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The role of heparan sulfate in the glomerular basement membrane

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Recent studies, including those by van den Hoven and colleagues, have challenged the classic negative-charge theory of glomerular filtration. However, the possibility remains that heparan sulfate in the glomerular basement membrane plays a role in maintaining the glomerular filtration barrier.

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In the glomerulus, transport of circulating albumin into the urinary space is strictly regulated by a filtration barrier that consists of size- and charge-selective filters. For decades, the issue of which component of the glomerulus constitutes the filtration barrier has been a subject of investigation by nephrologists. Ultrastructural studies with cationic/neutral/anionic tracers performed in the 1960s, 1970s, and 1980s led us to believe firmly that the glomerular basement membrane (GBM) functions as the filtration barrier;[1] that circulating albumin is repelled by the negative charge of the GBM; that heparan sulfate (HS) carries the negative charge in the GBM; and that loss of HS in the GBM results in massive proteinuria. Some investigators in the mid-1990s questioned the interpretation of earlier tracer studies.[2] The 'negative-charge theory' described above was favored until the slit diaphragm molecule nephrin was discovered in 1998.[3] Subsequent studies of rare human families and genetically altered animals led to the identification of other slit diaphragm molecules that play important roles in glomerular filtration. Some of these molecules interact with signaling molecules that regulate the actin cytoskeleton of podocyte foot processes. Attention shifted from the GBM to podocytes, not because the negative-charge theory was disproved, but because podocytes became so attractive. Although knowledge regarding the function of podocytes has increased vastly in the past decade, it is time for us to revisit the negative-charge theory. Molecular techniques are now available to examine decisive questions; recent studies have suggested that the glomerular filtration barrier is complex. The following fundamental questions can now be addressed: (1) does the GBM function as a filtration barrier? and (2) is HS in the GBM essential for maintaining the barrier?

With respect to the first question, no one doubts the importance of podocytes in maintaining the filtration barrier. However, the fact that podocytes are important does not exclude a role of the GBM. Laminin β2-chain, which is encoded by the *LAMB2* gene, is an essential constituent of the GBM. In 1963, Pierson reported that two sisters with congenital nephrotic syndrome progressed to end-stage renal disease and microcoria. Forty years later, *LAMB2* mutations were found in patients from five unrelated families that showed clinical manifestations similar to those described by Pierson.[4] *LAMB2* mutations were found in 2.5% of patients in a European cohort of 89 children from 80 families with nephritic syndrome, which appeared within the first year of life. In *Lamb2*$^{-/-}$ mice, anionic sites in the GBM were disorganized.[5] Albuminuria preceded podocyte foot process effacement, which was visible about 2 weeks after birth. Ferritin permeability of the GBM was markedly increased in *Lamb2*$^{-/-}$ mice, but not in nephrotic *Cd2ap*$^{-/-}$ mice, which showed a primary podocyte defect and no alterations in the distribution of anionic sites in the GBM. These findings indicate that the GBM and the slit membrane act as filtration barriers.

To address the question of whether HS in the GBM is necessary to maintain the filtration barrier, we must first discuss the biochemical features of HS. HS is a polysaccharide classified as a form of glycosaminoglycan, a polysaccharide that contains amino sugars (glycosamines). The basic glycosaminoglycan structure is a simple repeat of two sugar (disaccharide) units. In the case of HS, D-gluconate (GlcA) and *N*-acetyl-D-glucosamine (GlcNAc) units joined in 1→4 linkages are repeated (Figure 1). However, a variety of chemical modifications can be introduced, resulting in enormous structural complexity. HS is subject to several modifications, including conversion of D-GlcA to L-iduronate (IdoA) through C5-epimerization, formation of *N*-sulfated GlcN ($GlcNSO_3$) units through *N*-deacetylation/*N*-sulfation, and formation of *O*-sulfated (-OSO_3) GlcA, IdoA, or GlcNAc through *O*-sulfation at positions 2, 3, and 6. For example, 2-*O*-sulfated IdoA (*O*-sulfated IdoA at position 2, or IdoA[2-OSO_3]), 6-*O*-sulfated GlcNAc (*O*-sulfated GlcNAc at position 6, or GlcNAc[6-OSO_3]), and 6-*O*-sulated $GlcNSO_3$ (*O*-sulfated $GlcNSO_3$ at position 6, or $GlcNSO_3$[6-OSO_3]) are major constituents of HS (Figure 1). The large numbers of *N*- and *O*-sulfation in this macromolecule yield very strong anionic charges. In fact, the negative-charge densities of heparin and HS, which have very similar molecular structures, are stronger than those of any other macromolecules *in vivo*. HS is suggested to be the molecular source of the negative charges in the GBM.

Van den Hoven and colleagues[6] (this issue) now describe the kidney phenotypes of mice overexpressing human heparanase (*HPSE*-tg), which has been a useful tool in studies of the nature of HS in the GBM. They prepared a large panel of anti-HS monoclonal antibodies to show not only that different HS epitopes are present in the

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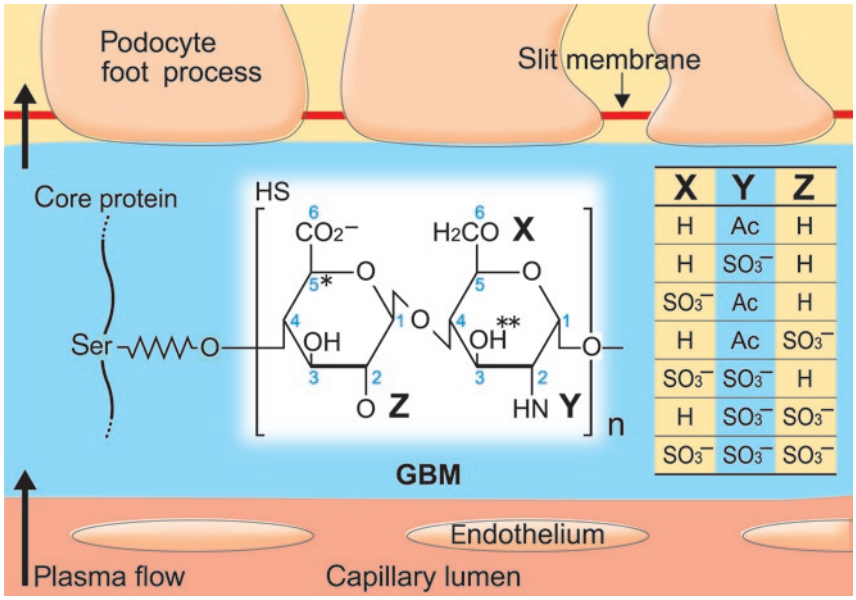

Figure 1 | Heparan sulfate (HS) in the glomerular basement membrane (GBM). HS is covalently bound to a serine residue of a proteoglycan core protein via a tetrasaccharide linkage region (sawtooth line). The polysaccharide of HS is a simple repeat of D-glucuronate and *N*-acetyl- (or sulfo-)D-glucosamine joined by 1→4 linkages. The combination of X, Y, and Z as well as 'n' in the figure varies. Furthermore, the conversion of D-glucuronate (asterisk) to L-iduronate by C5-epimerization and 3-*O*-sulfation of D-glucosamine (double asterisk) can occur.

GBM but also that some of the HS epitopes differ from those in tubular basement membranes or the basement membranes of Bowman's capsule. These results are in agreement with the concept that tissue- and function-specific expression of HS structures exists as originally suggested by basic carbohydrate researchers. The immunohistochemical data from van den Hoven and colleagues[6] clearly show that some HS epitopes in the GBM are lost in *HPSE*-tg mice. Their ultrastructural analysis with a cationic dye indicates that anionic sites were reduced in the GBM of *HPSE*-tg mice. Morphology of the glomerulus was unchanged, and massive proteinuria was not observed.

Two groups of HS-degrading enzymes are known. One is bacterial heparan sulfate lyase, a heparitinase that catalyzes the eliminative cleavage of the α-*N*-acetyl-D-glucosaminidic linkage in HS. The other is bacterial or mammalian endoglucuronidase, a heparanase that catalyzes the hydrolytic cleavage of glycosidic bonds in HS. Mammalian cells express a single major heparanase. In humans, the gene *HPSE*-1 maps to 4q21.3, contains 14 exons, and encodes a 65-kilodalton precursor protein. Proteolysis yields active heparanase, a heterodimer of 50- and 8-kilodalton proteins. Substrate specificity of mammalian heparanase was investigated on a biochemical basis. HexUA(2S)-GlcN(NS,6S)-IdoA-GlcNAc(6S)-GlcA-GlcN(NS,+/−6S)-IdoA(2S)GlcN(NS,6S) may be a candidate physiologic target octasaccharide sequence for human heparanase.[7] In van den Hoven and colleagues' work,[6] HS epitopes recognized by the NAH46 and AO4B08 antibodies were resistant to heparanase digestion. The authors deduced from cationic dye staining that approximately 20% of the GBM HS was resistant to heparanase digestion in *HPSE*-tg mice. However, biochemical analysis of the GBM HS is necessary to determine whether this value is underestimated, although such an analysis is technically difficult to achieve.

In findings similar to those of van den Hoven *et al.*,[6] a recent study published in the *Journal of the American Society of Nephrology* showed that removal of GBM HS in rats by injection of HS-degrading enzyme did not lead to proteinuria.[8] In spite of this line of evidence, it is too early for us to conclude that GBM HS does not play an important role in maintaining filtration barrier. Van den Hoven and colleagues state that one cannot exclude the role of loss of GBM HS in the development of proteinuria. They hypothesize that development of proteinuria in human glomerulonephritis may be mediated by a multi-hit or multi-signal mechanism that consists of loss of GBM HS, release of various growth factors/cytokines bound to HS, and unfavorable activation of podocytes.[9] Experimental manipulation of only one signal may not be sufficient to mimic the condition present in glomerulonephritis with proteinuria in humans. Furthermore, there is evidence that expression of glomerular heparanase is increased in a variety of proteinuric disorders in mice, rats, and even humans.[9,10] Structure-oriented studies of HS might be unfamiliar, but they deepen our understanding of the roles of HS in the GBM and may facilitate generation of new HS-based drugs to treat proteinuria. In the light of recent findings, the classic negative-charge theory may need some modification. However, the possibility remains that HS in the GBM plays a role in maintaining the glomerular filtration barrier.

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