Tubulointerstitial heparan sulfate proteoglycan changes in human renal diseases correlate with leukocyte influx and proteinuria

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HEPARAN SULFATE PROTEOGLYCANs (HSPGs) are glycoconjugates present both in extracellular matrices and on the cell surface. These molecules are composed of a protein backbone, to which extended linear carbohydrate chains [glycosaminoglycans (GAGs)] are attached. Heparan sulfate (HS)-GAG chains are attached (50). Heparan sulfate proteoglycan changes in glomerular filtration (14, 21, 36), although this paradigm is currently under debate (17, 18, 20). In addition, HSPGs can bind the leukocyte adhesion molecule 1-selectin and various chemokines, implicating a role in inflammation as well (25, 30, 31, 49, 50). Our laboratory has previously shown that 1-selectin binding to HSPGs is dependent on the presence of a specific binding domain on the HS-GAG chains (3). Under normal conditions, this domain is only present on a subset of renal HSPGs (3). Recently, we demonstrated that, upon renal ischemia-reperfusion, HSPGs associated with the interstitial microvasculature become major ligands for 1-selectin and monocyte chemoattractant protein-1 (MCP-1/CC chemokine ligand-2) and affect the inflammatory response (4). Although alterations in glomerular HSPGs have been shown in primary kidney diseases (13, 35, 43, 45, 47, 48) and the role of 1-selectin and chemokines in renal disease is well established (1, 8, 27, 39, 41), few studies have addressed a possible interaction between HSPGs and 1-selectin or chemokines in primary kidney diseases (37, 41). In this study, we examined a panel of human renal biopsies from patients diagnosed with various renal diseases for the presence of HSPGs binding to 1-selectin and MCP-1. We chose to detect direct binding of these proteins to HSPGs in various renal structures, because this likely reflects functional HSPG alterations, rather than using HS-specific antibodies, which detect usually well-defined, but not necessarily functional, epitopes.

Our results show that HSPG alterations, from a functional point of view, occur in the renal tubulointerstitial in various renal diseases, as well as in the urine.

METHODS

Reagents. 1-Selectin-IgM chimeric protein was produced as described (3, 5). Recombinant human MCP-1 was from Peprotech (London, UK); anti-human syndecan-1 (CD138) from Serotec (Oxford, UK); anti-HS antibody 10E4, heparitinase I (EC4.2.2.8), and chondroitinase ABC (EC4.2.2.4) from Seikagaku (Tokyo, Japan); anti-human IL-8 from BD Pharmingen (Erembodegem, Belgium); Ulex europaeus agglutinin I (UEA I) from Vector (Burlingame, CA); and Alexa fluor-labeled secondary antibodies from Molecular Probes (Eugene, OR). Recombinant human MCP-1 was from R&D Systems (Abingdon, UK). Antibodies directed against syndecan-4 (8G3) and epidermal growth factor (HGF) and anti-HGF were from R&D Systems (Abingdon, UK). Recombinant human MCP-1 was from Peprotech (London, UK).
immune extracapillary proliferative glomerulonephritis, lupus nephritis (LN), diabetic nephropathy (DN), membranous glomerulopathy (MGP), IgA nephropathy (IgAN), minimal change nephropathy (MCN), as well as biopsies without histological alterations, which were, however, obtained from patients with proteinuria [miscellaneous (MSC)] (Table 1). Histologically normal cortical tissue from kidneys removed because of renal adenocarcinoma served as controls. All human tissue material was used with the approval of the local medical ethics committee. Interstitial leukocyte count was determined in periodic acid Schiff/hematoxylin-stained tissue sections and is expressed as mean of 10 non-overlapping high-power fields ($\times 400$).

**Immunofluorescence.** In situ t-Selectin and MCP-1 binding was performed as described (4, 5). In some experiments, tissue sections were pretreated using heparitinase I (0.05 U/ml) or chondroitinase ABC (1 U/ml) in acetate buffer (50 mM C$_2$H$_3$O$_2$Na, 5 mM CaCl$_2$, 2H$_2$O, 5 mM MgCl$_2$·6H$_2$O, pH 7.0 for heparitinase I, pH 8.0 for chondroitinase ABC) for 1 h at 37°C. Syndecan-1, syndecan-4, glypican-1, 10E4, and IL-8 stainings were performed by incubating primary and secondary antibodies for 1 h at room temperature (RT) on tissue sections. Nonrelevant isotype-matched antibodies served as background controls and proved to be negative. Sections were examined using a Nikon Eclipse E800 fluorescence microscope using identical microscopy conditions and exposure time to compare stainings between different samples. Scoring was performed by two independent investigators. Scale for t-selectin binding and syndecan-1 expression ranged from 0 (no binding/expression), 1 (<30% of microvascular/tubular epithelial cells (TECs) involved), 2 (30–60% of microvascular/TECs involved), 3 (>60% of microvascular/TECs involved), to 4 (>60% of microvascular/TECs involved with marked higher intensity). For IL-8, a low level of uniform staining was observed in control kidneys, which could not be attributed to background, and therefore scoring was based on intensity alone ranging from 1 (weak), 2 (moderate), and 3 (intense).

**Cell culture.** The human TEC cell line HK-2 (38) was kindly provided by Dr. C. van Kooten, Leiden University Medical Center, Leiden, the Netherlands. Cells were cultured in Dulbecco’s modified Eagle’s medium/F-12 (Gibco, Invitrogen, Breda, the Netherlands), supplemented with insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml), hydrocortisone (36 ng/ml), epidermal growth factor (10 ng/ml) (Sigma-Aldrich, Zwijndrecht, the Netherlands), 50 U/ml penicillin, 50 μg/ml streptomycin, and 2 mM l-glutamine (Cambrex, Verviers, Belgium).

**Table 1. Proteinuria, interstitial leukocyte count, and detection of HSPGs and IL-8 in a panel of human renal diseases**

<table>
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<th>Patient</th>
<th>Proteinuria, g/24 h</th>
<th>Interstitial Leukocyte Count/Field</th>
<th>t-Selectin Binding HSPGs in Perivascular Interstitial Matrix*</th>
<th>Syndecan-1 Expression in TECs*</th>
<th>IL-8 Expression in TECs*</th>
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HSPG, heparan sulfate proteoglycan; TEC, tubular epithelial cell; C, control; MSC, miscellaneous; TIN, tubulointerstitial nephritis; PGN, pauci-immune extracapillary proliferative glomerulonephritis; LN-IV, lupus nephritis class IV; DN, diabetic nephropathy; MGP, membranous glomerulopathy; IgAN, IgA nephropathy; MCN, minimal change nephropathy; ND, not determined. *Scale ranges from 0 (no binding/expression), 1 (<30% of microvascular/TECs involved), 2 (30–60% of microvascular/TECs involved), 3 (>60% of microvascular/TECs involved), to 4 (>60% of microvascular/TECs involved with marked higher intensity). **Scale is based on intensity of staining ranging from 1 (weak), 2 (moderate), to 3 (intense). *No histological alterations; swollen podocytes without effacement of foot processes. °No histological alterations; no effacement of podocyte foot processes. °Diagnosed as LN class IV and V. °Kimmelstiel-Wilson noduli identified.
Short-hairpin RNA silencing. Short-hairpin RNAs (shRNAs) targeting syndecan-1 (synd1-shRNA; target sequence 3′-GGTCTTTT-GCAAGATATCA-5′) and renilla luciferase (control vector; target sequence 3′-AACATGCAGAAAAATGCTG-5′) were cloned into the pTER expression vector (kindly provided by H. Clevers, Hubrecht Laboratory, Centre for Biomedical Genetics, Utrecht, the Netherlands). HK-2 cells were transfected with synd1-shRNA vector or control vector using lipofectamine (Invitrogen), according to manufacturer’s protocol, and selected based on zeocine resistance.

Flow cytometry. Cells were detached (10 mM EDTA, 150 mM NaCl, 6 mM KCl, 1.2 mM KH₂PO₄, 20 mM HEPES, 5 mM glucose, 0.5% BSA; pH 7.4), washed with PBS/0.5% BSA, and incubated with anti-syndecan-1 (1 μg/ml) in PBS/0.5% BSA (30 min on ice). Non-relevant mouse IgG served as isotype control. After washing, cells were incubated with FITC-labeled anti-mouse IgG (30 min on ice), washed, and analyzed by flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA). Similarly, cells were incubated with L-selectin-IgM diluted in Tris-buffered saline (TBS)/1% BSA, and incubation of L-selectin-IgM in the presence of 10 mM EGTA, and incubation of nonrelevant IgM antibody, all resulting in signals reduced to background levels (not shown).

Urine analysis. Early morning first-void urine samples were collected from healthy volunteers (controls; n = 7) and from patients diagnosed with various renal diseases (n = 10) after informed consent was obtained. Patients with known proteinuria (>1 g/24 h) were included, irrespective of underlying renal disease. Characteristics of both groups were as follows: controls, mean age 42 yr (range 25–57 yr), male/female ratio 5/2; patients, mean age 47 yr (range 26–79 yr), male/female ratio 5/5, mean proteinuria at diagnosis 5.6 ± 24 h (range 2.3–12.2), diagnosis included LN (n = 2), MG (n = 2), DN (n = 1), MSC (n = 1), focal segmental glomerulosclerosis (n = 1), renal sclerosis (n = 1), reflux nephropathy (n = 1), and Alport glomerulonephritis (n = 1). Samples were centrifuged for 15 min at 300 g, and supernatants were stored at −20°C. Urinary protein and creatinine were determined at 450 nm.

Statistical analysis. For statistical analysis, control kidneys were presumed to be nonproteinuric (<0.2 g/24 h). Data were analyzed using two-tailed Spearman’s rank correlation test and Student’s t-test, with P < 0.05 considered as statistically significant. For correlation analyses, all cases presented in Table 1 were included.

RESULTS

Interstitial perivascular matrix HSPGs bind L-selectin and MCP-1 in renal diseases associated with interstitial leukocyte influx. In human control kidneys, L-selectin binding was mainly detected in tubular basement membranes and in interstitial matrix that was sparsely present (Fig. 1A). No L-selectin binding was observed in the glomerulus, and little binding was detected in association with peritubular capillaries (identified using endothelial marker UEA I; Fig. 1A). The majority of L-selectin binding in control kidneys was sensitive to pretreatment of the tissue sections with heparitinase I, an enzyme that specifically degrades HS, demonstrating that these ligands are HSPGs (Fig. 1B). These observations are in line with the previously reported L-selectin binding pattern in normal rat, mouse, and human kidneys (3–5). In a number of renal biopsies, we observed an evident increase of L-selectin binding to interstitial matrix, as shown for a patient with TIN (TIN2; Fig. 1C). Double staining using endothelial marker UEA I revealed that this L-selectin binding matrix was partly associated with interstitial capillaries (Fig. 1C). Heparitinase I pretreatment reduced this perivascular binding, showing that these L-selectin ligands are predominantly HSPGs (Fig. 1D). Scoring for the presence of L-selectin binding HSPGs in perivascular interstitial matrix revealed that this pattern of binding is observed in biopsies of patients diagnosed with various renal diseases, however, most prominently in TIN and pauci-immune extra-capillary proliferative glomerulonephritis (Table 1). As TIN is clearly associated with interstitial leukocyte influx, we counted the amount of leukocytes per interstitial field in our panel of biopsies to correlate this count to the score for L-selectin binding HSPGs in perivascular interstitial matrix. Indeed, a positive correlation was found between leukocyte count and L-selectin binding HSPGs in the perivascular matrix in our panel of biopsies (Spearman’s ρ = 0.536, P = 0.001).

In addition to L-selectin binding in perivascular matrix, we observed intense binding in fibrotic regions in a number of biopsies (shown for TIN2; Fig. 1E). This binding was not sensitive to heparitinase I pretreatment (not shown). As fibrotic regions in the kidney are known to contain CS/DS proteoglycans (DSPGs) (26, 32), we used chondroitinase ABC, an enzyme that specifically degrades CS/DS-GAGs. Pretreatment using this enzyme diminished L-selectin binding to fibrotic regions, demonstrating that these L-selectin ligands are CS/ DSPGs (Fig. 1F).

To examine whether also chemokine binding is altered in biopsies showing increased L-selectin binding, we detected binding of MCP-1, known to be involved in renal inflammation (1, 39), in control kidneys and TIN biopsies (shown for TIN1; Fig. 2, A and C). In control kidneys, MCP-1 binding was only observed in a number of interstitial cells, possibly leukocytes (Fig. 2A). No binding was detected in interstitial matrix or glomeruli. In TIN, strikingly pronounced MCP-1 binding to interstitial matrix, including perivascular capillaries, was observed, as well as some binding in the glomerulus (Fig. 2C). Both interstitial and glomerular MCP-1 binding in TIN biopsies were reduced after preincubation with heparitinase I, showing that binding is mediated predominantly by HSPGs (Fig. 2D). Incubation with the anti-MCP-1 antibody alone did not result in any staining in these biopsies (data not shown), which could well be explained by the possibility that the anti-MCP-1 antibody does not recognize endogenous MCP-1 in formalin-fixed tissue. Together, these data show an increased binding of L-selectin and MCP-1 to HSPGs in the perivascular interstitial matrix in various renal diseases, which is associated with leukocyte influx. In addition, L-selectin binding to CS/DSPGs in fibrotic areas was detected.

Basolateral L-selectin and MCP-1 binding HSPGs in tubular epithelial cells associated with proteinuria. In addition to L-selectin binding to interstitial matrix, also evident binding to TECs was observed in a number of biopsies, which was
typically not that pronounced in control tissue (Fig. 3, A, C, and E; Table 1). High-power magnification revealed that binding was localized to the basolateral aspect of the cells (Fig. 3C, inset). L-Selectin binding to TECs was completely abrogated after heparitinase I pretreatment, demonstrating that L-selectin binds to cellular HSPGs (Fig. 3, D and F). L-selectin ligands in interstitial fibrotic regions (E) are shown to be chondroitin sulfate (CS)/dermatan sulfate proteoglycans (DSPGs) by chondroitinase ABC pretreatment of sequential sections (F). Bar = 50 μm.

We next set out to determine whether the L-selectin binding HSPGs on TECs were also able to bind MCP-1. Apart from a few individual cells in the interstitium, no evident binding of MCP-1 was detected in control renal tissue (Fig. 4A). However, in biopsies showing basolateral TEC L-selectin binding HSPGs, basolateral MCP-1 binding was also observed, in addition to binding to the interstitial matrix (Fig. 4, C and E). MCP-1 binding was completely sensitive to heparitinase I pretreatment, demonstrating that binding is mediated by HSPGs (Fig. 4, B, D, and F). Interestingly, preliminary data show no basolateral TEC binding of HGF [a growth factor known to bind HSPGs on multiple myeloma cells (9)], indicating that the HSPGs expressed do not bind all potential HSPG binding partners (data not shown).
These findings demonstrate that cellular HSPGs expressed on TECs are induced to bind \( \lambda \)-selectin and MCP-1 in proteinuric kidney diseases.

\( \lambda \)-selectin and MCP-1 binding HSPGs are induced on activated TECs. Several studies have reported that TECs become activated under proteinuric conditions, resulting in increased expression of chemokines, including IL-8 (6, 16, 44, 51). To examine whether the proteinuria-associated induction of \( \lambda \)-selectin and MCP-1 binding HSPGs is associated with activation of TECs in our panel of biopsies, we stained for IL-8 protein. In control kidneys, very weak staining for IL-8 was observed in TECs (Fig. 5A). In IgAN biopsies with increased TEC \( \lambda \)-selectin binding HSPGs, tubular IL-8 staining was modestly increased compared with control (Fig. 5B). In MCN biopsies, a more pronounced increase of tubular IL-8 staining was observed (Fig. 5C). In addition to tubular IL-8, individual cells in the interstitium and some glomeruli were also highly positive for IL-8 in a number of biopsies (Fig. 5, B and C). TEC IL-8 was detected in a cytoplasmic pattern, suggesting that we are detecting IL-8 production rather than IL-8 being presented on (induced basolateral) HSPGs. Indeed, heparitinase I pretreatment did not alter IL-8 detection (not shown).

Statistical analysis revealed a significant correlation between tubular IL-8 expression and TEC \( \lambda \)-selectin binding (Table 2; Spearman’s \( \rho = 0.530, P = 0.002 \)), as well as with proteinuria (Table 2; Spearman’s \( \rho = 0.352, P = 0.044 \)). This supports the hypothesis that the increased capacity of TEC HSPGs to bind \( \lambda \)-selectin and MCP-1 is associated with epithelial cell activation in the context of proteinuria.

Basolateral TEC syndecan-1 expression is increased in proteinuric kidney diseases and colocalizes with increased anti-HS antibody 10E4 staining. We further characterized the TEC HSPGs induced in proteinuric kidney diseases by staining the tissue sections using antibodies directed against cell-surface HSPGs expressed in the kidney (syndecans-1 and -4, glypican-1), as well as the anti-HS antibody 10E4 (34, 46, 52). Although no basolateral TEC staining was observed for syndecan-4 and glypican-1 (not shown), a low level of syndecan-1 staining was observed in control kidneys (Fig. 6A). Strikingly, a pronounced increase in staining for syndecan-1 in a basolateral pattern was observed in IgAN and MCN biopsies compared with control kidneys (Fig. 6, A, C, and E). Using the anti-HS antibody 10E4, no staining could be detected in control kidneys, although staining was evident in IgAN and MCN biopsies and colocalized with basolateral syndecan-1 (Fig. 6, B, D, and F). Scoring the whole panel of biopsies for syndecan-1 staining in TECs (Table 1) revealed a statistically significant correlation between syndecan-1 staining in TECs and TEC \( \lambda \)-selectin binding HSPGs (Table 2; Spearman’s \( \rho = 0.753, P < 0.001 \)), and between syndecan-1 and IL-8 expression (Table 2; Spearman’s \( \rho = 0.689, P < 0.001 \)). These data indicate that the induced basolateral TEC HSPG could be syndecan-1 decorated with HS-GAGs, recognized by anti-HS antibody 10E4, \( \lambda \)-selectin, and MCP-1.

Syndecan-1 mediates binding of \( \lambda \)-selectin to TEC cell line HK-2. To prove that \( \lambda \)-selectin is able to bind syndecan-1 on tubular epithelial cells, we employed syndecan-1-specific RNA silencing. Under normal culture conditions, the human TEC cell line HK-2 expresses syndecan-1 and is able to bind \( \lambda \)-selectin. To specifically reduce syndecan-1 expression in these cells, we targeted this molecule using shRNA transfection (synd1-shRNA). As shown in Fig. 7, this resulted in a significant decrease in cell-surface expression of syndecan-1 compared with cells transfected with control vector. \( \lambda \)-selectin binding to synd1-shRNA cells was also reduced compared with control vector (Fig. 7).
showing that syndecan-1 mediates L-selectin binding to HK-2 cells.

_Urinary syndecan-1 from proteinuric patients is less capable of binding L-selectin._ We next examined whether the increase in syndecan-1 expression observed in renal biopsies of proteinuric patients is reflected in the urine. Therefore, we collected urine samples from healthy controls (n/7) and proteinuric patients (n/10) (Fig. 8A; for patient characteristics see METHODS). As shown in Fig. 8B, no difference in urinary syndecan-1 concentration between controls and proteinuric patients could be observed. When corrected for creatinine, syndecan-1 concentration was also not different between healthy controls and proteinuric patients (not shown). To determine whether urinary syndecan-1 is able to bind L-selectin, we performed an ELISA-based L-selectin binding assay (3) on captured syndecan-1 from the same urine samples. As shown in Fig. 8C, significantly less binding of L-selectin to urinary syndecan-1 could be detected in samples obtained from proteinuric patients (Student’s t-test; P/0.012). In fact, values for L-selectin binding to urinary syndecan-1 in urine samples obtained from proteinuric patients approximated background levels (omitting urine samples; OD 450-nm/0.126). This shows that, although the concentration of syndecan-1 shed in urine is not different between controls and proteinuric patients, the binding capacity of urinary syndecan-1 for L-selectin is decreased in proteinuric patients.

**DISCUSSION**

Historically, research on renal HSPGs has mainly focused on the glomerulus, using antibodies directed against specific, but not necessarily functional, HS epitopes (35, 43, 45, 47, 48).
In this study, we examined changes in renal HSPGs using the known, immunologically relevant binding partners L-selectin and MCP-1. We show increased binding of both recombinant human L-selectin and MCP-1 to HSPGs in the tubulointerstitial compartment in various renal diseases.

In the first part of this study, we demonstrated an increased binding of L-selectin and MCP-1 to perivascular interstitial matrix HSPGs, which was significantly associated with interstitial leukocyte influx. These data are in line with our recent findings in experimental renal ischemia-reperfusion and human

**Fig. 4.** Binding of MCP-1 to basolateral TEC HSPGs in proteinuric kidney diseases. A–F: MCP-1 binding was detected in tissue sections of control kidney (A and B; patient C4), IgAN (C and D; patient IgAN2), and MCN biopsies (E and F; patient MCN5). Heparitinase I treatment shows that basolateral TEC MCP-1 binding molecules in IgAN and MCN are HSPGs (B, D, and F). Bar = 50 μm.

**Fig. 5.** Increased expression of IL-8 protein in proteinuric kidney diseases. IL-8 protein was detected in tissue sections of control kidney (A; patient C1), IgAN (B; patient IgAN3), and MCN biopsies (C; patient MCN5). Bar = 50 μm.
renal allograft transplantation, showing a similar modification of microvascular matrix HSPGs, resulting in L-selectin binding and facilitating monocyte/macrophage influx (4). Our observations reinforce the concept that (vascular) matrix HSPGs are not merely static structural components, but can be actively regulated to influence cellular processes, including inflammation. Apart from HSPGs, we also detected L-selectin binding CS/DSPGs, mainly located in areas of interstitial fibrosis. Although we did not aim to identify the CS/DSPG ligand for L-selectin in this study, likely candidates include versican, a renal CSPG known to bind L-selectin, and collagen type XV, a matrix CSPG that is upregulated in human fibrotic kidney tissue (15, 23, 24).

The mechanism behind the increased binding of L-selectin and MCP-1 to perivascular matrix HSPGs could be based on upregulated core-protein expression of matrix HSPGs, which include agrin, perlecan, and collagen type XVIII (19). In addition, binding properties of expressed HSPGs can be altered by modification of HS-GAG chain composition (50). Several different enzymes, acting both intracellularly and extracellularly, are involved in HS-GAG biosynthesis and modification (12, 29). At least one of these enzymes is under the control of the proinflammatory transcription factor NF-kB (2). Further research will provide more insight into the pathways involved in the increased binding of L-selectin and MCP-1 to HSPGs observed in this study.

In the second part, we showed that binding of L-selectin and MCP-1 to cellular HSPGs is induced on TECs in association with proteinuria. In addition, increased expression of the cell surface HSPG syndecan-1 on TECs was demonstrated, which colocalized with the L-selectin and MCP-1 binding HSPGs. Using shRNA-mediated silencing, we showed that syndecan-1 can indeed mediate L-selectin binding to a TEC cell line in vitro. However, although shRNA-mediated silencing of syndecan-1 resulted in reduction of expression to background levels, binding of L-selectin was not completely abolished. This may be due to differences in detection efficiency, but may also implicate an additional ligand for L-selectin on this TEC cell line. Additional evidence that syndecan-1 can mediate L-selectin binding was provided by our studies using urine samples from controls and proteinuric patients. We showed that urinary syndecan-1 from controls was able to bind L-selectin, whereas
binding to urinary syndecan-1 from proteinuric patients was significantly reduced, which cannot be explained by differences in syndecan-1 concentration in these samples. Apparently, the increase in syndecan-1 expression on TECs in proteinuric diseases is not reflected in the urine, and, even more strikingly, urinary syndecan-1 from proteinuric patients is less capable of binding L-selectin, although binding to TECs in the biopsies is increased. Possibly, L-selectin and MCP-1 binding HSPGs are retained under proteinuric conditions, resulting in an apparently increased expression on TECs. Alternatively, HS-GAG degrading enzymes could be released in the urine of proteinuric patients, resulting in loss of detection of L-selectin binding (22, 40). In addition, the L-selectin binding site on HSPGs could be masked in proteinuric samples by binding of other cationic proteins (36). Future experiments in a more large-scale study may provide more insight.

Although the changes in proteoglycan binding properties observed in this study are evident, binding was detected using exogenously added recombinant proteins, and we, therefore, cannot conclude that binding of endogenous L-selectin or MCP-1 will also occur in vivo. However, the fact that we detect proteoglycan changes in the tubulointerstitial compartment in various renal diseases is interesting in itself.

The increased syndecan-1 expression on TECs in the context of proteinuria may well be a result of TEC activation due to increased (or altered) protein content in the tubular lumen, as the correlation with increased IL-8 expression suggests. It is known that various renal diseases, if they persist, proceed to follow a common final pathway leading to renal failure (16). It is possible that TEC HSPGs could play a role in this process, although more research has to be done to address this point. For example, activation of TECs by luminal protein combined with exposure to specific cytokines could result in basolateral shedding of syndecan-1 (which could bind L-selectin and MCP-1) (7), providing directional cues for the migration of leukocytes into the interstitium (28). Alternatively, the TEC HSPG changes may not play a role in disease progression, but could rather reflect an attempt of the renal epithelium to
recover from injury. Indeed, both syndecan-1 and anti-HS antibody 10E4 are associated with wound healing, epithelial cell activation, and proliferation (10, 11, 42, 46). Future research may provide more insight into the mechanisms involved in modification of tubulointerstitial HSPGs and their potential role in renal disease progression.

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