The Bittersweet Taste of Tubulo-Interstitial Glycans

Ditmer T. Talsma, Mohamed R. Daha, Jacob van den Born

Summary

Recently, interesting work is published by Farrar et al. (1) showing the interaction of fucosylated glycoproteins on stressed tubular epithelial cells with collectin-11 leading to complement activation via the lectin route of complement. This elegant work stimulated us to evaluate the dark side (bittersweet taste) of tubulo-interstitial glycans in kidney tissue damage. As will be worked out glycans not only initiate tubular complement activation, but also orchestrate tubulo-interstitial leukocyte recruitment and growth factor responses. In this review we restrict ourselves to tubulo-interstitial damage mainly by proteinuria, ischemia/reperfusion injury and transplantation, and we discuss the involvement of endothelial and tubular glycans in atypical and \textit{E. coli}-mediated hemolytic uremic syndrome. As will be seen, fucosylated, mannosylated, galactosylated and sialylated oligosaccharide structures along with glycosaminoglycans comprise most important glycans related to kidney injury pathways. Up to now, therapeutic interventions in these glycan-mediated injury pathways are underexplored and warrant further research.

Glycans – An introduction

All cells and extracellular matrices carry an array of covalently attached sugars (monosaccharides) or sugar chains (oligo- and polysaccharides) which are referred to as carbohydrates or glycans. Glycoconjugates consist of glycoproteins (carrying N-asparagine and O-serine/threonine linked sugars), proteoglycans (carrying O-serine linked unbranched sugars called glycosaminoglycans) and glycosphingolipids or glycolipids (carrying ceramide linked sugars). Monosaccharides form the basic building blocks of all glycans. Glycanation is mostly a post-translational modification and is predominantly confined by a large number of different glycosyltransferases within the endoplasmatic reticulum and the Golgi compartment. Monosaccharide units of glycans can be modified by methylation, esterification (mostly sulfation), deoxygenation and amino group addition. The large number of different monosaccharides, its synthesis into oligosaccharides, number and position of branching points, and modifications of the saccharide units create an enormous heterogeneity in glycans (2,3). It is important to realize that glycan structures are not encoded directly in the genome, since, in contrast to DNA, RNA and proteins, there is no template for sequence order and linkages of monosaccharide units, nor for branching of polysaccharide structures and the modification reactions. This is why glycobiology is lagging behind the molecular biology of nucleic acids and proteins. Because many glycans are on the outer surface of cells, they are in a position to modulate cell-cell, cell-matrix, and cell-molecule interactions, and can serve as regulatory switches by rapid turnover and changes in glycosyltransferase expression patterns (4). Two major classes of glycan-binding
proteins are known, the lectins and glycosaminoglycan-binding proteins. C-type lectins (such as the selectin and collectin family members) are calcium-dependent lectins, most other mammalian lectins and glycosaminoglycan-binding proteins are calcium-independent (5,6). As part of the humoral arm of the innate immune system, the collectins and ficolins, mostly circulatory lectins, function as pattern recognition molecules, binding glycans on a wide range of bacteria, viruses, fungi and protozoa, but also on altered self-structures on apoptotic and necrotic cells and are aimed at elimination of these microbes and damaged cells (7). The collectins and ficolins initiate the lectin route of complement as will be outlined below.

Glycosaminoglycans are linear polysaccharides covalently bound to a core protein, forming a proteoglycan. Based on the composition of glycosaminoglycan chains, proteoglycans are categorized as heparan sulfate, chondroitin sulfate, keratan sulfate or dermatan sulfate proteoglycans (8). The sulfation pattern on these glycosaminoglycan chains affects activities of proteoglycans (9). Proteoglycans are found in extracellular matrices and on almost all mammalian cell types and they can interact with many factors among which are growth factors, cytokines and chemokines. Proteoglycans are involved in cell proliferation, differentiation, inflammation, development, cell-cell adhesion and signalling (10). Although proteoglycans play a role in mammalian physiology, under certain conditions they can be also involved in pathophysiology as will be outlined below. The most abundant glycosaminoglycan found in renal tissue is heparan sulfate. These heparan sulfate polysaccharide side chains display variations in sulfation and the expression pattern in renal tubulointerstitium of various renal diseases (11). The involvement of tubulo-interstitial glycans in complement activation, leukocyte recruitment, growth factor responses, and in the haemolytic uremic syndrome is discussed in more detail in this review.

Glycans initiate tubulo-interstitial complement activation

The complement system

The complement system consists of a set of inactive liver-derived plasma components that are linked and activated in a cascading manner. The complement system has more than 60 components and activation fragments, which comprise the nine central components of the cascade (C1-C9), multiple activation products with diverse biological functions (eg C3a and C5a), regulators and inhibitors (eg factor H), proteases (eg factors B and D), and complement receptors (eg C1qR and C5aR). Complement is activated by three major pathways, namely i) the alternative pathway (spontaneously and constantly activated on biological surfaces); ii) the classical pathway (triggered by immune complexes); and iii) the lectin pathway (initiated by glycans on microbial surfaces). Activation of each of these pathways results in assembly of the so-called C3 convertase, followed by formation of the C5 convertase, and finally the terminal C5b-9 membrane attack
complex. Basically, the complement system has three major functions: Via C3b opsonisation the elimination of cells/microbes by phagocytosis, via the terminal C5b-9 complex lysis of cells/microbes, and via C3a and C5a the recruitment of neutrophils and macrophages towards sites of injury (12). Renal production of complement factors might function as danger signals to initiate and amplify inflammatory reactions (13). In general, the complement system is aimed as a first defense mechanism against invading microbes and elimination of damaged cells.

The lectin pathway (LP) of complement
The LP is initiated by a number of different well-characterized pattern recognition receptors that form macromolecular complexes with the mannan-binding lectin serine proteases 1, 2 and 3 (MASP 1-3), which, upon activation will cleave C2 and C4 and form the classical C3 convertase. The most important LP-associated pattern recognition receptors are mannan binding lectin (MBL), and various members of the ficolin and collectin families. Altogether these lectins recognize glycan structures on microbial surfaces, but also on modified-self structures, eg on stressed or activated renal tubular cells. As indicated in the April issue of J. Clin. Invest. of the year, Farrar et al. published on the detrimental role of L-fucosylated glycoproteins on stressed tubular cells with collectin-11 as the initiation of LP of complement (1). This concept is worked out in the context of murine renal ischemia/reperfusion and in vitro hypoxia and hypothermia of proximal tubular epithelial cells. First observation was a cellular distribution change of fucosylated glycoproteins upon tubular stress. Normally these glycoproteins are exclusively found in the cytoplasm of tubular cells, however upon cellular injury, tubular cells partly exteriorize these fucosylated glycans, which now become expressed in the baso-lateral cell membranes. Second observation was a strong induction of collectin-11 in proximal tubular epithelial cells upon stress, which was partly excreted and now able to bind with the fucosylated glycans, leading to activation of the complement system via the LP. Convincing control experiments using collectin-11 KO mice, and specific removal of L-fucose residues by fucosidase treatment underline this concept. Interestingly however, they also show the collectin-11 mediated LP complement activation to be MASP-2 dependent, however C4 independent. This has been described before, but this C4 bypass route is not yet identified (14). Next to collectin-11, also MBL is described to bind to tubular cells upon ischemia/reperfusion. Binding is mediated via the highly glycosylated meprins within the brush borders and unidentified mannosylated glycoproteins on proximal tubular cells. However, in contrast to collectin-11, MBL did not elicit activation of the LP of complement, rather induced cell injury and cell death independent of complement activation (15). No glycan-based therapeutic interventions have been performed yet in order to interrupt tubular collectin-11 and/or MBL binding in ischemia/reperfusion or renal transplantation setting.

The alternative pathway (AP) of complement
It has been known for decades that AP complement components are
present on the luminal side of tubular epithelium under proteinuric conditions (16). Later, it was shown in vitro that activation of the AP of complement on proximal tubular cells is dependent on the binding of properdin, an activator of the AP (17). Moreover these authors found properdin deposition on the brush borders of proximal tubular epithelium in biopsies of proteinuric patients and reported that urinary properdin excretion is associated with urinary C5b-9 excretion and reduced renal function (more severe albuminuria and lower creatinine clearance) in proteinuric patients (17, 18). It has been shown independently that tubular C5b-9 formation mediated tubulo-interstitial injury during proteinuria (19).

After the discovery that properdin played a significant role in the activation of the AP under proteinuric conditions, Zaferani and colleagues showed that properdin binds heparan sulfate structures in vitro and showed apical co-localization with syndecan-1, a major epithelial HSPG, in vivo in proteinuric rats. C3 deposition on cultured tubular epithelial cells was shown to be dependent of the binding of properdin to heparan sulfate as shown by heparitinase pretreatment of the cells and heparinoid competition experiments (20). Although properdin is the only initiator of the AP, there are a number of AP inhibitors. One of them, factor H, is long known for its binding to heparan sulfates and is present in the urine under proteinuric condition (21). Factor H has multiple glycosaminoglycan binding sites, but only recently it has been shown that factor H requires a relatively high sulfation content of glycosaminoglycans, mostly seen in N-sulfated domains of heparan sulfates. From this observation it was discovered that properdin and factor H recognize different non-overlapping epitopes on tubular epithelial heparan sulfates. Finally, the authors showed that certain low-anticoagulant heparinoids can inhibit properdin binding to tubular heparan sulfate, with minor effect of factor H binding to tubular heparan sulfate. As a result, these heparinoids prevented C5b-9 deposition on the epithelial cells and thus control the AP of complement (22). Buelli and colleagues showed that protein overloading of cultured HK-2 cells led to a reduced heparan sulfate expression and reduced factor H binding. This suggests that protein overload induces changes in heparan sulfate proteoglycan density and/or sulfation on tubular epithelial cells, accounting for a loss of factor H binding sites and subsequent activation of the AP of complement by properdin binding (23). Not only during proteinuria, but also upon acute kidney injury (at least ischemia/reperfusion in the mouse) both properdin and factor H determine tubular injury via AP (24, 25). In contrast to apical/luminal deposition of both factors during proteinuria, upon ischemia/reperfusion both factors deposit at the baso-lateral membranes of tubular cells. Whether this binding is also via heparan sulfates is not demonstrated. Treatment of renal ischemia/reperfusion and renal transplantation in the rat with heparins improved outcome measures, however, whether this was achieved via inhibition of complement activation is not known (26), since heparin interact with a substantial number of complement factors and non-complement mediators (27). It has also been shown that certain sialylated glycans can bind factor H and could therefore modulate the alternative pathway of complement (28). However it has been shown that the most prominent sialic
Chapter 2

acid subtype on the tubular cell membrane, α2-6 linked sialic acid glycans, does not bind factor H (29). In fact factor H prefers binding to α2-3 linked sialic acid glycans, which are more abundant on glomerular endothelium and podocytes. The interaction of tubular glycans with the pattern recognition molecules of the complement system is schematically depicted in Figure 1.

The classical pathway (CP) of complement

C1q, the initiator of the CP, binds mostly to immobilized immunoglobulins on target structures, and is involved in (auto)immune mediated renal diseases such as lupus nephritis, and have been shown to bind with natural IgM antibodies that recognizes phosphomonoesters on hypoxic human epithelial cells (30). Besides, it has been shown that C1q directly can bind with heparan sulfates, the glycan side chains of heparan sulfate proteoglycans (31). C1q also directly binds with advanced glycation end product-modified proteins (32). However, pathophysiological involvement of these C1q – glycan interactions in the tubulo-interstitium have not been described.

Glycans mediate the hemolytic uremic syndrome (HUS)

Tubular globotrihexosylceramide interaction with Shigatoxin 2

Infection with Shigatoxin 2 producing E. coli sometimes progress to hemolytic uremic syndrome, which is characterized by glomerular thrombotic angiopathy and acute tubular damage as a consequence of binding of Shigatoxin 2 with the glycolipid globotrihexosylceramide (Gb3 or CD77) on glomerular endothelium and tubular epithelium. The involvement of complement in Shigatoxin-in-induced HUS is under debate (33). Tubulo-specific depletion of Gb3 protected mice from acute renal failure upon a challenge with Shigatoxin 2 (34). Various receptor mimics that display multiple copies of the Gb3 trisaccharide have been developed, however have not reached the clinic yet (35).

Endothelial sialic acids and heparan sulfate mediate cellular binding of factor H

In contrast to E. coli-mediated HUS, atypical HUS is not induced by Shigatoxin-2 rather by reduced regulation of the alternative pathway of complement, in about half of the cases due to mutations in factor H. As outlined before, factor H is able to bind with cellular sialic acid residues and/or heparan sulfate glycosaminoglycans. This interaction is mainly via domains 6-8 and 19-20 of factor H (36). Once bound, factor H effectively inhibits alternative pathway activation by three different mechanisms, namely inhibition of factor B binding to C3b, decay dissociation of Bb from the C3 convertase, and promotion of the inactivation of C3b by factor I (37). Mutations in domains 6-8 and/or 19-20 of factor H prevent effective cellular binding. Whereas mutations in domains 6-8 are associated with dense deposit disease and age-related macular degeneration (36), mutations is domains 19-20 are associated with atypical HUS. Impaired interaction of these
mutated factor H variants with sialic acids and/or heparan sulfate glycosaminoglycans in the glycocalyx of endothelial cells allow alternative pathway activation via uncontrolled C3bBb alternative convertase formation, leading to endothelial complement activation, followed by platelet binding and activation, and finally to the disastrous thrombotic microangiopathy (38,39). Inhibition of the complement cascade by anti-C5 (eculizumab) is a very powerful treatment for atypical HUS (40).

Figure 1. Schematic representation of the interaction of tubular glycans with the pattern recognition molecules of the complement system. During ischemia/reperfusion injury and transplantation (left part of the scheme), mannosylated and/or fucosylated glycans become expressed on stressed and activated tubular cells. MBL binds with the mannosylated glycans and induces cell death independently of complement activation (5). The fucosylated glycans are recognized by injury-induced collectin-11 and activate the complement system via the lectin route, however independent of C4 (1). Under proteinuric conditions (right part of the scheme), heparan sulfate polysaccharides, mainly present as side chains of syndecan-1 become expressed at the apical membranes of epithelial cells, being predominantly, but not exclusively, proximal cells. These heparan glycans binds both properdin and factor H on different epitopes along the polysaccharide. Depending on the structural composition of the glycan the properdin/factor H balance might be different, thus the heparan sulfate structure (sulfation) orchestrate the activation of the alternative pathway of complement (10,12).
Glycans orchestrate tubulo-interstitial leukocyte recruitment

Upon inflammation in general, leukocytes migrate from the bloodstream through the vessel wall towards the site of inflammation. The process of leukocyte trans endothelial migration involves sequentially acting molecules that mediate leukocyte rolling, activation, firm adhesion and transmigration. After passing the endothelial cell layer, extravasating leukocytes cross the vascular basement membrane and migrate through interstitial matrix towards the site of injury. As will be outlined, glycans, in particular proteoglycans, are highly involved in various steps of these processes and the kidney is no exception to this rule (41).

Sialylated selectin ligands
Leukocyte rolling over activated endothelium is largely selectin mediated. E- and P- selectins are expressed on activated endothelium, whereas L-selectin is constitutively expressed on leukocytes. All three selectins bind glycoproteins decorated with tetrasaccharides comprising (sulfated and/or fucosylated) sialylated Lewis\(^x\) motifs. Indeed, neutrophil influx was significantly impaired in I/R kidneys of mice deficient for both fucosyltransferases IV and VII, enzymes involved in the synthesis of fucosylated glycoprotein ligands for L-selectin (42). A sialyl Lewis\(^x\) inhibitor improved outcome of renal I/R (43) and improved renal transplant survival in rats (44).

Heparan sulfate proteoglycans
P- and L-selectins also recognize heparin-like glycosaminoglycans, identifying vessel wall heparan sulfate proteoglycans as potential physiological ligands (45). Upon activation, endothelial cells synthesize chemokines and present these via the heparan sulfate glycan chains of heparan sulfate proteoglycans to rolling leukocytes. These endothelial heparan sulfate proteoglycan thus not only bind with L-selectin on rolling leukocytes, but at the same time also present chemokines, such as CCL2 (MCP-1) towards rolling leukocytes, and activate the corresponding chemokine receptor, leading to integrin activation and firm adhesion of the leukocyte with (sub) endothelial ligands for integrins, such as ICAM-1 and VCAM-1, fibronectin and laminins. Support for a functional role of the interaction between L-selectin and MCP-1 on one hand and heparan sulfate proteoglycans on the other hand is provided by Wang et al. (46) who elegantly showed that reducing endothelial heparan sulfate sulfation by genetic manipulation impairs neutrophil extravasation. Using the same mouse model, Rops et al. showed reduced neutrophil influx and dampened inflammatory response in the anti-GBM nephritis model (47). Importantly, in healthy kidneys, vascular heparan sulfates do not bind with L-selectin and chemokines, however, upon inflammation convert from silent to pro-inflammatory heparan sulfates now able to bind with L-selectin and chemokines and thus mediate renal leukocyte recruitment. This heparan sulfate
conversion is most likely mediated by a down-regulation of SULF1, an enzyme that normally cleaves off 6-O sulfate groups from heparan sulfates, (rendering them biologically inactive). Inflammatory downmodulation of SULF1 thus allow stable positioning of 6-O sulfates in endothelial heparan sulfates, which thereby allow L-selectin and chemokine-mediated leukocyte entry into the kidney (48). Another interesting observation is that both in experimental and human renal inflamed tissues, L-selectin and MCP-1 binding heparan sulfates are detected at the abluminal side of peritubular capillaries and not at the endothelial surface. These heparan sulfate proteoglycans are thus likely to be perlecan, agrin or one of the basement membrane associated collagen/proteogycan hybrids, such as collagen XV and XVIII. Indeed, mice deficient for both perlecan and collagen XVIII or double deficient for collagen XV and XVIII showed reduced/delayed I/R induced neutrophil and monocyte influx, along with improved kidney morphology and improved kidney function (48,49). In human renal biopsies of inflammatory kidney diseases such as tubulo-interstitial nephritis, the induction of perivascular L-selectin/MCP-1 binding heparan sulfates correlate with increased leukocyte counts, indicating that these subendothelial heparan sulfate proteoglycans do contribute to leukocyte extravasation (11). Likely, inflammation-induced loss of endothelial integrity exposes the vascular basement membranes to extrusions of rolling and adherent leukocytes. The potential of heparan sulfate – chemokine inhibitors as therapeutic agents in inflammatory diseases is increasingly recognized (50,51). The involvement of tubulo-interstitial glycans in leukocyte recruitment is depicted in Figure 2.

Glycans modulate tubulo-interstitial growth factor responses

Upon acute or chronic kidney injury, a cellular response is initiated being either fibrogenic, resulting in progressive renal scarring or regenerative, resulting in renal tissue repair. Both fibrogenic and regenerative responses are largely driven by growth factors. Binding of those growth factors to their cognate high affinity receptor is modulated by glycans as will be outlined below.

Core fucosylation of growth factor receptors

Fucosyltransferase-8 is an enzyme that mediates core alpha1,6-fucosylation of glycoproteins, among which a number of growth factor receptors. Knock down of this enzyme results in loss of receptor core fucosylation and receptor activation. This is shown for the TGF-beta receptors ALK5 and TGF-βRII, but also for the EGF receptor, the HGF receptor and the PDGF receptor. Interestingly, in cultured tubular epithelial cells, blocking of Fucosyltransferase-8 by small interfering RNA technology greatly reduced the phosphorylation of Smad2/3 protein, caused by the inactivation of TGF-β/Smad2/3 signaling, and resulted in remis- sion of epithelial mesenchymal transition (52). Also in vivo, adenoviral-mediated knockdown of Fucosyltransferase-8 mRNA inhibited the core fucosylation of the
TGF-β receptors ALK5 and TGF-βRII, and ameliorated the progression of renal fibrosis induced by ureteral obstruction (53). In contrast to alpha1,6 fucosylation (by fucosyltransferase-8) which promotes growth factor signaling, alpha1,3 fucosylation (by fucosyltransferases-4 and -6) suppress receptor dimerization and activation of EGF receptor (54). This suggests that switching the various isotypes of the fucosyltransferases strongly modulate growth factor responses. Understanding of the regulation of these fucosyltransferases is very limited.

**Heparan sulfate proteoglycans as coreceptors for growth factor**

Heparan sulfate proteoglycans are highly abundant, low affinity co-receptors for heparin-binding growth factors. Via their heparan sulfate polysaccharide side chains, these proteoglycans bind the growth factors at the plane of the cell membrane, and form functional ternary complexes of heparan sulfate proteoglycan, growth factor with its high affinity receptor. Within the kidney FGF2 have been identified as a fibrogenic growth factor. The proliferative response of renal interstitial fibroblasts to FGF2 is completely dependent on cell surface heparan sulfate proteoglycans (55). Also epithelial mesenchymal transition of renal tubular cells by FGF2 proved to be dependent on syndecan-1, a major epithelial membrane heparan sulfate proteoglycan (56). Increased 6-O sulfation of heparan sulfates of cultured tubular cells and within transplanted kidneys promoted FGF2 binding and signaling (57). Our group showed increased binding of perlecan (large matrix heparan sulfate proteoglycan) with FGF2 in the glomeruli and arterial neointima of kidneys suffering from chronic transplant dysfunction. Indeed, also the proliferative response of mesangial cells was fully heparan sulfate sulfation dependent (58). FGF2-driven fibrogenic responses could be ameliorated by interrupting the heparan sulfate – growth factor interaction by preventing sulfation of the heparan sulfate glycan chains of heparan sulfate proteoglycans and/or by competitive blocking with heparin-like glycans (58), showing potential for anti-fibrotic therapy. Tubular epithelial heparan sulfate proteoglycans however, also contribute to regenerative responses. Tubular overexpression of CD44V3 (the CD44 variant decorated with heparan sulfate glycans to the V3 domain) promotes HGF-driven renal repair (59). We showed tubular syndecan-1, as coreceptor for regenerative growth factors such as HB-EGF, to be involved in tubular repair in transplanted kidneys (60,61); see Figure 3. Indeed, the tubular regenerative response to I/R injury is retarded in syndecan-1 KO mice (60). Apparently, the modulatory role of heparan sulfate proteoglycans in growth factor responses is highly context and growth factor dependent, exerting opposing roles from progression of renal fibrosis to renoprotection by tubular regeneration.

**Summary and future perspectives**

In this review we showed the interaction of different types of tubulo-interstitial glycans with complement factors, selectins, chemokines and growth
The Bittersweet Taste of Tubulo-Interstitial Glycans

factors. In most conditions, the modified-self glycan structure is induced upon tubular epithelial activation such as ischemia/reperfusion, renal transplantation or proteinuria. Often, also the corresponding ligand is induced by these noxi. Such injury-induced glycan-ligand interaction often leads to innate immune and

Figure 2. The involvement of abluminal endothelial heparan sulfate proteoglycans in leukocyte recruitment. Both leukocyte adhesion molecule L-selectin (A and B) and chemoattractant MCP-1 (D and E) are able to bind with endothelial basal lamina associated heparan sulfate proteoglycans, especially in renal allografts (B and E), here a one-year protocol biopsy. White arrows indicate the L-selectin/MCP-1 binding to endothelial basement membranes. In solid phase binding assays we further demonstrate the interaction of L-selectin (C) and MCP-1 (F) with collagen XVIII/heparan sulfate proteoglycan fragments. N-terminal non-collagenous domains of short collagen XVIII (comprising amino acids 1-325) and including the TSP1 domain with long (-●-), intermediate (-Δ-) or without (-◊-) heparan sulfate side chains were immobilized to wells and tested for binding with L-selectin (C) and MCP-1 (F). Binding is compared with full length short collagen XVIII (-●-). The binding of L-selectin and MCP-1 with collagen XVIII fragments is stronger when the heparan sulfate chains are longer. MCP-1 induced monocyte migration is studied in a transwell system (G) where monocytic RAW 264.7 cells were added into the top compartment and chemoattractant MCP-1 into the lower compartment. Spontaneous migration over albumin-coated membrane in the absence of MCP-1 was set as 1 and the other values were calculated accordingly. Immobilization of heparin-albumin onto the porous membrane, mimicking an artificial basement membrane heparan sulfate proteoglycan, promotes monocyte migration (H). Transmigration of monocytes towards MCP-1 (10 ng/ml) was increased in the presence of filter-immobilized heparin-albumin and collagen XVIII fragment with long heparan sulfate chains (I). The error bars represent SEM. *: p<0.05, **: p<0.01. Magnifications in A, B, D and E are at 200x. Data is a compilation of work that have been published before and is reproduced with permission of the respective journals (30,31).
tissue remodeling responses, which, during chronic activation conditions worsen renal performance. Despite tremendous research on glycan mimetics in order to interrupt abovementioned interactions, clinical application of such drugs is disappointing. This is related to the fact that most glycan-protein interactions are in the mid-micromolar range of affinity, whereas protein-protein interactions are in the desired nanomolar range. A few promising examples however are the pan-selectin inhibitor Rivipansel (GMI-1070 by Pfizer) which is currently in phase III clinical trial to treat exacerbations of sickle cell disease; and some heparan sulfate mimetics (such as M402 by Momenta, PI-88 and PG545 by Progen), which are in Phase I-III clinical trials of neoplastic disorders. None of these glycan blockers are in renal clinical trials yet. However, in the opinion of the authors, small

![Image of Figure 3](image_url)

**Figure 3.** Tubular heparan sulfate proteoglycan syndecan-1, as coreceptor for regenerative growth factors such as HB-EGF, is involved in tubular repair in transplanted kidneys. Both the regenerative growth factor HB-EGF as well as its co-receptor syndecan-1 are upregulated in one-year protocol biopsies of renal allografts (A and B; left two panels) and co-localize at the baso-lateral cell membranes of proximal tubular epithelial cells (C). The heparan sulfate side chains of syndecan-1 are able to bind the growth factor HB-EGF in the allografted kidney in contrast to control renal tissue (A and B, third panel). Together with the high affinity EGF receptor, HB-EGF and syndecan-1 form a functional ternary signaling complex evidenced by phosphorylation of the EGF receptor in the allograft, but not in control renal tissue (A and B, right panel). The significance of syndecan-1 as co-receptor for tubular proliferation and repair is evidenced by the presence of Ki67-proliferation marker in syndecan-1 positive tubular cells, but not present in syndecan-1 negative tubular cells (D). All magnification are 200 x, except the HB-EGF staining in A and B which is 400x. Data is a compilation of work that have been published before and is reproduced with permission of the respective journals (43,44).
oligosaccharide-based drugs are insensitive to proteolytic degradation, are relatively easy – thus cheap- to produce and offer excellent pharmacodynamics, as exemplified by the widely used low molecular weight heparins. Largest issues of these polysaccharide-based drugs are the molecular affinity for their target structures and the specificity of ligand recognition. Promising candidate polysaccharide-based drugs might be non-anticoagulant heparinoids able to modulate alternative pathway of complement by preventing properdin from binding to cells, however without interfering with factor H binding, and Gb3 trisaccharide analogs in order to prevent Shigatoxin-induced HUS. Also small compounds blocking enzymatic activity of certain fucosyltransferases might prevent fucosylation of collectin-11 ligands, selectins and growth factor receptors, thereby improving ischemia/reperfusion injury, inflammation and fibrosis respectively.
Chapter 2

References


(19) He C, Imai M, Song H, Quigg RJ, Tomlinson S. Complement inhibitors targeted to the proximal tubule prevent injury in experimental nephrotic syndrome and demonstrate a
The Bittersweet Taste of Tubulo-Interstitial Glycans


Part 1

Endothelial heparan sulfates in renal leukocyte migration