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Heparanase Is Essential for the Development of Acute Experimental Glomerulonephritis

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Heparanase, a heparan sulfate (HS)—specific endoglucuronidase, mediates the onset of proteinuria and renal damage during experimental diabetic nephropathy. Glomerular heparanase expression is increased in most proteinuric diseases. Herein, we evaluated the role of heparanase in two models of experimental glomerulonephritis, being anti-glomerular basement membrane and lipopolysaccharide-induced glomerulonephritis, in wild-type and heparanase-deficient mice. Induction of experimental glomerulonephritis led to an increased heparanase expression in wild-type mice, which was associated with a decreased glomerular expression of a highly sulfated HS domain, and albuminuria. Albuminuria was reduced in the heparanase-deficient mice in both models of experimental glomerulonephritis, which was accompanied by a better renal function and less renal damage. Notably, glomerular HS expression was preserved in the heparanase-deficient mice. Glomerular leukocyte and macrophage influx was reduced in the heparanase-deficient mice, which was accompanied by a reduced expression of both types 1 and 2 helper T-cell cytokines. *In vitro*, tumor necrosis factor-α and lipopolysaccharide directly induced heparanase expression and increased transendothelial albumin passage. Our study shows that heparanase contributes to proteinuria and renal damage in experimental glomerulonephritis by decreasing glomerular HS expression, enhancing renal leukocyte and macrophage influx, and affecting the local cytokine milieu. *(Am J Pathol 2016, 186: 805—815; http://dx.doi.org/10.1016/j.ajpath.2015.12.008)*

Proteinuria is a hallmark of many glomerular diseases and an independent risk factor for the progression of renal failure.[1] Heparan sulfate (HS) is a highly negatively charged glycosaminoglycan that is covalently attached to a core protein, so-called HS proteoglycans. In seminal articles, researchers demonstrated the presence of HS in the glomerular filtration barrier (GFB), which is composed of glomerular endothelial cells covered by a glycocalyx, the glomerular basement membrane (GBM), and podocytes.[2–4] Because of its negative charge, HS seems to play an important role in the charge-selective permeability of the GFB.[5] Removal of HS with bacterial heparinase leads to a dramatic increase in glomerular permeability for neutral and cationic macromolecules.[6] Genetic targeting of HS in the GFB compromised permselectivity and barrier function to a lesser extent,[7–9] although the development of proteinuria has been associated with a reduced expression of HS in the GFB.[3,5,10] The HS chain is composed of up to 150 α(1-4)-glucuronate-β(1,4)-*N*-acetylglucosamine disaccharide units that can be modified extensively. HS is characterized by an enormous structural diversity, which dictates the binding of several soluble ligands, such as cytokines, chemokines, and growth factors.[11,12] In addition, specific endothelial HS domains mediate the trafficking of leukocytes.[13]

In many human and experimental glomerular diseases, the reduced expression of HS in the GFB is associated with an increased expression of heparanase.[3,14] Heparanase is an endo-β(1,4)-D-glucuronidase that can cleave HS side chains.

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Heparanase is synthesized as a preproheparanase of 68 kDa. To gain its biological activity, preproheparanase is processed in the endoplasmatic reticulum, where the signal peptide is removed, and further processed in lysosomes, where cathepsin L cleaves off a linker domain to form the active form of heparanase.[14,15] Outside the kidney, heparanase is involved in cancer progression, in particular metastasis and neovascularization.[16–18] Heparanase is also involved in the pathogenesis of several inflammatory disorders, such as inflammatory lung injury, rheumatoid arthritis, and chronic colitis.[19–22] Recently, we demonstrated that heparanase is essential for the development of proteinuria in experimental diabetic nephropathy.[23] In streptozotocin-induced diabetes, heparanase-deficient mice failed to develop proteinuria and renal damage, in contrast to their wild-type (WT) littermates. In addition, proteinuria was reduced and renal function improved by treatment with the heparanase inhibitor SST0001.[23] In a follow-up study, we showed that heparanase contributes to the inflammatory cascade during the pathogenesis of diabetic nephropathy.[24] Although heparanase plays a crucial role in the development of diabetic nephropathy, the exact role of heparanase in inflammatory glomerular diseases, such as glomerulonephritis, is still unknown.

Glomerulonephritis is characterized by the influx of inflammatory cells, proteinuria, hematuria, and a decline in renal function. Previous studies revealed that heparanase may be involved in the development of proteinuria in passive Heymann nephritis and in a model of accelerated anti-GBM disease, because treatment of rats with a polyclonal antibody against heparanase or the heparanase inhibitor PI-88 reduced proteinuria in both experimental diseases.[25–27] In addition, it has been recently described that lipopolysaccharide (LPS)–induced glomerulonephritis involves an increased heparanase expression and a damaged glomerular endothelium, which is, in large part, mediated by tumor necrosis factor (TNF)-α.[28] Interestingly, heparanase inhibition prevented glycocalyx loss and neutrophil adhesion during sepsis-induced acute lung injury, indicating that heparanase may also be involved in the influx of inflammatory cells.[19] In another recent study, severe systemic sepsis was induced by cecal ligation and puncture, and it was suggested that heparanase mediated early renal dysfunction. Unfortunately, in the latter study, there was no direct proof for a reduced HS expression in the GFB mediated by heparanase, whereas the applied anti-HS antibody (3G10) suggests involvement of bacterial-derived heparinases instead of mammalian heparanase.[29]

To elucidate the role of heparanase in the development of glomerulonephritis, we evaluated the involvement of heparanase in two experimental glomerulonephritis models, anti-GBM and LPS-induced glomerulonephritis, in WT and heparanase-deficient mice. Our results indicate that heparanase drives anti-GBM and LPS-induced glomerulonephritis by enhancing the renal influx of inflammatory cells and by influencing the local cytokine production.

Materials and Methods

Animals

C57Bl/6 (Harlan Laboratories, Jerusalem, Israel) and heparanase knockout mice[30] in a C57Bl/6 background were kept under pathogen-free conditions, housed in a temperature-controlled room with a 12-hour light/dark cycle, and had *ad libitum* access to food and water. All animal experiments were performed in accordance with, and approved by, the Hebrew University (Jerusalem, Israel) Institutional Animal Care and Use Committee.

Induction of Anti-GBM and LPS Glomerulonephritis and Determination of Albuminuria and Blood Urea Nitrogen

Experimental anti-GBM glomerulonephritis was induced as previously described.[31] WT and heparanase-deficient mice, 14 to 15 weeks old, were injected in the tail vein with 7 mg rabbit anti-mouse GBM IgG serum. Mice were sacrificed after 2 hours, 1 day, and 4 days to collect kidneys and blood. LPS glomerulonephritis was induced in 14- to 15-week-old WT and heparanase-deficient mice by an i.p. injection with 80 μg LPS (O111:B4; Sigma-Aldrich Chemie, Zwijndrecht, the Netherlands). Mice were sacrificed after 2 days to collect kidneys and blood. Eight mice were used per time point. Urine was collected through a bladder punction or after 24 hours in metabolic cages. Collected kidneys were snap frozen in liquid nitrogen. Urinary albumin was measured by radial immunodiffusion (Mancini), and blood urea nitrogen and urinary creatinine concentrations were determined routinely in our clinical diagnostic facility.

Immunofluorescence Staining

Immunofluorescence staining was performed on cryosections (2 μm thick), as described.[31] Directly labeled antibodies included rat anti-mouse GR-1 (RB6.8C5)–fluorescein isothiocyanate (BD Biosciences, Alphen aan de Rijn, the Netherlands) and rat anti-mouse CD41-Alexa 488 (ITK Diagnostics, Uithoorn, the Netherlands). Unlabeled primary antibodies included CD68 (MCA1957) (Serotec, Oxford, UK) and the VSV-tagged anti-HS antibodies HS4C3, AO4B08, EW4G2, and EW3D10.[31,32] Appropriate secondary antibodies include Alexa 488 antibodies (Invitrogen Life Technologies, Breda, the Netherlands) or anti–VSV-Cy3 antibody (Sigma-Aldrich). Capillary loops were visualized with the hamster anti-agrin antibody (MI91),[33] recognized by a Cy-3–labeled antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). Sections were post-fixed with 1% paraformaldehyde–phosphate-buffered saline and embedded in Vectashield mounting medium H-1000 (Brunschwig Chemie, Amsterdam, the Netherlands). HS was scored semiquantitatively for staining intensities on a scale between 0 and 10 (0 indicates no staining; 5, 50% staining; and 10, 100% staining) by two investigators (M.G. and A.L.W.M.M.R.). Glomerular influx of granulocytes

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