The adherence of uropathogenic Escherichia coli to the urothelial surface, a critical first step in the pathogenesis of urinary tract infection (UTI), is controlled by three key elements: E. coli adhesins, host receptors, and host defense mechanisms. Although much has been learned about E. coli adhesins and their urothelial receptors, little is known about the role of host defense in the adherence process. Here we show that Tamm-Horsfall protein (THP) is the principal urinary protein that binds specifically to type 1 fimbriated E. coli, the main cause of UTI. The binding was highly specific and saturable and could be inhibited by N-mannose and abolished by endoglycosidase H treatment of THP, suggesting that the binding is mediated by the high-mannose moieties of THP. It is species-conserved, occurring in both human and mouse THPs. In addition, the binding to THP was much greater with an E. coli strain bearing a phenotypic variant of the type 1 fimbrial FimH adhesin characteristic of those prevalent in UTI isolates compared with the one prevalent in isolates from the large intestine of healthy individuals. Finally, a physiological concentration of THP completely abolished the binding of type 1 fimbriated E. coli to uroplakins Ia and Ib, two putative urothelial receptors for type 1 fimbriae. These results establish, on a functional level, that THP contains conserved high-mannose moieties capable of specific interaction with type 1 fimbriae and strongly suggest that this major urinary glycoprotein is a key urinary anti-adherence factor serving to prevent type 1 fimbriated E. coli from binding to the urothelial receptors.

The adhesion of Escherichia coli, the most common cause of urinary tract infection (UTI), to urothelial cells is a crucially important first step in UTI pathogenesis (1–5). This adhesion process frequently requires filamentous surface appendages of uropathogenic E. coli that are called fimbriae, or pili. Epidemiological studies have shown that >90% of all E. coli isolates from UTI patients elaborate type 1 fimbriae (also named mannosensitive fimbriae) (4, 6, 7). Although controversies exist for several years, recent investigations have unequivocally documented the importance of type 1 fimbriae as a major urovirulence factor. For instance, of the nine most common E. coli virulence factors, the genes for type 1 fimbriae emerged as the only trait common in all 203 UTI isolates examined (8). In addition, 26% of the 203 strains were positive only for type 1 fimbrial genes and were negative for the eight other urovirulence factors tested, including P, S, and Dr fimbriae. In experimental mouse models, type 1 fimbriae were shown to be indispensable for bladder colonization and infection (9). Conversely, systemic immunization of mice with the FimH tip adhesin of type 1 fimbriae reduced bladder colonization of E. coli by 99% even in neutropenic mice, suggesting that blocking type 1 fimbriae could completely abolish E. coli adhesion (10). Further evidence supporting an important role of type 1 fimbriae in UTIs has come from the identification of phenotypic variants of the type 1 fimbrial FimH adhesin. On the basis of the receptor binding specificity for monomannose residues, type 1 fimbriae can be divided into the low monomannose-binding variant (ML) and the high monomannose-binding variant (MH) (4, 11–13). Interestingly, the ML phenotype predominates in the large intestine, whereas the MH phenotype predominates in the UTI isolates, suggesting a selective advantage for certain subtypes of type 1 fimbriated E. coli in the urinary tract (14). These recent results, along with numerous previous studies demonstrating the direct binding between type 1 fimbriae and the urothelium (15–17), clearly established the functional importance of type 1 fimbriae in UTI.

The established importance of type 1 fimbriae in urovirulence prompted us to search for their urothelial receptors. The apical surface of mammalian urothelia is covered by numerous rigid-looking plaques whose luminal leaflets are twice as thick as the cytoplasmic ones, hence the name asymmetric unit membrane (AUM) (18–20). Since AUM constitutes >90% of the luminal surface of the urinary tract, including the proximal urethra, bladder, ureters, and renal pelvis, we explored the role of AUM in E. coli adherence. Using sucrose gradient followed by detergent washing, we isolated milligram quantities of highly purified AUMs (21, 22). Such isolated AUMs contain four major integral membrane proteins that were designated as uroplakins Ia (27 kDa), Ib (28 kDa), II (15 kDa), and III (47 kDa) (21, 23, 24). Together, these four proteins form natural two-dimensional crystals arranged in hexagonal arrays (25–28). By generating antibodies to each of these proteins, we have

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The adhesion of E. coli to urothelial cells is a critical first step in the pathogenesis of urinary tract infection (UTI), controlled by three key elements: E. coli adhesins, host receptors, and host defense mechanisms. Although much has been learned about E. coli adhesins and their urothelial receptors, little is known about the role of host defense in the adherence process. Here we show that Tamm-Horsfall protein (THP) is the principal urinary protein that binds specifically to type 1 fimbriated E. coli, the main cause of UTI. The binding was highly specific and saturable and could be inhibited by N-mannose and abolished by endoglycosidase H treatment of THP, suggesting that the binding is mediated by the high-mannose moieties of THP. It is species-conserved, occurring in both human and mouse THPs. In addition, the binding to THP was much greater with an E. coli strain bearing a phenotypic variant of the type 1 fimbrial FimH adhesin characteristic of those prevalent in UTI isolates compared with the one prevalent in isolates from the large intestine of healthy individuals. Finally, a physiological concentration of THP completely abolished the binding of type 1 fimbriated E. coli to uroplakins Ia and Ib, two putative urothelial receptors for type 1 fimbriae. These results establish, on a functional level, that THP contains conserved high-mannose moieties capable of specific interaction with type 1 fimbriae and strongly suggest that this major urinary glycoprotein is a key urinary anti-adherence factor serving to prevent type 1 fimbriated E. coli from binding to the urothelial receptors.
demonstrated that all these proteins are urothelium-specific, are confined to the apical surface of the urothelium (21, 29), and are highly conserved morphologically and biochemically in all these mammalian species (22). Using an in vitro adherence system, we recently showed that type 1 fimbriated \textit{E. coli} can bind to uroplakins Ia and Ib, two major high mannnose-type glycoproteins of apical urothelial plaques (30). The binding is highly specific, saturable, and species-conserved and can be inhibited by D-mannose. We further showed that the alllic variants of type 1 fimbriated \textit{E. coli} that are prevalent in UTIs, but not those prevalent in feces, bind to the uroplakins (14).2 These results strongly suggest that the uroplakins can serve as the urothelial receptors for type 1 fimbriae and that these receptors can provide a selective advantage for certain \textit{E. coli} strains to survive in the urinary niche. Our in vivo data showing the functional interaction between type 1 fimbriated \textit{E. coli} and uroplakins have been recently confirmed morphologically by an in vivo infection model. Using quick-freeze and deep-etch electron microscopy of infected mouse bladders, Mulvey et al. (31) demonstrated that the tip of type 1 fimbriae interacted directly with the central depression (3.7 nm in diameter) of hexagonal uroplakin particles. This finding parallels our previously proposed structural model of uroplakin particles in which either uroplakin Ia or Ib, the two in vitro urothelial receptors for type 1 fimbriae, occupies the inner ring surrounding the central depression of hexagonal uroplakin particles (32). Together, these in vitro and in vivo data strongly indicate that uroplakins Ia and Ib can serve as the major urothelial receptors for type 1 fimbriae.

Despite these developments, little attention has been paid to the host defense factors that may interfere with \textit{E. coli} adhesion to the urothelial receptors. Previous studies suggested that Tamm-Horsfall protein (THP; also named uromodulin), the most abundant protein in mammalian urine, can bind to type 1 fimbriated \textit{E. coli}, thus implying a role for THP in urinary defense (33–36). These studies were, however, not performed in the context of well defined \textit{E. coli} strains or in the presence of the cognate urothelial receptors. It was also unclear whether THP possesses a single, well conserved, high-mannose chain that is necessary for type 1 fimbrial binding (37, 38) and whether soluble urinary THP is capable of interacting at all with the fimbriae (39). There have also been questions regarding the specificity of the THP-\textit{E. coli} interaction as non-mannose-specific P fimbriae were found to bind THP (39). In addition, it remains uncertain what the relative contribution of THP would be to urinary defense compared with other urinary proteins. Finally, it is unclear how THP interacts with the two recently identified major phenotypic FimH variants (14). We have undertaken this study to address some of these questions and to examine the potential role of THP in host urinary tract defense.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Culture, and Metabolic Labeling**—The fimbrial expression of clinical and recombinant \textit{E. coli} strains was determined by yeast aggregation and hemaggulination as previously described (14, 30). Thus, J96 (O4, K6), a human pyelonephritis isolate, expresses both type 1 (MH variant of FimH) and P (PapG1 and PapG3) fimbriae (34, 40). P678-54, a minicell-producing \textit{E. coli} K12 derivative, expresses no FimH but can express the high mannose-binding variant (ML), respectively (14). KB18, a negative control strain, expresses a nonfunctional FimH mutant.

All strains were cultured in Luria-Bertani medium at 37 °C for 16 h in methionine- and cysteine-free Dulbecco’s modified Eagle’s medium (glucose-free; Life Technologies, Inc.) for 2 h and then in Dulbecco’s modified Eagle’s medium containing 0.58 mM NaCl and solubilization and dialysis in distilled water (43). Mouse THP was purified from pooled fresh urine of BALB/c and 129/svj strains using the same method. The yield and purity of THP were assessed by SDS-PAGE followed by silver nitrate staining (see below), and both human and mouse THPs migrated as a single band.

AUM was purified from bovine urinary bladders by first isolating total urothelial membranes with gradient centrifugation, and then treating them with 2% Sarkosyl followed by 25 mM NaOH (21, 22). AUM was quantified using bicinchoninic acid reagent (Pierce) in the presence of 1% SDS. On SDS gel, purified AUM contained four major proteins: 27-kDa uroplakin Ia, 28-kDa uroplakin Ib, 15-kDa uroplakin II, and 47-kDa uroplakin III (21, 24, 29, 44).

**Binding Assays**—For the bacterial overlay assay, total urinary proteins or purified THP was resolved by SDS-PAGE (15% acrylamide) onto nitrocellulose membrane, and reacted with [35S]methionine-labeled \textit{E. coli} strains reconstituted in 3% bovine serum albumin and 0.1% NaNO3 in PBS. After washing in PBS, the binding was visualized by autoradiography.

For the microtiter well binding assay, purified THP was dissolved in distilled water and incubated in 96-well polystyrene microtitre plates at room temperature for 30 min and at 4 °C overnight. After washing, the microtiter wells were blocked with 3% bovine serum albumin in PBS for 2 h, and immobilized THP was then incubated with [35S]methionine-labeled \textit{E. coli} strains reconstituted in 3% bovine serum albumin and 0.1% NaN3 in PBS for 2 h. After washing four times in PBS, the bound bacteria were dissolved in 1% SDS and quantified using scintillation counting. All binding was performed in triplicate.

**Deglycosylation of THP**—Purified human THP was reduced and S-carboxymethylated according to van Rooijen et al. (45). THP was dissolved in 1 m Tris-HCl (pH 8.25) containing a final concentration of 50 μg/ml iodoacetic acid (Sigma) and incubated in 0.02% formaldehyde (46) for 30 min in the dark for 2 h. After incubation at 37 °C for 2 h, the mixture was supplemented with iodoacetic acid (Sigma) to a final concentration of 100 μM. The reaction was performed in the dark for 30 min and was stopped by the addition of 200 μM β-mercaptoethanol followed by dialysis against distilled water. For deglycosylation, reduced and carboxymethylated THP was digested with endoglycosidase H (0.05 units) or with N-glycosidase F (2500 units) in 50 mM phosphate buffer containing 0.5% SDS, 1% Nonidet P-40, 1% β-mercaptoethanol, 10 mM EDTA, and 0.05% NaN3 at 37 °C for 16 h.

**Silver Nitrate Staining and Western Blotting**—After electrophoresis, the polyclonal antibody was prefixed with 50% methanol and 7% acetic acid and then incubated with 10% glutaraldehyde for 30 min. After extensive washing with distilled water, the gel was exposed to a solution containing 20% silver nitrate, 0.4% NaNO3, 0.1% NH4OH, and 2% ethanol for 6 min. The gel was washed with distilled water for 1 h and developed in a solution containing 0.005% citric acid, 0.02% formaldehyde, and 10% ethanol. The reaction was stopped by incubating the gel with 10% acetic acid. For Western blotting, proteins resolved by SDS-PAGE were electrophoretically transferred onto nitrocellulose membrane and incubated first with an anti-human THP polyclonal antibody (BIODESIGN International) and then with a secondary antibody conjugated with peroxidase. The membrane was developed in a diamobenidine/H2O2 solution.

**RESULTS**

**Identification of Urinary Proteins That Bind to Type 1 Fimbriated \textit{E. coli}**—To identify urinary proteins that can potentially serve as defense factors against \textit{E. coli} adherence, we examined the interaction between type 1 fimbriated \textit{E. coli} and total urinary proteins using the gel overlay assay. Pooled fresh urine samples from healthy male donors were immediately denatured and reduced in SDS/β-mercaptoethanol solution to

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2 J. Pak, D. L. Hasty, and X.-R. Wu, unpublished data.
minimize protein degradation and aggregation and subsequently analyzed by SDS-PAGE. Silver nitrate staining revealed two major protein species at 90 and 65 kDa, along with many minor proteins at a lower molecular mass range (Fig. 1A). When duplicate samples resolved by SDS-PAGE were electrotransferred onto nitrocellulose membrane and reacted with [35S]methionine-labeled type 1 fimbriated E. coli (strain SH48, MH type), the bacteria reacted specifically with the 90-kDa protein band, with very little binding to any other urinary proteins (Fig. 1B). On the basis of the predominance and the molecular mass range, we speculated that the 90-kDa protein was THP (46). This was proven to be the case, as immunoblotting using a polyclonal antibody raised against human THP specifically reacted with the 90-kDa band (Fig. 1C). The fact that as little as 20 µl of the unconcentrated urine contained sufficient amounts of THP to bind a detectable number of type 1 fimbriated E. coli cells suggested that this protein is the major urinary protein that can potentially block E. coli adherence to urothelial receptors (see below).

**Binding of Two Major Phenotypic Variants of Type 1 Fimbriae to Purified THP**—It has been recently documented that two major phenotypic variants exist for type 1 fimbriae based on their binding specificity for the monomannose residues: the low monomannose-binding (ML) and high monomannose-binding (MH) variants (14). They predominate in different niches, with 80% of fecal E. coli expressing the ML phenotype and >70% of UTI isolates expressing the MH phenotype. A previous in vitro adherence assay showed that the MH (UTI) variant bound to the purified urothelial plaques containing uropakinins Ia and Ib, two putative urothelial receptors for type 1 fimbriae, in significantly greater numbers than the ML (focal) variant (14). This provides an explanation for the selective advantage of the urothelium for the MH variant and raises the interesting possibility that the two variants might bind differentially to THP (47).

To further study this possibility, we performed an in vitro adherence assay to test the binding between a panel of E. coli strains and purified human THP. THP, shown to be a single species by silver nitrate staining (see Figs. 4A and 6A), was immobilized on microtiter wells and incubated with equivalent numbers of each [35S]methionine-labeled E. coli strain (Fig. 2). The first experiment tested isogenic strains representing the two major phenotypic variants of type 1 fimbriae. Although KB91 and KB54, which express the ML and MH adhesins, respectively, aggregated Saccharomyces cerevisiae equally well (data not shown), KB54 bound to THP four times better compared with KB91. This result suggests that it is the unmodified terminal mannose, most likely a moiety within the high-mannose residues, but not the bulky complex-type sugars, of THP that is responsible for the binding. Not surprisingly, the negative control strain, KB18, which expresses a nonfunctional mutant adhesin, showed little binding (Fig. 2A). The second experiment examined strains generated in the E. coli P678-54 background, with SH48 expressing type 1 fimbriae (MH type), HU849 expressing PapG1-type P fimbriae, IA2 expressing PapG2-type P fimbriae, and P678 expressing no fimbriae. High-level binding to THP was observed only with the type 1 fimbriated SH48 strain, with no significant binding of the P or non-fimbriated E. coli strains (Fig. 2B). With both sets of strains, the binding to bovine serum albumin was consistently at a background level. These results strongly suggest that the binding of E. coli to THP is specific for type 1 fimbriated E. coli. THP preferentially binds to the MH variant of type 1 fimbriae, which is prevalent in UTIs, this lends further support that THP may play an important role in urinary tract defense.

**Characteristics of Type 1 Fimbrial Binding to THP**—To further examine the binding specificity between type 1 fimbriated E. coli and THP, we performed two saturation binding assays (Fig. 3). In the first, we incubated increasing numbers of [35S]methionine-labeled E. coli cells with immobilized THP. Again, of five strains tested, only type 1 fimbriated E. coli...
(SH48, MH type) bound strongly to immobilized THP. The binding of J96, which expresses both type 1 and P fimbriae, was most likely due to its type 1 fimbriae, as isogenic strains expressing P fimbriae (HU849 or Ia2) alone failed to bind. With both type 1 fimbria-expressing strains, the binding to THP was linearly proportional to the E. coli input and was saturable. H denotes FimH of type 1 fimbriae, and G1, G2, and G3 denote the three PapG adhesins of P fimbriae. B, a fixed amount of 35S-labeled type 1 fimbriated E. coli (5 × 10^6 cpm) was incubated with increasing amounts of immobilized THP. Note the saturable binding of E. coli to THP.

To directly establish that the binding between type 1 fimbriated E. coli and THP was mannose-mediated, we carried out a gel overlay assay in the presence of various synthetic carbohydrates (Fig. 4, A–C). Binding could be inhibited in a concentration-dependent manner by s-mannose (Fig. 4B), but not by s-galactose (Fig. 4C). In addition, deglycosylation of THP using endoglycosidase H (Fig. 4D–F), which specifically cleaves the high-mannose residues, completely abolished the binding of type 1 fimbriated E. coli to THP (Fig. 4E). Moreover, type 1 fimbriated E. coli was found to bind equally well to equal moles of immobilized THP and bovine RNase B, the latter of which is known to possess a single high-mannose chain (48). These results establish that human THP contains the high-mannose moiety responsible for the binding of type 1 fimbriated E. coli (Figs. 4 and 5).

To determine whether the high-mannose moiety of THP is conserved in different species, we isolated THP from mouse urine using a protocol designed for isolating human THP. A total of 30 ml of pooled mouse urine yielded ~3 mg of THP (0.1 mg/ml), a concentration comparable to that of human urinary THP. THP from two different mouse strains (BALB/c and 129/svj) had a similar yield and purity, and both samples showed a slightly higher molecular mass compared with human THP (Fig. 6A). This might be related to the fact that the mouse THP sequence contains two more Asn-linked glycosylation consensus sites and therefore is more heavily glycosylated by complex-type moieties. Microtiter well assays showed that type 1 fimbriated E. coli bound equally well to both human and mouse THPs (Fig. 6B), indicating that the high-mannose glycosylation of THP is evolutionarily conserved.

Competitive Inhibition of Type 1 Fimbrial Binding to Uroplakin Ia by Soluble THP—To determine whether THP can inhibit the binding of type 1 fimbriated E. coli to the uroplakin Ia and Ib urothelial receptors, we performed an in vitro adherence interaction.

**Fig. 3.** Saturation binding of E. coli to purified human THP. A, a fixed amount (1 μg) of immobilized THP was incubated with increasing amounts of 35S-labeled E. coli as indicated. Note that only the FimH-expressing type 1 fimbriated E. coli strains (SH48 and J96) bound to THP. Also note that the binding was linearly proportional to the E. coli input and was saturable. H denotes FimH of type 1 fimbriae, and G1, G2, and G3 denote the three PapG adhesins of P fimbriae. B, a fixed amount of 35S-labeled type 1 fimbriated E. coli (5 × 10^6 cpm) was incubated with increasing amounts of immobilized THP. Note the saturable binding of E. coli to THP.

**Fig. 4.** Binding of type 1 fimbriated E. coli (SH48, MH type) to THP is mediated by high-mannose moieties of THP. A, purified THP shown as a single band by SDS-PAGE and silver nitrate staining (lane 1) reacted with anti-THP antibody upon immunoblotting (lane 2). B, gel-resolved THP was electrotransferred onto nitrocellulose and incubated with 35S-labeled type 1 fimbriated E. coli in the absence (lane 1) or presence of increasing concentration of β-mannose (lanes 2–7, 0.0001, 0.001, 0.01, 0.1, 1, and 10%, respectively). C, shown are the results of E. coli binding to THP in the presence of β-galactose (lanes 1–3, 0.1%, 1%, and 10%, respectively). Note that E. coli binding to THP was inhibited by β-mannose, but not by β-galactose. D, purified human THP was treated with buffer only (lane 1), endoglycosidase H (lane 2), or N-glycosidase F (lane 3); resolved by SDS-PAGE; and immunoblotted with anti-THP antibody. Note the slight decrease in molecular mass with endoglycosidase H treatment and the larger decrease in molecular mass with N-glycosidase F treatment. E and F, duplicated nitrocellulose blots were reacted with the type 1 fimbriated SH48 strain and the non-fimbriated P678-54 control strain, respectively. Note the complete loss of reactivity of deglycosylated THP with type 1 fimbriated E. coli (E, lanes 2 and 3).
E. coli binding to different substrata

**FIG. 5.** Comparative binding of type 1 fimbriated *E. coli* to immobilized THP and bovine RNase B. Note that 35S-labeled type 1 fimbriated *E. coli* bound equally well to equal moles (10 pmol) of THP and bovine RNase B (bRNase B), the latter of which contains a single high-mannose chain, indicating the high mannose-mediated binding of THP. The P value indicates the significance level of the binding to SH48 between the two proteins.

**FIG. 6.** Type 1 fimbriated *E. coli* binds to both human and mouse THPs. A, SDS-PAGE analysis of purified human and mouse THPs. THP was isolated from BALB/c (lane 1) and 129/65 (lane 2) mouse urine using the NaCl precipitation method; 1 μg of each sample was analyzed by SDS-PAGE and silver staining. Lane 1 is a human THP sample (control). Note that the molecular mass of mouse THP is slightly higher than that of human THP. B, comparative binding of *E. coli* strains to purified human and mouse THPs. 35S-Labeled isogenic *E. coli* strains expressing no fimbriae (None), type 1 fimbriae (Type 1: SH48, MH type), PapG1-type P fimbriae (P (G-1)), or PapG2-type P fimbriae (P (G-2)) (2 x 10^5 cpm/strain) were incubated with 1 μg of immobilized human or mouse THP. The number of *E. coli* cells bound to THP is indicated as (counts/min) x 10^-4. Note that type 1 fimbriated *E. coli* bound to THP isolated from both species equally efficiently, suggesting that the high-mannose residues in THP are highly conserved.

**FIG. 7.** THP blocks the binding of type 1 fimbriated *E. coli* (SH48, MH type) to uroplakins. A fixed amount of *E. coli* (2 x 10^6 colony-forming units/ml) was incubated, in the presence of increasing amounts of THP, with immobilized AUMs (2 μg/well). Note that the binding of type 1 fimbriated *E. coli* to AUMs was greatly inhibited by THP.

**DISCUSSION**

**Tamm-Horsfall Protein as a Major Urinary Defense Factor: A Working Model**—Although direct interaction between bacterial adhesins and host receptors is critical for bacterial adherence to host epithelial cells, almost all mucosal epithelial cells are naturally resistant to bacterial adhesion due to potent host defense mechanisms (49–52). Much has been learned about such antibacterial defense mechanisms in the secretory epitelia of the respiratory and intestinal tracts. In these tissues, a powerful mucus secretion covers the epithelium, thus shielding the underlying receptors from invading bacteria (50). Less is known, however, about the biochemical basis of the anti-adhesion mechanisms of the urinary tract. Unlike other epithelia, the urothelium is not known to have a major secretory function because it lacks secretory goblet cells and glands and because the apical urothelial cells do not contain secretory granules, but are covered by rigid-looking plaques (AUM) that are seemingly incompatible with a secretory function (53, 54). Furthermore, quick-freeze and deep-etch microscopic studies of unperturbed mouse bladder surfaces showed that the uroplakins, the putative receptors for type 1 fimbriae, are not covered by a detectable glycocalyx or mucus layer, but appear to be directly exposed to the urinary space (28). Similar observations were recently made using a murine infection model in which type 1 fimbriae appeared to bind directly to the naturally exposed uroplakin particles of the bladder luminal surface (31). The absence of detectable protective layers covering the uroplakin *E. coli* receptors of the mouse urinary bladder suggests that the urinary tract defense must rely largely on the physical forces imparted by micturition and, perhaps as important, by the soluble factors present in urine.

In this study, we presented several lines of evidence strongly implicating THP as the major urinary defense factor. These include the following. 1) THP is the major urinary protein that binds to type 1 fimbriated *E. coli*. 2) A strain expressing a UTI-prevalent phenotypic variant of type 1 fimbriae, but not one expressing a feces-prevalent variant, binds avidly to THP. 3) Type 1 fimbriated *E. coli*, but not P fimbriated *E. coli*, binds to THP in a saturable and mannose-dependent manner. 4) Type 1 fimbriated *E. coli* binds equally well to THP and to bovine RNase B, the latter of which is known to possess a single high-mannose chain. 5) The high-mannose moiety of THP is evolutionarily conserved. 6) At physiological levels, THP completely abolishes the binding of type 1 fimbriae to the urothelial receptors. These results strongly suggest that THP, the most abundant glycoprotein in mammalian urine, may serve as a
Highly conserved high-mannose residues, to type 1 fimbriated E. coli. This binding can effectively block the attachment of the glycan by 1H NMR spectroscopy (57). The relative proportion of high-mannose to polyantennary complex moieties was measured by isolating chemical (50 mg) amounts of high-mannose glycopeptides. Serafini-Cessi et al. (56) separated a high-mannose glycopeptide from the THP digest and analyzed its oligosaccharides by endoglycosidase digestion and thin-layer chromatography. They showed that native urinary THP bears one unprocessed high-mannose chain. Other investigators have identified high-mannose glycan not only from human THP, but also from bovine THP, indicating the highly conserved structure of THP. However, proteolytic digestion of human urinary THP consistently yielded high mannose-bearing glycopeptides. Serafini-Cessi et al. (56) previously found that the MH variant is a better colonizer in the bladder and why this variant predominates in UTI isolates. It is therefore of interest to note that the binding of the two FimH variants to THP is different, favoring the MH variant. Although this seems to be paradoxical, the phenomenon can be readily explained from an evolutionary standpoint, as the selective advantage of the urinary tract for the MH variant will likely be countered by an effective defense mechanism, specifically the modification of THP by the high-mannose residues. The intricate balance among E. coli adhesions, urothelial receptors, and urinary tract defense factors, both on quantitative and qualitative levels, may well determine whether an infection occurs. For example, when urothelial receptor expression and host defense factors are within normal range, E. coli inoculum will be a critical factor. Similarly, if host urinary defense is compromised, even a small E. coli inoculum could cause infection. By the same token, overexpression of urothelial receptors, which could conceivably occur in certain disease states, could increase the chance of E. coli bladder colonization. Further studies to better define each of these conditions in humans should no doubt enhance our understanding of how multiple factors interact in contributing to the pathogenesis of UTIs.

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