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FUNGAL AND PARASITIC INFECTIONS

Tamm-Horsfall Protein Protects the Urinary Tract against *Candida albicans*

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ABSTRACT Urinary tract infections (UTIs) caused by the human fungal pathogen *Candida albicans* and related species are prevalent in hospitalized patients, especially those on antibiotic therapy, with indwelling catheters, or with predisposing conditions such as diabetes or immunodeficiency. Understanding of key host defenses against *Candida* UTI is critical for developing effective treatment strategies. Tamm-Horsfall glycoprotein (THP) is the most abundant urine protein, with multiple roles in renal physiology and bladder protection. THP protects against bacterial UTI by blocking bacterial adherence to the bladder epithelium, but its role in defense against fungal pathogens is not yet described. Here we demonstrate that THP restricts colonization of the urinary tract by *C. albicans*. THP binds to *C. albicans* hyphae, but not the yeast form, in a manner dependent on fungal expression of the Als3 adhesion glycoprotein. THP directly blocks *C. albicans* adherence to bladder epithelial cells *in vitro*, and THP-deficient mice display increased fungal burden in a *C. albicans* UTI model. This work outlines a previously unknown role for THP as an essential component for host immune defense against fungal urinary tract infection.

KEYWORDS Als3, *Candida albicans*, Tamm-Horsfall protein, urinary tract infection

Urinary tract infections (UTIs) are a common hospital-acquired infection associated with increased morbidity and cost of care (1). UTI incidence by the fungal pathogen *Candida albicans* and related species ranges from 6.5% to 22% depending on the population and location studied, with the highest prevalence in hospitalized patients with predisposing factors, such as indwelling catheters, antibiotic treatment, diabetes, and immunodeficiency, some of which have been reflected in murine models (2–6). Remarkably, the clinical significance of *Candida* in the urine (candiduria) is frequently unclear. While patients with symptomatic cystitis and invasive renal infection display candiduria, asymptomatic bladder colonization or even sample contamination can occur, making candiduria a poor diagnostic factor for determining appropriate treatment (6, 7). Candiduria is self-resolving in many patients when the driving factors are eliminated, such as catheter removal or cessation of antibiotic treatment; however, a subset of patients develop persistent infection that is refractory to antifungal treatment (8). While several studies indicate that intensive care unit (ICU) patients with candiduria have higher morbidity and mortality rates than those with sterile urine, data interpretation is confounded by comorbidities (3, 5). Currently, incomplete clinical understanding and absence of diagnostic tools hamper differentiation of candiduria that necessitates treatment from candiduria as a result of benign colonization. Indeed, 30% to 70% of healthy adults are colonized with *Candida* in the gut and vaginal tract (9, 10) and successfully prohibit *Candida* dissemination to the urinary tract through largely unknown mechanisms.

Renal access by *Candida* can occur either through the bloodstream (hematogenous)

Received 8 June 2018 **Returned for modification** 3 July 2018 **Accepted** 1 October 2018

Accepted manuscript posted online 8 October 2018

Citation Coady A, Ramos AR, Olson J, Nizet V, Patras KA. 2018. Tamm-Horsfall protein protects the urinary tract against *Candida albicans*. Infect Immun 86:e00451-18. https://doi.org/10.1128/IAI.00451-18.

Editor George S. Deepe, University of Cincinnati

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or by ascension from a focal colonization of the ureter (retrograde). While renal infection (pyelonephritis) via bloodstream seeding has been well characterized, the factors driving the pathogenesis of retrograde infection are not as clear. Early studies with human urothelial cells and rat bladder epithelial cells suggest that *Candida* yeasts adhere less readily to bladder epithelial cells than other epithelial cells (11, 12), although a rabbit bladder explant model demonstrated that *Candida* hyphae adhere more avidly than yeasts (13). Certain urine conditions, such as glucosuria, increase susceptibility to *Candida* UTI (14). Healthy mice (like humans) are generally resistant to ascending infection, suggesting a strong conserved mechanism preventing *Candida* infection in this host niche.

Tamm-Horsfall glycoprotein (THP), the most abundant protein in mammalian urine, is produced exclusively by the kidneys and plays a significant role in kidney function, including sodium reabsorption, formation of kidney stones, and protection against tubulointerstitial nephritis (15, 16). Also known as uromodulin, THP modulates responses of a variety of immune cells, including T cells, monocytes, and neutrophils (17–20). In the context of bacterial UTIs, THP directly binds multiple uropathogenic bacteria, including *Escherichia coli* (UPEC), *Proteus mirabilis*, and *Klebsiella pneumoniae*, preventing bacterial adherence to the bladder epithelium (21–24). However, in certain contexts, THP can instead promote bacterial infection. For instance, in catheterized patients, THP aggravates *Pseudomonas aeruginosa* UTI by coating catheter material and promoting the adherence and subsequent growth of bacteria on the catheter (25, 26). While mice deficient in THP have confirmed an important role for THP in preventing bacterial UTIs (21, 22), whether THP also modulates fungal colonization of the urinary tract is unknown.

In this study, we investigated the role of THP in controlling *C. albicans* colonization of the urinary tract. We found that mice deficient in THP are more susceptible to *C. albicans* UTI and that THP binds to *C. albicans* hyphae but not yeasts, preventing adherence to bladder epithelial cells. Contrary to THP engagement of *E. coli*, *C. albicans* binding is independent of THP glycosylation; rather, THP binding depends on the major fungal adhesion protein agglutinin-like sequence 3 (Als3). Collectively, our results reveal a previously unknown role for THP in protecting against ascending *C. albicans* colonization of the urinary tract.

RESULTS

THP-deficient mice are more susceptible to *C. albicans* colonization. Given the central role of THP in both kidney function and resistance to bacterial UTIs, we sought to determine the function of THP during fungal infection of the urinary tract. To address this, we measured *C. albicans* colonization of wild-type (WT) and THP-deficient ($THP^{-/-}$) mice in a UTI model. Female mice, 2 to 4 months of age, were infected with 1×10^7 CFU of *C. albicans* strain SC5314, and urine was collected 24 h postinfection. $THP^{-/-}$ mice displayed increased fungal burden in the urine (median: 3,300 CFU/ml) compared to that in WT mice (median: 100 CFU/ml; $P = 0.0104$) (Fig. 1A). Moreover, infections in $THP^{-/-}$ mice persisted longer, with detectable CFU in the urine 3 days postinfection (median: 50 CFU/ml), a time when most WT mice had cleared the infection (median: 0 CFU/ml; Fisher's exact test, $P = 0.0572$) (Fig. 1B).

THP blocks *C. albicans* interaction with bladder epithelial cells. Adherence to epithelial cells is a critical component driving microbial colonization and infection of the urinary tract. In response to urinary bacteria, THP coats bacteria and limits colonization, promoting bacterial flushing from the urinary tract and preventing infection. To determine if THP imparts similar antagonism between *C. albicans* and the bladder epithelium, we measured *C. albicans* adherence after 45 min of incubation with immortalized human bladder epithelial cells (HTB-9) in the presence of physiological concentrations of THP (50 μg/ml). THP treatment reduced *C. albicans* adherence to bladder epithelium (median: 1.4×10^5 CFU) compared to that in untreated controls (median: 2.2×10^5 CFU; $P = 0.0079$) (Fig. 1C). THP was not directly antimicrobial under the assay conditions (see Fig. S1A in the supplemental material).

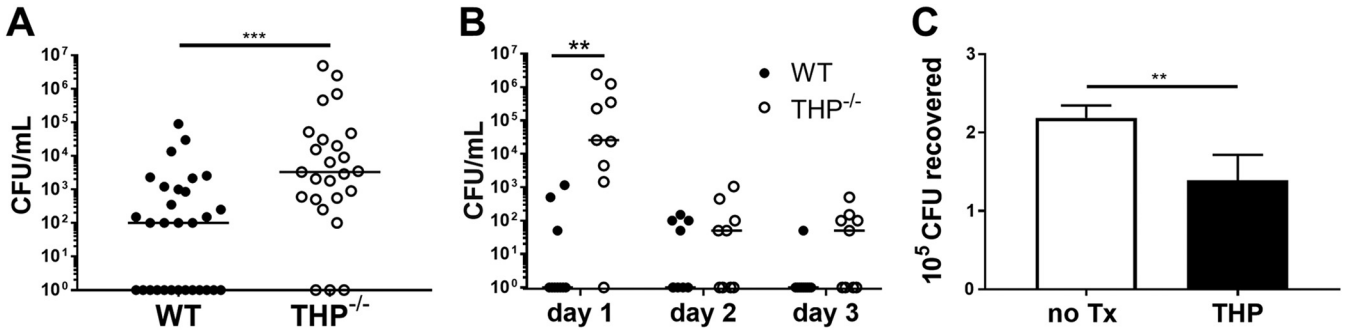

FIG 1 THP reduces *C. albicans* colonization of mice and adhesion to human bladder epithelial cells. WT and THP$^{-/-}$ mice were infected with 1×10^7 yeasts via transurethral inoculation. Twenty-four hours later, urine (A) was harvested and assessed for fungal growth. Circles represent individual mice, and lines represent the median for each group. Experiments were done three times independently and data combined ($n = 30$ WT mice and 25 THP$^{-/-}$ mice). (B) WT and THP$^{-/-}$ mice were infected as for panel A, and urine was harvested every day for 3 days and assessed for fungal growth. Experiments were performed once ($n = 10$ mice per group). (C) Adherence of *C. albicans* yeasts to human bladder epithelial cell line HTB-9 after treatment of HTB-9 cells with 50 μg/ml of THP. Data represent the means from 5 independent experiments performed in technical triplicate and are expressed as medians with interquartile ranges. Tx, treatment. Statistical analysis between experiments was performed using the Mann-Whitney test (A and C) or two-way ANOVA with Sidak's multiple-comparison test (B). Significant comparisons are indicated; all others are not significant. **, $P < 0.01$; ***, $P < 0.001$.

THP directly binds *C. albicans* hyphae. To determine if THP interacts directly with *C. albicans*, we measured binding of purified human THP (hTHP) to *C. albicans* strain SC5314 using a fluorescein isothiocyanate (FITC)-conjugated anti-hTHP antibody. Soluble THP (50 μg/ml) was incubated with *Candida* yeasts or hyphae, and binding was detected by flow cytometry. THP bound 79% (median) of *C. albicans* hyphae but only minimally bound the yeast form (1.23%) (Fig. 2A and B). To determine if THP-*Candida*

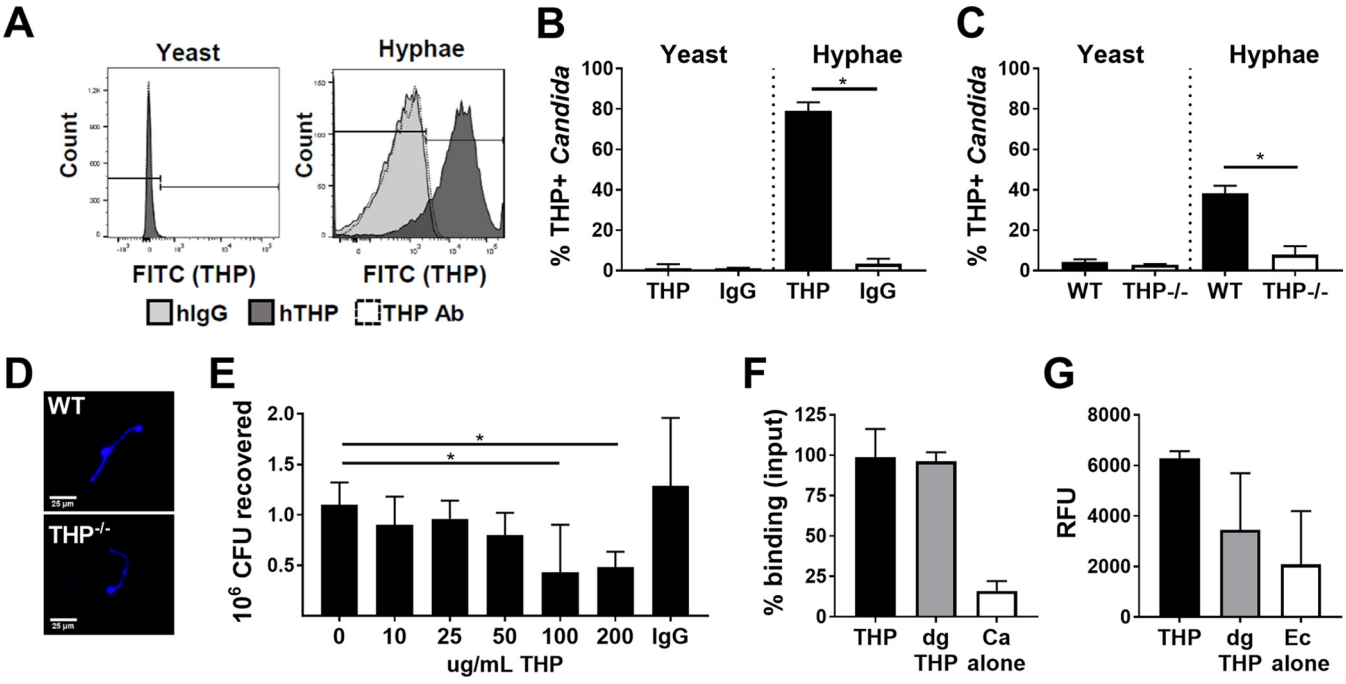

FIG 2 THP binds *C. albicans* hyphae but not yeasts, independent of THP glycosylation. THP binding to *C. albicans* yeasts and hyphae was assessed via flow cytometry. (A) Gating scheme for yeasts and hyphae; (B) quantification of flow cytometry data. (C) Hyphae were incubated in urine from WT or THP$^{-/-}$ mice and then assessed for THP binding via flow cytometry. (D) To confirm that hypha formation occurs *in vivo*, mice were infected via transurethral inoculation with 1×10^7 yeasts. Urine was expressed 4 h later, cytocentrifuged onto glass slides, and stained with calcofluor white. Yeast and hyphal structures were visualized via microscopy. Magnification, ×400. (E) Adherence of *C. albicans* hyphae to human bladder epithelial cell line HTB-9 after treatment of HTB-9 cells with the indicated concentrations of THP or 50 μg/ml of human IgG as a control. (F and G) Binding assay of *C. albicans* hyphae (F) and *E. coli* (G) with plate-bound human THP (hTHP), plate-bound hTHP treated with total deglycosylation enzyme mix (dgTHP), and pathogen-only control (Ec, *E. coli*; Ca, *C. albicans*). Data represent the means from at least 3 independent experiments performed in technical duplicate and are expressed as medians with interquartile ranges. Statistical analysis between experiments was performed using the Mann-Whitney test (B and C) and Kruskal-Wallis ANOVA with Dunn's multiple-comparison test (E to G). Significant comparisons are indicated; all others are not significant. *, $P < 0.05$.

interactions occur in urine, *C. albicans* was incubated with urine from WT or THP$^{-/-}$ mice and binding to native THP was measured by flow cytometry using an anti-murine THP (anti-mTHP) antibody followed by a FITC-labeled secondary antibody. In urine from WT mice, 38.2% of hyphae were FITC$^+$, compared to 7.9% in urine from THP$^{-/-}$ mice ($P = 0.0286$) (Fig. 2C). *C. albicans* yeasts incubated with urine from WT and THP$^{-/-}$ mice showed minimal FITC labeling (4.26% and 2.925%, respectively; $P = 0.400$) (Fig. 2C). To confirm that *C. albicans* forms hyphae in urine *in vivo*, WT and THP$^{-/-}$ mice were infected with 1×10^7 CFU of *C. albicans* strain SN250, and urine was collected 4 h postinfection, cytocentrifuged onto glass slides, and visualized using calcofluor white. For both WT and THP$^{-/-}$ mice, hyphal structures were observed (Fig. 2D). Together, these results demonstrate THP-*Candida* interactions are maintained under physiological conditions and are specific to the hyphal form.

Since THP preferentially bound *C. albicans* hyphae, we next verified that THP could also block hyphal adherence to HTB-9 cells. Indeed, coincubating hyphae with increasing concentrations of THP indicated that THP blocked *Candida* adherence, with 100 μg/ml of THP providing the maximal reduction in adherence (Fig. 2E). Induced hyphae bound to HTB-9 cells more strongly than yeasts (34% versus 19% of inoculum, respectively), and treatment with 100 μg/ml of THP reduced binding of both yeast and hyphae, while treatment with an equivalent amount of human IgG had no effect (Fig. S1B). As only hyphae specifically bound THP, all further experiments were performed with induced hyphae treated with 100 μg/ml of THP.

In *E. coli*, THP binding is dependent on the presence of high-mannose structures on the terminal end of protein glycans (27). Therefore, we investigated whether THP binding to *C. albicans* is dependent on the glycosylation of THP in a plate-based binding assay. Surprisingly, enzymatic deglycosylation of THP protein did not decrease binding to *C. albicans* hyphae compared to that with untreated THP (98.64% of input versus 96.18%; $P \geq 0.9999$) (Fig. 2F). In control experiments, deglycosylation of THP did impair binding to *E. coli* compared to binding with untreated THP (Fig. 2G). Deglycosylation of THP was confirmed via SDS-PAGE (Fig. S2). These data suggest that in contrast to *E. coli*, *C. albicans* hyphae engage THP through interactions with protein structure or amino acid sequence.

Als3 is required for *C. albicans* binding to THP. *C. albicans* expresses multiple proteins that facilitate adherence and invasion of epithelial cells. Since THP reduces adhesion of *C. albicans* to bladder epithelial cells (Fig. 1), we hypothesized that THP was binding and/or blocking the function of a fungal adhesion protein. One of the major groups of surface proteins known to mediate adhesion to host tissues is the agglutinin-like sequence (Als) family of cell surface glycoproteins. In particular, Als3 is hypha specific and mediates adhesion to buccal epithelium (28). Notably, Als3 has high sequence homology to Als1, a family member that is thought to be important during environmental transitions (29) and displays regulation and function similar to those of Als3 (30). Therefore, we reasoned that THP could bind to Als3 and/or Als1 to block *C. albicans* adherence to bladder epithelium.

We first tested the ability of Als3 and Als1 mutants to bind THP using mutants deficient for either one or both of these proteins. Strikingly, we found that THP binding was greatly reduced in the *als1/als1 als3/als3* double mutant (0.94% FITC$^+$ compared to 64.5% FITC$^+$ in WT, $P = 0.0806$); however, only complementation with *ALS3* (and not *ALS1*) rescued THP binding (68.6% FITC$^+$ and 3.3% FITC$^+$, respectively) (Fig. 3A). An independent *als3/als3* single mutant also showed significant reduction in THP binding (7.3% FITC$^+$ compared to 72.3% FITC$^+$ for the WT; $P = 0.0400$), and complementation of Als3 (65.5% FITC$^+$) resulted in a return of THP binding to WT levels (Fig. 3B). Binding of THP to WT hyphae, but not the *als3/als3* mutant, was visualized via fluorescence microscopy (Fig. 3C). We then tested whether Als1 or Als3 was required for THP to block adherence of hyphae to human bladder epithelial cells. Interestingly, not only was adherence to bladder cells decreased in the *als1/als1 als3/als3* double mutant (10% of inoculum adhered) compared to adherence of the WT strain (52% of inoculum ad-

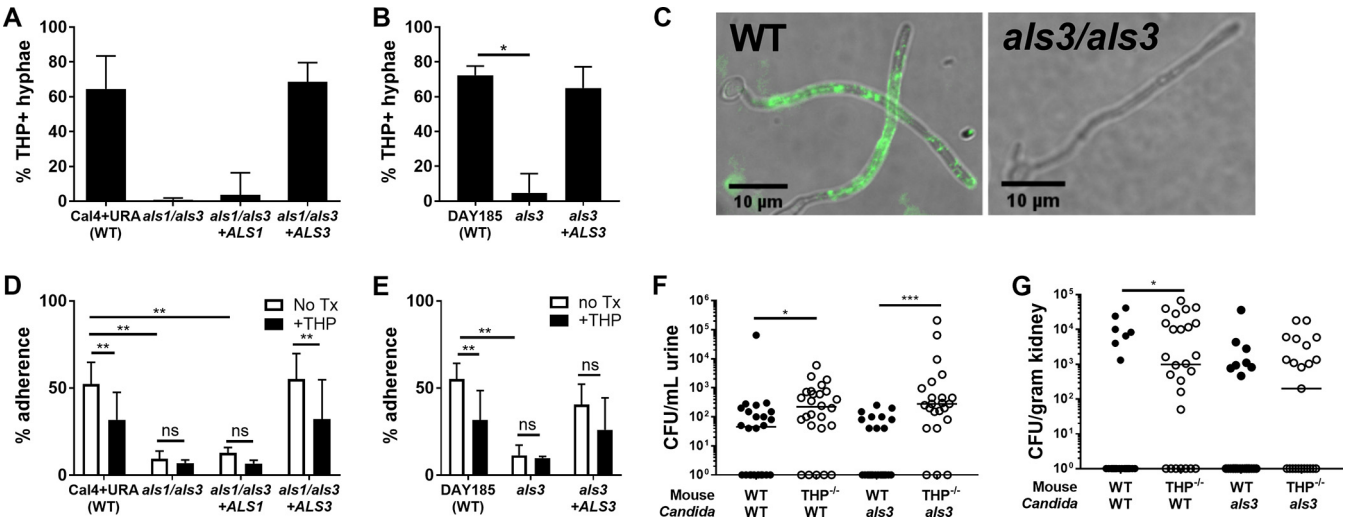

FIG 3 *C. albicans* adhesion protein Als3 mediates adhesion to bladder epithelial cells and THP. (A and B) Percent binding of THP to *C. albicans* mutant strains was assessed via flow cytometry. (C) THP binding to *C. albicans* hyphae was visualized using fluorescence microscopy. (D and E) Indicated strains of *C. albicans* pretreated with 100 μg/ml THP were assessed for adherence to the human bladder epithelial cell line HTB-9. Data represent the means from three independent experiments performed in technical duplicate or triplicate. (F and G) WT and THP$^{-/-}$ mice were infected with 1×10^7 yeasts via transurethral inoculation. Twenty-four hours later, urine (F) and kidneys (G) were harvested and assessed for fungal growth. Circles represent individual mice, and lines represent the median for each group. Experiments were done twice independently and data combined ($n = 24$ WT mice infected with WT *C. albicans*, 26 THP$^{-/-}$ mice infected with WT *C. albicans*, 26 WT mice infected with the *als3/als3* mutant, and 23 THP$^{-/-}$ mice infected with the *als3/als3* mutant). Statistical analysis between samples was performed using Kruskal-Wallis ANOVA with Dunn's multiple-comparison test (A, B, F, and G) or 2-way ANOVA with Sidak's multiple-comparison test (D and E). Significant comparisons are indicated; all others are not significant. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

hered), but also the blocking effect of exogenous THP was eliminated (Fig. 3D). Only complementation of the *als1/als1 als3/als3* mutant with *ALS3*, and not *ALS1*, restored adherence (55% of inoculum adhered) as well as the effect of exogenous THP (Fig. 3D). In support of this, the *als3/als3* single mutant also showed decreased adherence (10.9% of inoculum adhered) and loss of THP blocking effect, both partially restored with complementation (Fig. 3E). Collectively, these data demonstrate that THP blocks *C. albicans* colonization of the urinary tract by binding Als3 and is a critical adhesion protein for human bladder epithelial adherence.

These findings suggest that while Als3 loss in *Candida* would circumvent THP binding, any potential benefit for colonization of the urinary tract might be negated by inherently poor adherence of this mutant to bladder epithelium. To investigate this, we infected both WT and THP$^{-/-}$ mice with WT *C. albicans* or the *als3/als3* mutant. In WT mice, recovery of the *als3/als3* mutant was modestly attenuated in urine compared to that of WT *C. albicans* (median: 50 CFU/ml compared to 0 CFU/ml; Mann-Whitney U test, $P = 0.0952$), but no differences were observed in the kidneys (Fig. 3F and G). In line with our model, we found that in THP$^{-/-}$ mice the *als3/als3* mutant achieved colonization of the urinary tract similar to that of the wild-type strain (Fig. 3F and G), suggesting that Als3 is dispensable in the absence of THP in mice. In both the urine and kidneys, THP$^{-/-}$ mice displayed higher *C. albicans* burdens than WT mice (Fig. 3F and G). Collectively, our results demonstrate that THP is a soluble component of a host innate defense against *C. albicans* in the urinary tract.

DISCUSSION

Host factors contributing to host protection against fungal UTI have remained largely unknown. A murine catheter UTI model has previously shown that LysM plays a role in inflammatory responses (31). Here we provide some of the first evidence that Tamm-Horsfall protein is an important component in preventing *C. albicans* colonization of the urinary tract. Using purified human protein, we showed that THP binds *Candida* hyphae and antagonizes adherence to bladder epithelial cells and that loss of THP in mice increases fungal colonization of the urinary tract. Importantly, these observations expand the known role of THP as a major protective factor against

infection by multiple bacterial pathogens, including UPEC, *P. mirabilis*, and *K. pneumoniae* (21–24).

Our data support a model in which THP maintains distinct glycan and protein features to recognize and clear a variety of different pathogens. In the case of UPEC, THP acts to bind bacterial fimbrial proteins, blocking *E. coli* interaction with uroplakins on the bladder epithelium and preventing adherence and colonization of the bacteria (27). In particular, UPEC binds high-mannose structures of THP via the FimH adhesion molecule in a manner that is sensitive to D-mannose and reduced by endoglycosidase H treatment (27). Based on previously identified binding affinities, other bacteria, such as *Staphylococcus saprophyticus* and *P. mirabilis*, are predicted to bind THP via the GalNac residue on the Sda antigen, an abundant terminal structure on THP N-glycans (22, 23, 32). We show that in contrast to bacteria, *C. albicans* binds THP independent of glycosylation. Removal of THP glycans via a multienzymatic reaction failed to alter *C. albicans* binding to THP, suggesting that the THP-*Candida* interaction is protein mediated. Future studies will be needed to identify specific structural or sequence-dependent features that mediate this interaction.

A major determinant of *Candida* virulence is its ability to adhere to and invade epithelial cells, and *Candida* spp. express a number of adhesion molecules that enable interactions with a variety of host proteins and cell types. For example, in urinary tract infections *C. glabrata* epithelial adhesin (Epa) proteins promote adherence and colonization (33). Bladder epithelial receptors for *Candida* have not yet been identified, although *C. albicans* adherence to bladder epithelial cells is reduced in the presence of free mannose (11). In this study, we found that the fungal adhesion protein Als3 (agglutinin-like sequence 3) facilitates fungal adherence to bladder epithelium and is specifically blocked by THP. Additionally, an *als3/als3* mutant was recovered at lower levels in urine than was WT *C. albicans*; however, the overall poor colonization of *C. albicans* in WT mice limited the ability to distinguish the contribution of Als3-THP binding to ascending infection *in vivo*. Additionally, the high levels of colonization by the *als3/als3* mutant and WT *C. albicans* alike in THP-deficient animals suggest that Als3 is dispensable for ascending infection in mice. Als3 is a member of a large family of glycoproteins that are cell surface expressed and glycosylphosphatidylinositol (GPI) anchored to the fungal cell wall (30). Als3 interacts with multiple host proteins, including albumin, fibronectin, N-cadherin, E-cadherin, laminin, and collagen (34–36). This diversity of binding partners is possible due to a wide and flat protein-binding cavity which recognizes the free C termini of proteins (30). While the best-characterized role of Als3 is in adhesion to cell surfaces, Als3 also plays a role in invasion of cells via its interaction with host cadherins, as well as iron scavenging due to its ability to bind ferritin (34, 37). In this study, we found that Als3 is critical for adhesion to the bladder epithelium but did not distinguish the bladder epithelial component(s) which engages Als3. Furthermore, although THP treatment reduced *C. albicans* hyphal adherence to the bladder epithelium by approximately 40%, this effect was not as dramatic as the reduction observed in Als3-deficient strains (~80% reduction in adherence). THP may prevent *C. albicans* adhesion by either transiently blocking the interaction of Als3 with its host targets or engaging specific cell surface receptors which bind the fungal pathogen.

Interestingly, in different *C. albicans* infection models, Als3 is thought to share functional redundancy with other Als family members (38). In this study, our data indicate that Als3 is required for effective adherence to bladder epithelium but Als1 is not under the conditions tested (Fig. 3); however, we cannot rule out contributions of Als1 and other Als family members to urinary tract colonization. In this regard, THP is well suited for protection against *C. albicans* UTIs, as it not only coats fungal hyphae but also does so by recognizing and blocking the major adhesion molecule *C. albicans* uses to bind bladder epithelium. The pathogenicity of *C. albicans* can vary not only by host genetic background but also by strain; thus, more virulent strains may utilize alternative adhesins to adhere to bladder epithelium and potentially overcome THP control of fungal colonization. Additional analysis, particularly of clinical isolates, will be needed

TABLE 1 Wild-type and mutant strains used in this study

Strain	Source or reference
SC5314 (wild type)	ATCC
SN250 (reference)	42
SN-*als3/als3*	Gift from Suzanne Noble
DAY185 (reference)	43
CAI4-URA3 (reference)	44
CAYF178U (*als3/als3*)	44
CAQTP178U (*als3/als3* + pALS3)	44
CJN1348 (*als1/als1 als3/als3*)	43
CJN1352 (*als1/als1 als3/als3* + ALS3)	43
CJN1353 (*als1/als1 als3/als3* + ALS1)	43

to shed light on the possible compensatory role of differentially expressed Als family members in dictating the pathogenicity of *C. albicans* strains during UTI.

In general, healthy humans do not present with *Candida* UTI and candiduria is a concern only for hospitalized patients with underlying disease (6, 39). How the host prevents *Candida* colonization of the urinary tract is not well studied, and this work suggests that THP contributes to the prevention of *Candida* colonization. However, while THP may prevent *Candida* colonization in a healthy host, a significant risk factor associated with *Candida* UTI is the presence of a urinary catheter. THP binds efficiently to silicone catheter material and promotes bacterial adherence and colonization of catheters (26). In fact, patient catheters colonized with common urinary pathogens, including *Candida*, are more heavily deposited with THP than culture-negative catheters (26). Thus, it is conceivable that THP may likewise support *Candida* biofilm formation on catheters in hospitalized patients, increasing the risk of catheter-associated infection. Interestingly, THP levels in patients most susceptible to complicated candiduria are frequently reduced, such as in diabetic patients and patients with renal failure (40). Although the exact sequence of events in hospitalized patients is not known, our work suggests that the reduced levels of THP in these patients may contribute to an increase in *Candida* pathogenesis in the urinary tract.

Collectively, these data suggest that in a healthy host, THP acts in preventing fungal adhesion to the bladder epithelium, resulting in the flow of urine washing away *Candida* before it establishes bladder infection. Additionally, these data support THP as a potential target of interest in preventing catheter-associated UTI (CAUTI) in hospitalized individuals.

MATERIALS AND METHODS

***C. albicans* strains used.** Strains and sources used in this study are listed in Table 1. Strains were cultured from frozen stock onto yeast-peptone-dextrose (YPD) agar plates. For experiments, strains were inoculated from YPD agar into complete synthetic medium (CSM; 1× yeast nitrogen base plus amino acids plus 2% glucose) and grown at 30°C. Strains were passaged twice in CSM and then grown overnight (14 to 16 h) at 30°C and washed twice in phosphate-buffered saline (PBS). Cell numbers were determined by counting on a hemacytometer.

Bacterial strains used. Wild-type uropathogenic *E. coli* (UPEC) strain UTI89 (O18:K1:H7) (41) was grown for at least 20 h in shaking culture to stationary phase at 37°C in Luria-Bertani (LB) broth prior to experiments.

Murine urinary tract infections. All animal experiments were conducted under veterinary supervision and approved by the University of California, San Diego (UCSD), IACUC. $THP^{+/+}$ (WT) and $THP^{-/-}$ mouse breeding pairs, described previously, were bred and maintained at UCSD (24). All animals used in this study were females aged 2 to 4 months. Mice were allowed to eat and drink *ad libitum*. All efforts were made to minimize suffering of animals employed in this study. *C. albicans* strains (SC5314, SN250, and SN-*als3/als3*) were prepared as described above. Once counted, 1×10^7 cells in 50 to 100 μl of PBS were prepared and inoculated in mice via transurethral inoculation, as described previously (17). Twenty-four hours after inoculation, mouse urine was expressed and plated for CFU on Sabouraud dextrose agar with 75 mg/liter of chloramphenicol. At the desired time points, mice were euthanized and kidneys collected for CFU enumeration.

Adherence assay. The human bladder epithelial cell line HTB-9 (ATCC 5637) was grown in RPMI 1640 with glutamine and 10% fetal bovine serum (FBS) (RPMI-10). Cells were seeded in 24-well plates and grown to confluence overnight. For experiments in which HTB-9 cells were pretreated (Fig. 1), HTB-9 cells were washed once with PBS and the medium was replaced with 400 μl of RPMI-10 ± 50 μg/ml of human THP (BBI solutions). For experiments in which *C. albicans* was treated (Fig. 2 and 3; see also Fig. S1), 100

μg/ml of human THP was added to 1×10^6 *C. albicans* cells in 100 μl of RPMI-10. Both HTB-9 cells and *C. albicans* were incubated with THP for 30 min at 37°C with CO_2 before addition of *C. albicans* (multiplicity of infection [MOI] of 10) to cells. After addition of *C. albicans*, cells were centrifuged at 300 $\times$ *g* for 5 min and then incubated at 37°C with CO_2 for 30 to 45 min. After incubation, cells were washed 6 times with PBS, incubated in 0.25% trypsin for 5 min, and then lysed with 0.025% Triton X-100 in water with vigorous pipetting. The lysate was plated on YPD agar and incubated at 30°C for 24 h before enumeration.

Flow cytometry-THP binding assay. For yeasts, 1×10^7 yeasts per ml were incubated in SCM for 3 h. For hyphae, 1×10^7 yeasts per ml were incubated in RPMI 1640 at 37°C with CO_2 to induce hyphal formation. After 3 h, cells were centrifuged at 3,220 $\times$ *g*, washed with PBS, and blocked in SynBlock (Bio-Rad) for 30 min at room temperature. To prevent clumps and standardize the size of hyphae, all samples were passed through 70-μm FlowMi tips prior to incubation with THP and transferred to a 96-well round-bottom plate for all subsequent steps. Cells were incubated with 50 μg/ml of human THP in PBS at room temperature for 30 min. For determining binding to murine THP, urine was collected from wild-type and $THP^{-/-}$ mice, and yeasts and hyphae were incubated in undiluted urine for 30 min. After incubation with THP or urine, cells were washed twice with PBS and incubated in the appropriate antibody in SynBlock: for hTHP, 5 μg/ml of FITC-conjugated anti-hTHP (Origene; AM31843FC-N), and for mTHP, 5 μl/ml of anti-mTHP (R&D; MAB5176) followed by FITC-conjugated anti-rat secondary antibody at 1:500 (Jackson Immunolabs). After incubation with antibody, cells were washed twice and resuspended in 200 μl of PBS and then analyzed for THP binding (FITC-positive cells) on a BD Canto flow cytometer equipped with a high-throughput sequencer (HTS) system.

Deglycosylation of THP and binding assay. Five micrograms of THP/well in PBS was added to enzyme-linked immunosorbent assay (ELISA) high-binding plates and incubated at room temperature for 2 h and then at 4°C for at least 4 h. Plates were washed 3 times with PBS and then treated with protein deglycosylation mix II (New England BioLabs [NEB]) per the manufacturer's instructions. Briefly, each well contained 50 μl of 1$\times$ protein deglycosylation buffer 1 with or without 2.5 μl of enzyme mix, incubated at room temperature for 30 min and then 37°C for 16 h. After incubation, the plate was washed 3 times with PBS and blocked for 1 h with SynBlock. Meanwhile, 1×10^7 cells/ml of *C. albicans* hyphae and 2×10^9 cells/ml of *E. coli* strain UTI89 were washed 1$\times$ with PBS, then incubated in 2.5 μM Syto13 for 30 min at room temperature (*C. albicans*) or 10 min (*E. coli*) at 37°C, and then washed 3 times in PBS to remove excess dye. After labeling, 100 μl of *C. albicans* or *E. coli* was added to the plate, centrifuged for 5 min at 3,220 $\times$ *g*, and then incubated at room temperature for 30 min in the dark. After incubation, the plate was washed 4 times with PBS, and fluorescence (excitation wavelength, 488 nm; emission wavelength, 510 nm) was measured using a PerkinElmer Enspire Alpha plate reader. Percent binding was determined by comparing fluorescence left after washing (output) to the initial fluorescence (input). To confirm deglycosylation of THP, 5 μg of soluble THP was incubated with protein deglycosylation mix II (NEB) as per the manufacturer's instructions for both native and denatured protein deglycosylation for 16 h. Treated and untreated protein was run on a 4% to 12% NuPAGE bis-Tris protein gel before being stained with InstantBlue protein stain (Sigma) for visualization.

Visualization of THP binding *C. albicans* via fluorescence microscopy. A total of 1×10^5 cells of *C. albicans* yeast in RPMI were seeded into a 24-well plate containing poly-L-lysine-coated coverslips (Corning; BioCoat). Yeasts were incubated at 37°C with CO_2 for 2 h to induce hyphae, washed once in PBS, and then blocked for 30 min in SynBlock (Bio-Rad). After blocking, 100 μg/ml of THP in PBS was added and cells were incubated at room temperature for 30 min. After incubation, cells were washed twice with PBS and then fixed with 4% paraformaldehyde for 15 min. After fixation, the coverslips were blocked with 3% bovine serum albumin (BSA) in PBS for 30 min and incubated in 1 μg/ml of anti-hTHP (R&D; AF5144) followed by Alexa Fluor 488-conjugated anti-sheep secondary antibody at 1:1,000 (Invitrogen; A11015). After staining, samples were washed in double-distilled water (ddH_2O) and mounted with ProLong Diamond antifade mountant (Invitrogen). Slides were allowed to cure overnight prior to visualization on a Zeiss AxioObserver D1 microscope.

Visualizing *C. albicans* in mouse urine. Mice were infected as described above, and urine was expressed 4 h postinfection. Urine collected was diluted 1:10 and 1:50, and then 100 μl was cytocentrifuged onto glass slides. Samples were allowed to dry, briefly heat fixed, and then stained with 1 g/liter of calcofluor white stain (Sigma) for 3 min. After staining, samples were washing in ddH_2O and mounted with ProLong Diamond antifade mountant (Invitrogen). Slides were allowed to cure overnight prior to visualization on a Zeiss AxioObserver D1 microscope.

Statistical analyses. All *in vitro* experiments were performed in at least technical duplicate and repeated in at least three independent replicates. All *in vivo* experiments were performed using at least 8 mice per group and repeated in at least two independent replicates except for the time course in Fig. 1B, which was conducted once with 10 mice per group. Statistical analyses were conducted using GraphPad Prism, version 7.03 (GraphPad Software Inc., La Jolla, CA). Mean values from technical replicates were used for statistical analyses, with independent experiment values or biological replicates represented in graphs with medians with or without interquartile ranges as indicated in figure legends. All data sets were subjected to the D'Agostino and Pearson normality test to determine whether values displayed Gaussian distribution before selecting the appropriate parametric or nonparametric analyses. Statistical analyses performed include nonparametric Mann-Whitney tests, Kruskal-Wallis one-way analysis of variance (ANOVA) with Dunn's multiple-comparison test, or two-way ANOVA with Sidak's multiple-comparison test as indicated in figure legends. For *in vivo* experiments, the nonparametric Mann-Whitney test was performed between infected cohorts.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/IAI.00451-18.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

ACKNOWLEDGMENTS

A.C. was supported through a postdoctoral fellowship from the A. P. Giannini Foundation. K.A.P. was supported through a postdoctoral fellowship from University of California Chancellor's Postdoctoral Fellowship Program. Studies were supported by NIH/NHLBI Program of Excellence in Glycosciences (P01 HL107150 to V.N.).

We thank Satish Kumar for the generous gift of the THP mouse lines and Michael Florio and the UCSD vivarium staff for breeding and maintaining the mice; we are grateful to Suzanne Noble and Scott Filler for providing the *C. albicans* strains used in this study.

We have no conflicts of interest to declare.

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