The glycobiology of uropathogenic E. coli infection: the sweet and bitter role of sugars in urinary tract immunity

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REVIEW

The glycobiology of uropathogenic *E. coli* infection: the sweet and bitter role of sugars in urinary tract immunity

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Abstract

Urinary tract infections (UTI) are among the most prevalent infectious diseases and the most common cause of nosocomial infections, worldwide. Uropathogenic *E. coli* (UPEC) are responsible for approximately 80% of all UTI, which most commonly affect the bladder. UPEC colonize the urinary tract by ascension of the urethra, followed by cell invasion, and proliferation inside and outside urothelial cells, thereby causing symptomatic infections and quiescent intracellular reservoirs that may lead to recurrence. Sugars, or glycans, are key molecules for host–pathogen interactions, and UTI are no exception. Surface glycans regulate many of the events associated with UPEC adhesion and infection, as well as induction of the host immune response. While the bacterial protein FimH binds mannose-containing host glycoproteins to initiate infection and UPEC-secreted polysaccharides block immune mechanisms to favour intracellular replication, host glycans on the urothelial surface and on secreted glycoproteins prevent or limit infection by inhibiting UPEC adhesion. Given the importance of glycans during UTI, here we review the glycobiology of UPEC infection to highlight fundamental sugar-mediated processes of immunological interest for their potential clinical applications. Interdisciplinary approaches incorporating glycomics and infection biology may help to develop novel non-antibiotic-based therapeutic strategies for bacterial infections as the spread of antimicrobial-resistant uropathogens is currently threatening modern healthcare systems.

KEYWORDS

glycobiology, Urinary tract infections, uropathogenic *E. coli*

AN INTRODUCTION TO URINARY TRACT INFECTION AND BLADDER BARRIER DEFENCES

Urinary tract infections (UTI) are among the leading causes of bacterial infections, worldwide, affecting nearly 150 million people [1]. Whereas UTI incidence steadily increases with age in men and decreases in postmenopausal women, the highest infection frequency peaks among women aged between 15 and 29 years [2]. In fact, UTI are about 40 times more prevalent in women than in men among adults under the age of 60, and the proportion of individuals with UTI, annually, is four to five times higher among women than among men in the United States [2]. This difference in incidence is

Abbreviations: GAG, glycosaminoglycans; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; IBCs, intracellular bacterial communities; Neu5Ac, N-acetylneuraminic acid; SPATE, serine protease autotransporters of *Enterobacteriaceae*; THP, Tamm–Horsfall protein; UP, uroplakin plaque; UPEC, uropathogenic *E. coli*; UTI, Urinary tract infections.

less apparent in infants, children and the elderly, due in part to sex-dependent factors, such as hormones, which impact host defences [3,4]. The main risk factors for community-acquired UTI are biological sex, age and history of UTI, whereas catheterization is the primary risk factor for healthcare-associated UTI [5,6].

The most prevalent pathogens of the urinary tract are Gram-negative or Gram-positive bacteria, collectively termed uropathogens [5,6]. Accounting for about 80% of reported UTI, uropathogenic *E. coli* (UPEC) are the principal infectious agents of the urinary tract [5,6]. Nonetheless, some fungi, such as *Candida* species, can proliferate in the genitourinary tract of patients with underlying urogenital abnormalities or indwelling catheters [5]. In addition, normally latent viruses, such as adenoviruses or human polyomavirus, may also colonize the lower urinary tract, potentially causing haemorrhagic cystitis in at-risk patients, such as immunocompromised children who have undergone allogeneic bone marrow transplantation [7].

Uropathogens can colonize the gastrointestinal tract as commensal bacteria, which may be a relevant reservoir for infection [5]. UPEC may spread among people through individual behaviours and close human relationships, such as cohabitation or sexual intercourse [8–11]. From the periurethral area, uropathogens ascend the urethra to the bladder to establish infection [5]. Ultimately, bacterial ascension via the ureters leads to kidney colonization, or pyelonephritis, which increases the risk of bloodstream infection [5,12]. In men, prostate infection frequently accompanies cystitis, supporting the case for bacterial prostatitis to be classified as a UTI [13,14].

Urinary tract infections most frequently affect the bladder, despite the virtual impenetrability of the urothelium barrier system [15]. The luminal surface of the bladder is covered with uroplakin (UP) plaques, rendering it impermeable to non-gaseous molecules and resistant to the mechanical stresses associated with expansion and contraction of the organ [16]. UPs are integral membrane glycoproteins that assemble first as heterodimers of UP1a/UPII and UP1b/UPIIIa and then come together to form the inner and outer domains of rosette-shaped plaques [17]. UPs are integrated into the apical membrane leaflet of hexagonally shaped umbrella or facet cells, which constitute the luminal-facing layer of the urothelium [18]. Umbrella cells allow the bladder to accommodate variable urine volumes and maintain barrier integrity by reorganizing apical junctional rings, cytoskeleton and surface area, via Rab GTPase-dependent exocytosis or endocytosis of a subapical reservoir of discoidal- and/or fusiform-shaped vesicles [19–23]. In addition to UPs, proteoglycans and membrane-tethered or secreted glycosaminoglycans (GAG) form a mucus layer that shields the urothelium from pathogens or harmful chemicals in urine [24]. The urine itself is integral to the bladder barrier because it protects the urothelium by flushing away metabolic waste and microbes. Urine also contains high concentrations of antimicrobial peptides, such as cathelicidin or lactoferrin, and opsonizing glycoproteins, such as antibodies or Tamm–Horsfall protein (THP or uromodulin), which can destroy uropathogens and block adhesion, respectively [25–27].

Glycoproteins, proteoglycans and GAG are present on outer membranes of cells as part of the cellular interface with the extracellular space [28]. The polysaccharide groups on these macromolecules, called glycans, are the master regulators of cell–cell interactions given their outermost location on cell surfaces [28]. Pathogenic bacteria, including UPEC, can bind tissue- or cell-specific glycoconjugates, supporting adhesion to host cells and playing a key role in determining pathogen tropism [29]. To resist urine flow and colonize the bladder, UPEC adhere to the urothelium via bacterial surface appendages, called pili or fimbriae [30]. Attachment to specific host glycans occurs through adhesins, a family of carbohydrate-binding proteins located at the pili tip [30]. In addition to adhesion, pili-mediated binding of surface glycans initiates UPEC invasion of urothelial cells via a zippering mechanism involving close associations between the bacteria and the host cell surface [31,32]. Glycans also contribute to the process by which bacteria proliferate into large intracellular bacterial communities (IBCs) protected by insulating capsular polysaccharides [29,30]. Host glycans present in the mucus layer, such as GAGs, and on the surface of secreted glycoproteins, such as THP, in turn, prevent bacterial attachment to the urothelium and promote bacterial elimination in urine [33,34].

Here, we bring together experimental evidence describing how glycans mediate or prevent UPEC infection of the bladder, highlighting the relevance of glycan-dependent interactions in UTI at different steps of the infection and immune response. Finally, we provide an updated view on the current state of sugar-based treatments for UTI as a valuable alternative to antibiotics in the wake of disseminated multidrug resistance.

CARBOHYDRATES DIRECTLY MEDIATE UPEC ATTACHMENT, INTERNALIZATION AND PROLIFERATION

UPEC interact with host glycans at very early stages of infection, in which the bacteria use filamentous adhesive organelles called type 1 pili to adhere to urothelial cells in the bladder [31,32,35,36]. The coiled polymeric structure found in these pili terminates at its distal tip with the adhesin FimH, which mediates binding of UPEC to the urothelium [37,38]. Type 1 pili or FimH mutants are unable to adhere to the urothelium in a mouse model of
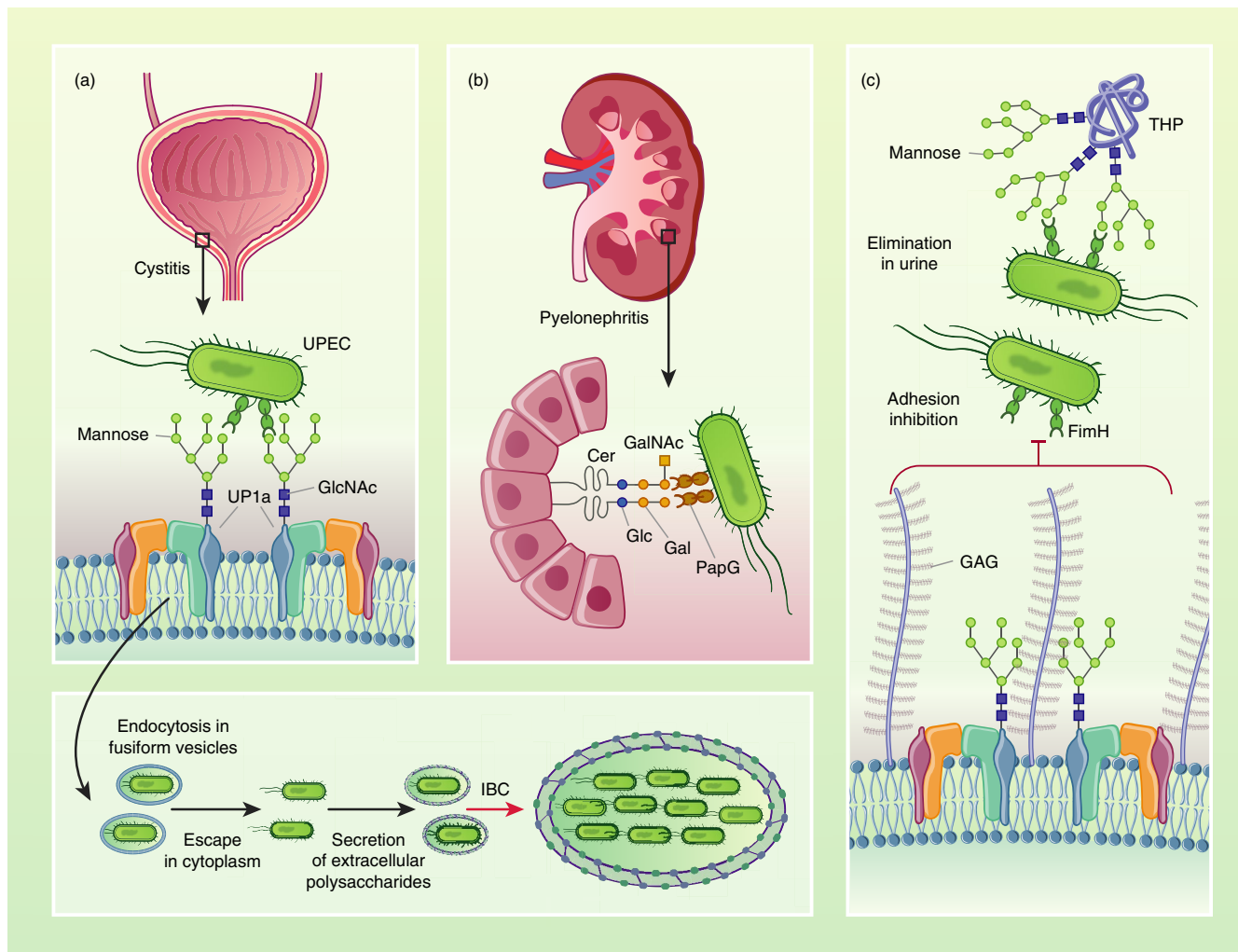
FIGURE 1 Glycan-dependent interactions that promote or prevent infection in the urinary tract. Uropathogenic *E. coli* (UPEC) colonize the bladder by adhering to the urothelium via hair-like extracellular appendages called pili, which have carbohydrate-binding proteins at their tips. (a) The major facilitator of colonization is the adhesin FimH, which binds mannose moieties of high-mannose structures on uroplakins (UPs) covering urothelial cell membranes. In the bladder, mannose-specific FimH-mediated UPEC binding induces bacterial internalization into umbrella cells via endocytosis into discoidal/fusiform vesicles. Internalized UPEC escape into the cytoplasm, where they aggregate and rapidly proliferate to form densely packed intracellular bacterial communities (IBCs). Extracellular bacterial polysaccharides, such as the K1 capsule, contribute to the formation, shape, and growth of IBCs. (b) UPEC can also ascend the ureters to the kidneys to cause pyelonephritis by invading and proliferating in renal tubular epithelial cells. To adhere to the kidney epithelium, UPEC typically use the galactose-specific PapG adhesins on P pili, which bind galactose-rich membrane glycolipids, including globotetraosylceramides and globotriaosylceramides. (c) Host glycans can prevent UPEC adhesion to the urothelium. Glycosaminoglycan (GAG)-rich proteoglycans form a gel-like mucus layer on the luminal surface of the bladder, which blocks FimH-mediated binding to mannosylated glycoproteins on the urothelium, inhibiting invasion of urothelial cells. In the lumen, highly glycosylated Tamm–Horsfall protein (THP) has uroplakin-like mannose glycans, which bind FimH with high affinity, preventing UPEC adhesion. Abbreviations: Cer – ceramide, Gal – galactose, GalNAc – *N*-acetylgalactosamine, Glc – glucose, GlcNAc – *N*-acetylglucosamine, Man – mannose

cystitis or invade bladder cells in vitro [32,39]. FimH has two protein domains: the pilus-linking pilin domain and the distal lectin domain, which binds carbohydrates [38,40]. The FimH lectin domain binds terminal mannose(α1,3)-mannose residues with high affinity and, to a lesser extent, mannose(α1,2)-mannose and mannose (α1,6)-mannose [41,42]. These constitute the terminal moieties of high-mannose N-glycans, which are particularly abundant on UP1a and integrin α3β1 [36,43,44].

As UPs almost entirely cover the luminal surface of umbrella cells, UP1a mannose groups are the most frequently encountered FimH receptor on the urothelium of many species, including humans, mice and cattle [15,36,43]. Interestingly, the presence of N-acetylglucosamine (GlcNAc), at the high-mannose core, increases FimH affinity towards mannosides [41]. Molecular docking models predict that the mannose(β1-4)-GlcNAc motif stabilizes the terminal mannose insertion in the cavity of the FimH mannose-binding pocket [41].

Considering that FimH preferentially binds terminally exposed mannoses [43], these results suggest that interactions with the N-glycan core of high-mannose glycans may stabilize FimH binding. Nevertheless, whether UPEC shows differential affinity towards terminal or core mannoses during UTI remains to be established.

After binding, FimH minimizes the probability of UPEC expulsion during micturition by enhancing its mannose-binding strength via a catch-bond mechanism, whereby the binding pocket affinity for mannosylated structures increases under the tensile mechanical force exerted by urine flow [45,46]. Given the prevalence of UPEC-mediated UTI, the abundance of UP on the urothelium and the requirement of FimH-mediated UPEC adhesion to initiate UTI, binding of FimH to mannose groups of UP1a exemplifies a key glycan target to disrupt host–pathogen interactions (Figure 1a).

Tissue glycosylation can vary over disease progression or following environmental changes, such as diet, smoking or age. It is therefore relevant to ask whether interindividual differences or long-lasting changes in urothelial glycosylation may differentially contribute to the risk of UTI. Interestingly, galactosyltransferase expression is upregulated in mouse bladders in UTI, and UPEC increase the expression of Fml pili with its associated FimH-like FmlH adhesin, which has high affinity for galactose-containing epitopes in chronic UTI models [47]. Microscopic analysis with fluorescence-tagged FmlH stains the GalNAc-rich intermediate urothelial layer of mouse bladders only in chronically infected mice, whereas staining in naïve bladders is scattered [47]. As exfoliation of bladder apical cells exposes the underlying intermediate urothelium, this observation suggests that the FmlH adhesin may help UPEC to resist elimination in urine by binding to the intermediate urothelium surface later in infection.

Once UPEC access the bladder, FimH-mediated binding to terminal mannoses on host UP or integrin α3β1 induces UPEC engulfment by umbrella cells [31,32,44,48]. Cross-linking of other FimH membrane receptors (reviewed elsewhere [30]) on adjacent lipid rafts activates downstream signalling pathways, such as Rho family GTPases, leading to actin cytoskeleton reorganization and UPEC endocytosis via intracellular trafficking proteins, such as dynamins [31,32,49]. The plasma membrane zippers around attached pili, facilitating the intimate binding of UPEC to the urothelium by engaging other coreceptors in lipid rafts [32,44,49,50]. Upon entry, some UPEC escape into the cytoplasm and proliferate rapidly to form clonal IBCs, which are biofilm-like, intracytoplasmic masses of bacteria that assume a coccoid shape [51–55]. A small number of UPEC may also invade intermediate cells and persist in late endosomal vesicles surrounded by a 'cocoon' of actin filaments, as quiescent intracellular reservoirs over periods of time [51,53,56].

UPEC induce host cell defence mechanisms arising from host recognition of pathogen-associated molecular patterns, such as lipopolysaccharides, or other bacterial factors not yet identified [48,57]. For example, FimH-mediated bacterial adhesion to mannosylated sites on umbrella cell membranes induces caspase-dependent apoptosis and exfoliation of these cells, leading to elimination of IBCs [31,48]. Additionally, umbrella cells can expel invading bacteria before IBC formation. UPEC hijack the bladder cell trafficking machinery to enter via Rab11b/Rab27b$^+$ cAMP-responsive fusiform vesicles; however, binding of intracellular TLR4 in infected urothelial cells induces exocytosis of UPEC-containing vacuoles via similar Rab27b$^+$ cAMP-dependent pathways, thus reducing the number of intracellularly proliferating bacteria [58–60].

Interestingly, UPEC require FimH not only for binding of mannosylated host surface proteins and cell invasion, but also for intracellular survival and proliferation into IBCs [39,61]. In a mouse model of UTI, UPEC mutants that do not express type 1 pili during the intracellular phase are decreased in number and do not organize into IBCs compared with infection with the control parental strain, demonstrating that type 1 pili aid formation of IBCs [39]. FimH specifically facilitates IBC development as mutations in two positively selected residues of the FimH pilin domain are sufficient to reduce IBCs in mice compared with animals infected with the wild-type isogenic UPEC strain [61]. Strikingly, the fitness reduction in UPEC mutants is specific to the urinary tract, as gut colonization is comparable to wild-type strain, and is apparent only at later time-points after initial intracellular colonization, when IBC development has already begun [61]. This is because positively selected residues of the pilin domain affecting the aggregation of intracellular UPEC into IBCs do not impair the mannose-binding capacity of FimH required for UPEC adhesion or invasion [61].

UPEC not only recognize host glycans, but they also synthesize their own set of carbohydrates to promote intracellular proliferation into IBCs [30] (Figure 1a). To limit their detection and favour proliferation, internalized UPEC secrete a protective polysaccharide-rich matrix that can incorporate host membrane-derived UPs [52]. The bacterial polysaccharide layer is poorly immunogenic and, as such, hides immunogenic molecular patterns from host intracellular recognition receptors [62]. In this way, IBC development may delay immune responses, such as caspase-3-mediated exfoliation of umbrella cells or phagocytosis [31,63]. As *E. coli* strains secrete multiple types of surface-enveloping or capsular extracellular polysaccharides, identifying the exact biochemical composition of the polysaccharide-rich matrix produced by intracellular UPEC at different time-points during UTI, including during formation of IBCs, will be challenging [53,62].

Most UPEC isolates produce capsular polysaccharides, such as the polysialic acid K1 capsule, and other negatively

charged polysaccharides that may polymerize into biofilm-like structures, such as colanic acid, β-1,6-N-acetyl-D-glucosamine or cellulose [62]. Specifically, K1 capsule and associated sialic acid signalling mediate a crucial structural role during intracellular proliferation in UTI [63]. Many IBC-forming UPEC strains express K1 polysaccharides in vivo, and deficiencies in K1 capsule synthesis or assembly, compromising capsule production, result in comparably reduced bacterial counts in mice 2 weeks post-infection [63]. K1-deficient UPEC numbers also decrease more quickly over time in mice than bacterial CFU of a cystitis patient-derived UPEC strain [63]. Similar to the type 1 pili or FimH pilin domain mutants [53,61], the numbers of intracellular K1 capsule-deficient UPEC bacteria are decreased because they fail to aggregate into IBCs. In fact, K1 capsule-deficient UPEC mutants further altered to upregulate sialic acid trafficking and metabolism display partially restored capacities of K1 capsule synthesis and IBC development compared with K1 capsule-deficient single-mutant strains, showing that secreted polymeric glycans facilitate UPEC proliferation into IBCs [63]. However, the exaggerated catabolism and dysregulated sensing of intracellular sialic acid in the UPEC double mutants used in this study may have decreased intracellular concentrations of sialic acids, such as N-acetylneuraminic acid (Neu5Ac) or N-acetylglucosamine (GlcNAc) [63], which would normally downregulate the OFF-to-ON phase variation switch that selectively induces type 1 pili expression in UPEC during UTI [64–67]. Therefore, it needs to be verified whether the partial restoration of IBC development capacity in these mutant UPEC is directly dependent on capsular polysaccharide metabolism rather than increased surface expression of type 1 pili. Future studies of the secreted polysaccharides of UPEC should also include investigation of their potential signalling functions to elucidate whether these bacterial glycans inhibit intracellular immune response mechanisms.

GLYCOLIPIDS SERVE AS SCAFFOLDS AND FOOTHOLDS FOR UPEC

Glycans are also attached to lipids on cell surfaces, creating a rich diversity of glycolipid structures. In the urothelium, glycolipids maintain the steep osmotic gradient between urine and plasma [15]. Biochemical and chromatographic studies of the bladder show that the luminal plasma membrane is made primarily of ceramides, which are glycosphingolipids consisting of a sphingosine backbone, one fatty acid tail and a polar head made of the monosaccharides D-glucose or D-galactose [68,69]. Umbrella cell membranes have a non-symmetrical ceramide distribution, with higher expression in the apical leaflet of the lipid bilayer compared with the

cytoplasmic side [68]. In addition to reduced permeability compared with phospholipids, the height of the ceramides and of the embedded uroplakins is very similar, enhancing the structural stability of UP in umbrella cell membranes [70]. Although glycolipids constitute 62% of the plaque three-dimensional structure, including the hollow centre with the FimH-targeted high-mannose structures [70], the bacterial factors governing UPEC interaction with bladder glycosphingolipids during UTI remain unknown.

Glycolipids play an important role when bacteria reach the kidneys, where UPEC preferentially bind to the galactose moieties of globotetraosylceramides (Gb4Cer) and globotriaosylceramides (Gb3Cer), two classes of ceramides bearing a combination of N-acetylgalactosamine, D-glucose, and D-galactose, which are highly expressed in the kidneys [69,71,72]. To mediate binding, UPEC use the attachment appendages called P pili. These are heteropolymeric fibres capped by the PapG adhesion molecule, which has high affinity for galactose(αl-4)galactose epitopes, such as those found in Gb4Cer and Gb3Cer [72,73]. Thus, UPEC strains that express P pili can bind the kidney tubular papillary epithelium and cause pyelonephritis [73] (Figure 1b). Similar analyses in umbrella cells will help to determine whether UPEC require the binding of specific membrane glycolipids after FimH-mediated adhesion to facilitate the engagement of lipid rafts initiating cell invasion.

BLADDER GLYCOSAMINOGLYCANS PREVENT UPEC ADHESION

As an added layer of complexity in the glycobiology of the bladder barrier, the urothelium is physically separated from harmful metabolites or invading uropathogens by a protective layer of secreted or membrane-tethered GAGs, as well as proteoglycans, which are linearized proteins decorated with a high number of different GAGs [24]. GAGs are long, negatively charged linear polysaccharides formed by polymerization of repeating disaccharide monomers made of amino sugars, such as sialic acids (N-acetylneuraminic acid [Neu5Ac] in humans), and a galactose or an oxidized monosaccharide, such as D-glucuronic acid [74]. Heparan sulphate (54%), chondroitin sulphate (29%) and dermatan sulphate (17%) are the most common GAGs in the human bladder [74]. In all cases, the long sugar chains in GAGs and proteoglycans give these molecules the capacity to strongly bind water molecules via intermolecular forces, thus trapping them into a gel-like structure that separates the bladder urothelium from urine and non-gaseous molecules.

GAG concentration in human urine samples or from pig bladder scrapings suggests that the mucus lining the bladder is thin and largely made of proteoglycans [75]. The bladder GAG layer is about 10 times thinner than the mucus in the

mouse colon [76], supporting the idea that the bladder surface requires different protection compared with the intestine. Bladder tissue is not absorptive like the gut and instead prevents the entry of non-gaseous material from the lumen through cell–cell junctional rings and surface UP [15,68]. Beyond the intrinsically different functions and structures of the bladder and gut, the presence of different numbers and types of commensal bacteria or metabolites likely requires different barriers in the two mucosal tissues [15,77]. Additionally, copious mucus secretion may pose an obstacle to urination.

However thin, the bladder mucus prevents bacterial adhesion to urothelium [78] (Figure 1c). Non-specific or GAG-specific chemically mediated depletion of the bladder mucus layer leads to increased attachment of radioactively labelled UPEC, *Klebsiella pneumoniae* or *Staphylococcus aureus* in a rabbit model, supporting that bladder mucus impedes adherence to the urothelium [78–80]. Interestingly, observation via scanning and transmission electron microscopy of bladder mucus in a rat model of UTI with UPEC shows a membrane-bound layer of hair-like GAGs entrapping large microcolonies of bacteria surrounded by a glycocalyx [81]. These micrographs support the idea that to reach mannosylated glycoproteins on umbrella cell surfaces with their pili, UPEC may adhere to and burrow through the mucus polymers. Although bladder mucus degradation by UPEC has never been formally demonstrated and no pathogenic K1 serotypes of extraintestinal *E. coli* express specific glycosaminolytic enzymes [82], UPEC may penetrate the GAG layer by secretion of serine protease autotransporters of *Enterobacteriaceae* (SPATE). Some SPATE, such as Pic, display dose-dependent glycosaminolytic activity in vitro [83]. However, mice inoculated with a pyelonephritis patient-derived UPEC strain or a Pic-deficient mutant show similar bacterial burden in bladder and kidneys 6 days post-infection, suggesting that Pic deficiency does not impair UPEC fitness in vivo [84].

Urinary tract microbiota may also influence UPEC fitness during infection by mucus consumption [77]. As microbiota-liberated GAG carbohydrates regulate the growth of enterohaemorrhagic *E. coli* in the intestine, the relative proportion of mucus-degrading bacteria species may favour UPEC infection by secretion of glycosaminolytic enzymes [85]. Oral administration of mucus-constituting glycans to mice tempers the detrimental perturbations of the gut microbial community by favouring the growth of mucus-consuming commensals that are prevalent in the healthy gut [86]. Given the differences between mucus layers of the gastrointestinal and urinary tract, it would be interesting to test whether modulating the relative abundance of urinary tract commensals feeding on bladder mucus impacts host susceptibility to UTI. Therefore, future studies should establish whether UPEC require the presence of a mucus-degrading commensal population to bind urothelium, or whether SPATE or other unknown virulence factors permit UPEC to penetrate the host mucus to reach the urothelial surface.

TAMM–HORSFALL PROTEIN, A CRITICAL HOST DEFENCE MOLECULE

In addition to GAGs, non-glycosylated cationic antimicrobial peptides, such as β-defensins or cathelicidin, contribute to protection of the bladder urothelium against uropathogens and toxins [27]. The most abundant glycoprotein in urine is Tamm–Horsfall protein (THP also called uromodulin), which is constitutively secreted in the kidneys, specifically by the renal cells in the thick ascending limb of the loop of Henle [34]. In urine, THP forms large multimeric aggregates with a flexible zigzag-shaped backbone and protruding hair-like filaments that physically prevent UPEC interaction with the urothelium by entrapping bacteria and clumping them together with its highly glycosylated surface [87]. Each THP monomer contains mannosylated and sialylated N-glycans that may antagonize glycan–uropathogen interactions, acting in urine as a multivalent molecular 'decoy' [87]. The high-mannose structures on the surface of THP strongly bind type 1-piliated UPEC in a mannose-dependent fashion, and THP deficiency results in greater bacterial numbers in mouse bladders, supporting that THP glycans are protective against UPEC infection in the bladder [88–90] (Figure 1c). Light microscopy or cryoelectron tomography analysis of urine from UTI patients infected with several uropathogens, including UPEC, reveals multiple bacterial aggregates associated with and surrounded by THP filaments, which even display clear contacts with pilus tips [87]. Consequently, changes in the glycosylation of THP may make the human urinary tract more permissive to infection. For instance, patients with type I diabetes have lower amounts of sialic acid in urinary THP compared with healthy volunteers, which may contribute to the higher risk of UTI among diabetic patients [91].

In addition to type 1 pili and P pili, which are associated with UPEC infection of the bladder and kidneys, respectively, another type of filamentous adherence structure, called S pilus, mediates binding in the urinary tract [34,92]. Whereas type 1 and P pili recognize and bind mannose and galactose disaccharides, respectively, S pili specifically adhere to sialic acids capping terminal galactosides [NeuAc(α2,3)Gal], which are found in the branches or antennae of THP N-glycans [34]. Consequently, THP may be protective against kidney infections by S-piliated UPEC as renal luminal cells of the distal tubules are also enriched with surface oligosaccharides containing NeuAc(α2,3)Gal [34]. However, the protective role of THP against S-piliated UPEC needs verification because the binding of THP by the adhesin of S pili has not been experimentally demonstrated, and the functional

role of S pili during UTI remains unclear [92]. Interestingly, atomic force microscopy experiments with an S-piliated UPEC strain reveal that the helix-like structure of the S pili shaft has the most rapid relaxation kinetics compared with type 1 and P pili, indicating that when pili unravel to absorb shear stress forces, such as urine flow, S pili are the first to recover their initial quaternary structure [92]. A faster relaxation of the rod may reduce the load exerted on the apical adhesin, potentially extending the duration of adhesion to the sialic acid-rich urothelium of the upper urinary tract, thereby favouring the ascension of S-piliated UPEC to the kidneys. Therefore, future glycobiology studies are needed to test the putative protection provided by THP against infection with S-piliated UPEC strains in vivo, as well as determine binding properties and the role of the S pilus during bladder and kidney infections.

Infected mice lacking THP have not only increased bacterial counts in the urine and bladder, but also elevated mortality that may be due to an exaggerated inflammatory response [89,90]. Indeed, significantly increased neutrophil numbers are present in the urine of THP-null mice 24 h after instillation of PBS into the bladder [93]. Greater neutrophil infiltration may be attributable to THP glycans as the surface cell protein sialic acid-binding immunoglobulin-type lectin-9 (Siglec-9, Siglec-E in mice) in human neutrophils directly binds to the structure Neu5Ac(α2,3)Gal(β1,4)GlcNAc on THP to decrease reactive oxygen species ex vivo [93]. These studies provide evidence to suggest that THP glycans not only directly protect against UTI as decoys for bacterial receptors, but may also regulate immunity to UTI by blunting the activation of infiltrating immune cells, such as neutrophils, where an exaggerated antimicrobial response may impair urothelial barrier integrity. Nonetheless, whether inhibition of neutrophil oxidative burst via THP-mediated engagement of Siglec-9/Siglec-E receptors has consequences on UTI needs to be verified in vivo. Altogether, the above studies highlight how integral THP is to bladder immunity and support a renewed translational research interest in THP glycosylation to explore its underappreciated barrier and immunomodulatory roles for clinical applications.

TRANSLATIONAL GLYCOBIOLOGY IN UTI: HOW TO SWEET TALK UPEC

More than 20 years ago, the finding that a FimH null mutant of UPEC had impaired colonization and induced less inflammation in mouse bladders, together with the widespread expression of FimH in UPEC strains, supported the idea that a FimH-targeted vaccine may provide positive therapeutic outcomes [35,94,95]. While this idea has not entirely borne out, as these vaccines have only shown preclinical efficacy

[95], understanding the glycobiology of UPEC infection and immunity may offer additional opportunities to target FimH and other glycan-dependent interactions in the clinic [96,97]. Administration of a mixture of selective sugar antagonists for UPEC adhesins via a catheter may be an inexpensive strategy to significantly decrease the risk of developing UTI [98]. By passively targeting invading uropathogens, sugar-derived drugs or glycomimetics have the potential to treat UTI and prevent recurrence regardless of most host-dependent factors.

As the mannose-specific recognition of FimH adhesin is well established, and nearly all UPEC strains express type 1 pili, many glycobiology studies focus on blocking this crucial interaction [41,96,99]. The earliest examples are soluble monosaccharides, such as D-mannose, which block lectin-mediated adhesion of uropathogens to urothelial cells in vitro [100]. Promisingly, daily oral intake of D-mannose supplements has the same efficacy as the antibiotic nitrofurantoin in reducing the frequency of infection in women with recurrent UTI [101]. Addition of D-mannose to UPEC incubated with human THP did not alter UPEC association with THP filaments or THP-mediated UPEC clumping in vitro [87], suggesting that prophylactic administration of D-mannose to patients with recurrent UTI may synergize with urinary THP to antagonize UPEC adhesion. To further test its therapeutic potential, an ongoing double-blinded placebo-controlled clinical trial in the UK has recruited 598 women to test whether daily intake of D-mannose reduces the rate of UTI recurrence within a 6-month period [102].

Aromatic alpha-mannosides, molecules containing a mannose linked to a benzene group, are a promising alternative to D-mannose due to their comparatively increased affinity for the mannose-binding domain of FimH [96]. Rationally designed, high-affinity mannoside antagonists of FimH block UPEC adhesion to cognate urothelial carbohydrates, preventing UPEC invasion [97,98]. Oral treatment with the high-affinity FimH antagonist mannoside M4284 reduces UPEC burden in the colon, bladder, and kidneys of mice intentionally colonized with UPEC in both the gut and urinary tract, compared with D-mannose control treatment [103]. This study demonstrates that mannosides not only treat UTI but may reduce the risk of new episodes by depleting tissue-associated UPEC reservoirs. Oral administration of a mannoside-containing compound to mice shortly before and after UPEC instillation, respectively mimicking prophylaxis and treatment, significantly reduces early colonization levels in the bladder, demonstrating that sugar-derived drugs have the potential to treat UTI [98]. Mannoside antagonists further reduce bacterial numbers in mouse bladders when administered in combination with a standard antibiotic regimen for UTI compared with either treatment alone, suggesting that sugar-based drugs may enhance bacterial killing by UPEC sequestration in urine. Therefore, glycan-based drugs could reduce the use of antibiotics, which will be of utmost

importance as UPEC strains exhibit diverse and widespread antibiotic resistance [5].

Based on promising experimental and clinical evidence collected so far, sugar-based mannose-specific FimH antagonists may be a safe and inexpensive drug to treat cystitis. Although their efficacy may not be as potent as antibiotics, carbohydrate-based anti-adhesives are not expected to select for resistance genes or have detrimental side effects on the microbiota, thus arguing in favour of their use for treatment of UTI and prophylaxis against recurrence [104]. Overall, promoting the prophylactic use of effective antimicrobial glycan-based therapeutics as an alternative to antibiotics, as well as determining the consequences of their long-term use, could be an efficient approach to limit the impact of antimicrobial resistance.

Learning from the successful clinical translation of mannose-based therapeutics for treatment of cystitis, future glycobiology studies may consider whether galactose-rich bioactive compounds that target PapG adhesins and are retained in the kidneys could inhibit the severity of pyelonephritis. For translational studies, some caution should be taken when using animal models. Basic glycosylation pathways are conserved in mammals, but some differences can be found between humans and common laboratory species. Understanding these structural differences in GAGs, glycoproteins, and glycolipids would facilitate the design of glycan-based drugs. Glycan structural complexity and diversity are major obstacles for the translation of basic discoveries to glycan-based therapeutics. Structural analysis relies on mass spectrometry or nuclear magnetic resonance spectroscopy, which are time-consuming, expensive and require large amount of biological material. However, recent breakthroughs in the field [105], such as novel arraying methods and improved carbohydrate synthesis, may foster translational glycobiology in the next years.

FINAL REMARKS

In conclusion, carbohydrates play a prominent role in host invasion and response during UTI. Following UPEC infection, FimH-mediated mannose binding induces adhesion and invasion of the urothelium, which is aided by bacterial capsular polysaccharides. However, host GAGs and proteoglycans protect the urothelium from bacterial adhesion, thereby reducing bacterial invasion. In addition, secreted, highly glycosylated glycoproteins, such as THP, inhibit UPEC attachment by binding to FimH or other bacterial adhesion proteins to reduce bacterial burden. Lectin antagonists show encouraging results in the treatment of UTI, revealing a promising horizon for translational glycobiology and supporting that the investigation of glycans involved in UTI will drive the discovery of targets for non-antibiotic therapies based on sugars.

CONFLICT OF INTEREST
The authors declare that no conflict of interest exists.

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