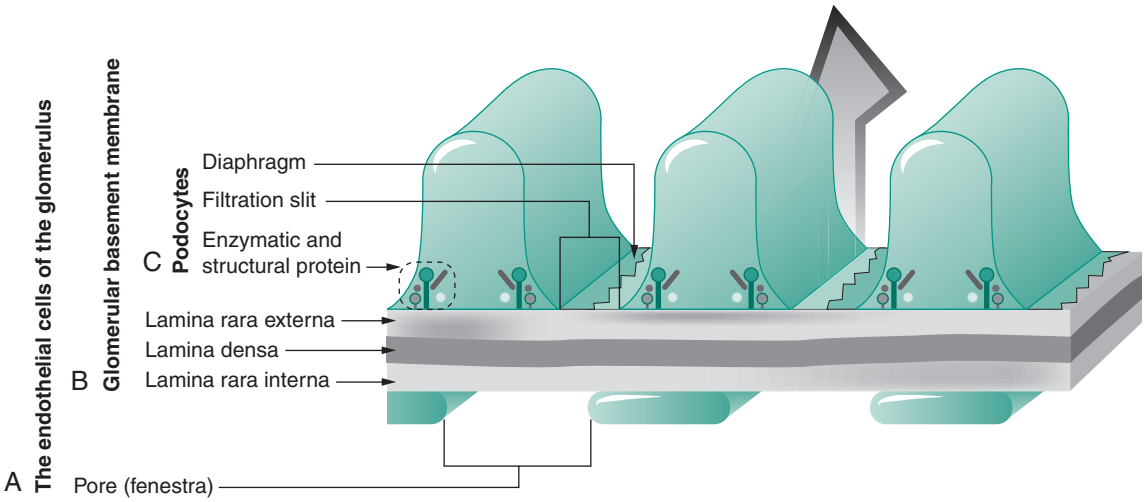

FIGURE 7.2 Glomerular basement membrane.

molecules depends on glomerular surface area and the glomerular capillary wall permeability. For example, studies with Dextrans show that molecules larger than 4.2 nm are essentially completely blocked[27] and molecules larger than 3.4 nm have limited filtration. Note, however, that molecules between 3.4 nm and 4.2 nm are filtered partially, and this includes some proteins. The traditional view that fluid entering the tubule from the glomerular capillaries is virtually protein free is being questioned. First, albumin (3.6 nm), like other proteins, is filtered and reabsorbed.[28] These filtered proteins normally are reabsorbed by proximal tubule cells using the megalin/cubilin complex, as well as possibly fluid phase endocytosis. Moreover, some renal diseases are explained by protein first passing through the glomerular filtration barrier and then reabsorbed by the proximal tubule. Fanconi syndrome caused by multiple myeloma is due to light chains passing through the glomerular filtration barrier and being endocytosed by proximal tubular cells.[29] In small quantities this protein endocytosis may not be harmful, but reabsorbing large amounts of free light chains may cause cell injury from crystal deposition in lysosomes as well as activating intracellular stress pathways such as nuclear factor-κB (NF-κB).

The filtration of any molecule across the glomerular filtration barrier is passive and driven by forces such as hydrostatics, oncotic pressure, and perhaps electrostatics. Hydrostatic pressure is nearly constant along the capillary path through the glomerulus. Empirically, this has been measured to be 46 mm Hg in the capillary, and 12 mm Hg in Bowman's space in Munich-Wistar rats.[30] As a result, there is a net force of 34 mm Hg driving plasma into the Bowman's space. In addition, the filtration is affected by the hydraulic conductivity or resistance of the capillary wall, basement membrane, and visceral epithelium.

Plasma oncotic pressure increases as more water is filtered out of the capillary, and the remaining blood increases in osmolality. Traditionally, oncotic pressure was assumed to be determined by the contents of blood, and the filtrate in Bowman's space was assumed to have virtually no oncotic pressure because it was assumed that there are no proteins. However, if we instead assume that some smaller proteins pass into Bowman's space, then we need to revisit these measurements.[31]

Endothelium

Endothelial cells in the glomerular capillaries are not a seamless barrier but instead have gaps within cells called fenestrations that allow water and molecules to pass through. Fenestrations are typically 70 to 100 nm in diameter and represent up to 20% of endothelium surface area. Fenestrations originally were considered more pores than filters; they were so large they let everything through and the basement membrane did the actual filtering. However, the endothelium is covered by a layer of anionic glycoproteins and proteoglycans, called the glycocalyx, that also seems to cover the fenestrations.[32] These glycoproteins absorb proteins from serum creating a 200-nm thick coat on the endothelial surface.[33] This is an anionic barrier, which may repel large macromolecules and anions.[34] The glycocalyx seems to contribute to protein filtration. Rupturing the proteoglycan cover with hyaluronidase and adriamycin causes proteinuria.[33] Similarly, in different experiments, progressive enzymatic breakdown of the endothelial surface layer leads to increased albuminuria.[35,36] The glycocalyx may be modified in diseases such as diabetes, which is associated with a nephrotic proteinuria,[37] and in humans diabetes proteinuria correlates with damage to the endothelium.[38]

Basement Membrane

The glomerular basement membrane is a trilaminar membrane consisting of the lamina densa in the middle, lamina interna next to the endothelial cells, and lamina externa next to the podocytes. The podocytes and endothelial cells secrete proteins to make up the basement membrane. There are 144 distinct proteins in the basement membrane, but about half of the proteins are collagen IV.[39] There are several subtypes of collagen IV, each made by a different gene, and the glomerular basement membrane largely uses a trimer of collagen IV subtypes, alpha3, alpha4, and alpha5. This is different from other basement membranes, which typically are made of collagen IV alpha1 and alpha2. These proteins form a network of fibrils, with pores that average 10 nm in diameter. Different proteins form layers in the membrane, so it is not a homogenous pile, but rather a laminated collection of layers.

Another common protein in the basement membrane is beta-2 laminin, which binds to alpha3beta1 integrin in the podocyte membrane. Alpha3beta1 integrin connects to the podocyte cytoskeleton inside the cell, so the bond between beta2 laminin and alpha3beta1 integrin connects the basement membrane to the podocyte cytoskeleton. Besides type IV collagen and laminin, other significant proteins include fibronectin, proteoglycans, and entactin connect collagen and laminin.

The glomerular basement membrane is anionic,[40] chiefly because of anionic proteoglycans. This previously was thought to cause a charge-selectivity: the anionic basement membrane may repel other anionic proteins, such as albumin.[41–44] Recent experiments, however, have challenged this. For example, Ficoll/Ficoll sulfate particles of different charges all passed through the basement membrane equally, whether they were anions or not.[45] Genetically modified mice with minimal anionic charge do not have proteinuria.[46] Treating the glomerular basement membrane with heparinase to strip out glycosaminoglycan anionic charge does not cause proteinuria.[47] Finally, genetically modified mice with reduced podocyte charge have only mild albuminuria, suggesting that charge is not a major factor in filtering anions like albumin from the urinary space.[48]

PODOCYTES

Podocytes encase the capillaries, forming a selectively permeable boundary between the fenestrated capillary and Bowman's space. They are also important to the filtration of blood and provide approximately 40% of hydraulic resistance of the filtration layer.[49]

If 40% of proteins are blocked by the glomerular basement membrane, then where do they go? Normally, there is no accumulation of proteins between the fenestrated endothelium and the basement membrane or between the basement membrane and the podocyte slit diaphragm. The filtration barrier seems to also have a process that continually removes proteins. Podocytes express general protein transporters such as cubilin/megalin, and podocytes may take up proteins that manage to traverse the basement membrane but then are blocked by the slit diaphragms.[50,51] Other theories propose that the basement membrane acts more like a gel than filter, and only proteins that travel further through the gel will pass.[52]

Podocyte Structure and Function

There are several distinct parts of the podocyte cell: the cell body, primary processes, secondary processes, and tertiary processes (foot processes) that anchor onto the basement membrane. Throughout these is a complex cytoskeleton, which includes microtubules and intermediate filaments in the body and primary processes, and actin microfilaments in podocytes.

The cytoskeleton is significant partly because the shape of the podocyte is complex and biologically important. Foot processes extend out from secondary processes, and there are slit-diaphragms between these foot processes. This gap between foot processes has similarities to epithelial cell tight junctions, although there is no E-cadherin, so this is not a true tight junction. Nonetheless, the space between podocytes is bridged by several types of proteins that extend into the podocyte interior and outside the cell into the mesangium. Some proteins span the cell membrane, including NEPH1, nephrin, and laminin. Nephrin has an intracellular domain and an extracellular domain and is part of an extracellular filtration pore and part of the intracellular cytoskeleton. Nephrin from adjacent podocytes may bind directly, or else indirectly through the NEPH1 molecules, and forms a filtration pore. The intracellular domain of nephrin binds with podocin, which enables podocin polymerization and so is part of controlling actin filament rearrangement to shape the foot processes. Similarly, alpha3beta1 integrin and beta2 laminin connect the podocyte cytoskeleton to the basement membrane. Proteins inside the cell include CD2-associated protein, which anchors nephrin to the cytoskeleton; podocin, which anchors nephrin to the plasma membrane; and zonula-occludens-1, which anchors nephrin to the cytoskeleton. In addition, zonula-occludens-1 positions nephrin and podocin, and loss of zonula-occludens-1 is associated with proteinuria and is reduced in diabetic nephropathy. Proteins outside the cell lie between adjacent foot processes and include NPHS2, which is a member of the stomatin family, P-cadherin and FAT, which are members of the cadherin family, and many others, including Kieel/Neph1, TRPC6, ACTN4, MYO1E, ARHGAP24, ARHGDIA, INF2, COQ2, COQ6, PLCE1, ANLN, PTPRO, and ADCK4.

Despite the large list of proteins, however, the true topology of the basement membrane is not understood. A different way to look at this catalog of proteins is to consider the diseases of the slit diaphragm, which shows what happens when one protein is defective. For example, congenital nephrosis and adult-onset FSGS is caused by mutation in the NPHS2 gene, which encodes podocin, and autosomal dominant focal segmental glomerular sclerosis is caused by a mutation in TRPC6, and congenital nephrotic syndrome (Finland) is caused by mutation the NPHS1 gene for nephrin.

Podocytes play a mechanical role that dynamically reacts to mechanical forces. The podocyte senses hydrodynamics and transduces changes in pressure into cell processes through several mechanisms, including changing membrane potential, activating protein kinases, and controlling gene expression.[53]

The podocyte also responds to systemic signals, and podocytes have receptors for vasoactive hormones, including angiotensin II, vasopressin, norepinephrine, adenosine, ANP, nitric oxide (NO), endothelin, and prostaglandins.[54] For example, the podocyte cell membrane contains ion channels such as Trpc5 and Trpc6, which respond to angiotensin II and allow Ca to enter the podocyte. Intracellular calcium causes actin reorganization by activating Rho GTPases.[55] Overstimulation of this process seems to cause podocyte injury, and gain of function mutations affecting TRPC6 leads to renal injury, whereas loss of TRPC5 is protective in some cases.[56–58]

MESANGIUM

The mesangial cells are a "stalk-like" support network that holds the coils and loops of capillaries in place, but they also play other roles. There are several types of mesangial cells. For example, the mesangium contains immune cells that are similar to monocytes/macrophages and make up 5% to 15% of the mesangium. However, more common are contractile cells, which make up 85% to 95% of mesangium and seem to provide structural support and contraction. This contraction may control capillary flow in a manner analogous to arteriole smooth muscle contraction and dilation.[59] Mesangial cells have hormone receptors,[60] and G-protein receptors will lead to constriction while cAMP receptors will lead to relaxation. The mesangial cell contains actin and myosin-based microfilaments inside the cell pass through the cell membrane and bind to laminin in the glomerular basement membrane.[61,62] The mesangium also contains the matrix, extracellular material that is made of several proteins including collagen III and IV, heparin sulfate proteoglycans, and fibronectin, laminin, entactin, and fibrillin.

Key Points

1. Blood pressure in the afferent and efferent arterioles is tightly regulated, which protects the kidney and controls filtration and diuresis under different physiologic conditions.
2. Glomerular capillaries are the site where plasma moves across a filtration barrier into the urinary space. The glomerular filtration barrier filters molecules based on size and charge and is made of three types of filters: the endothelial fenestrations in glomerular capillaries, the glomerular basement membrane, and the visceral epithelial cell podocyte slit diaphragm.
3. The glomerular filtration barrier filtration barrier will freely pass water and small molecules, but filtration of larger molecules also happens. The traditional view that fluid entering the tubule from the glomerular capillaries is virtually protein free is being questioned.
4. Podocytes' shape (regulated by its internal cytoskeleton) is complex and biologically important, and podocytes dynamically respond to mechanical forces as well as vasoactive hormones such as angiotensin II, vasopressin, norepinephrine, adenosine, and many more.

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A complete reference list can be found online at ExpertConsult.com.

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