Urol Res (1991) 19:301–304

Urological Research

Ability of uropathogens to bind to Tamm Horsfall protein-coated renal tubular cells

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Accepted: January 1, 1991

Summary. Epithelial cells were isolated from a healthy human kidney and found to be of normal, renal tubular origin. The cells were maintained in tissue culture and found to secrete Tamm Horsfall protein (THP). Three strains of uropathogenic *Escherichia coli,* and one each of *Proteus mirabilis* and *Pseudomonas aeruginosa* were found to adhere to the THP-coated tubular cells. Extraneous THP bound to all the organisms except P fimbriated *E. coli,* and caused a reduction in the adhesion of type 1 fimbriated *E. coli* and *P. mirabilis* to the renal cells. However, irrespective of reduced adhesion in three of five strains tested, there was adequate evidence to indicate that THP does not competitively exclude all uropathogenic adhesion. On the contrary, its presence on renal cells may act to mediate bacterial colonization.

Key words: Uropathogens – Adhesion – Tamm Horsfall protein

Tamm Horsfall protein (THP) is one of the most abundant proteins found in human urine, being produced in the thick ascending limb of Henles loop [1, 2, 13, 15]. There is some evidence to suggest that THP may have an antibacterial function in the urinary tract, achieved by binding via mannose to type 1 fimbriated *Escherichia coli,* and presumably preventing their adhesion to the uroepithelium [2, 7–9]. The reduced excretion of THP in elderly patients has been associated with an increased incidence of urinary tract infections [14]. However, the fact that type 1 fimbriated *E. coli* bind to mucopolysaccharide – coated uroepithelial cells suggests that THP coating could in fact mediate adhesion to the uroepithelia [11].

Pyelonephritis is a less common but more serious form of urinary tract infection, particularly harmful to newborns and small children [4]. According to animal experiments, ascending pyelonephritis without reflux could be caused by *E. coli* which express P and type 1 fimbriae [3, 12]. Struvite stones are also a significant urologic problem, with formation of the matrices being associated with *Proteus mirabilis* infection [6]. Other organisms, such as *Pseudomonas aeruginosa,* cause urinary tract infection in complicated cases, including catheterized and obstructed patients [5]. The adhesion of the latter two uropathogens to THP has not previously been investigated.

The present study was undertaken to examine whether or not THP coating on renal tubular cells and extraneous THP affect the adhesion of uropathogenic bacteria.

Materials and methods

Human kidney cells

Normal human kidney specimens were obtained from surgical biopsies. They were cut into small pieces measuring approximately 1 mm^2. These explants were grown organotypically in a defined medium composed of 90% Medium 199, 1% non-essential amino acids, 10% fetal bovine serum, 0.5% gentamicin sulphate, 1% amphotericin B (Gibco, Grand Island, NY), 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml selenium, 1 μg/ml hydrocortisone, 0.1% endothelial cell mitogen (Sigma, St. Louis, MO) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. Cells were passaged upon reaching confluence in 0.5 g/l trypsin, 0.2 g/l EDTA using a 1:4 ratio. Stock cultures were established and remained frozen at −80°C in nutrient medium and 5% dimethylsulphoxide. Cells were characterized by Dr. P. Van Nostrand, the Head Pathologist at Toronto General Hospital, as normal kidney epithelium of tubular origin, using the Papanicolaou test and morphological examination by electron microscopy.

Bacteria

The bacterial strains comprised type 1 fimbriated *E. coli* 2239, type 1 and P fimbriated *E. coli* Hu734, type P fimbriated *E. coli* 917, mannose-resistant *P. mirabilis* 28 cii and mucoid *Pseudomonas aeruginosa* SM1. All bacteria were frozen as stock cultures in brain heart infusion yeast extract (BYE) broth (Difco, Detroit, Mich.) and 20% glycerol. The strains were grown in BYE broth, except strain 917 which was grown on BYE agar to encourage expression of mannose-resistant adhesin. The organisms were washed three times in phosphate-buffered saline (PBS, pH 7.1), and resuspended to a concentration of 10^8 cells/ml in either PBS or THP solution. A luciferin-luciferase automated bioluminescence assay was per-

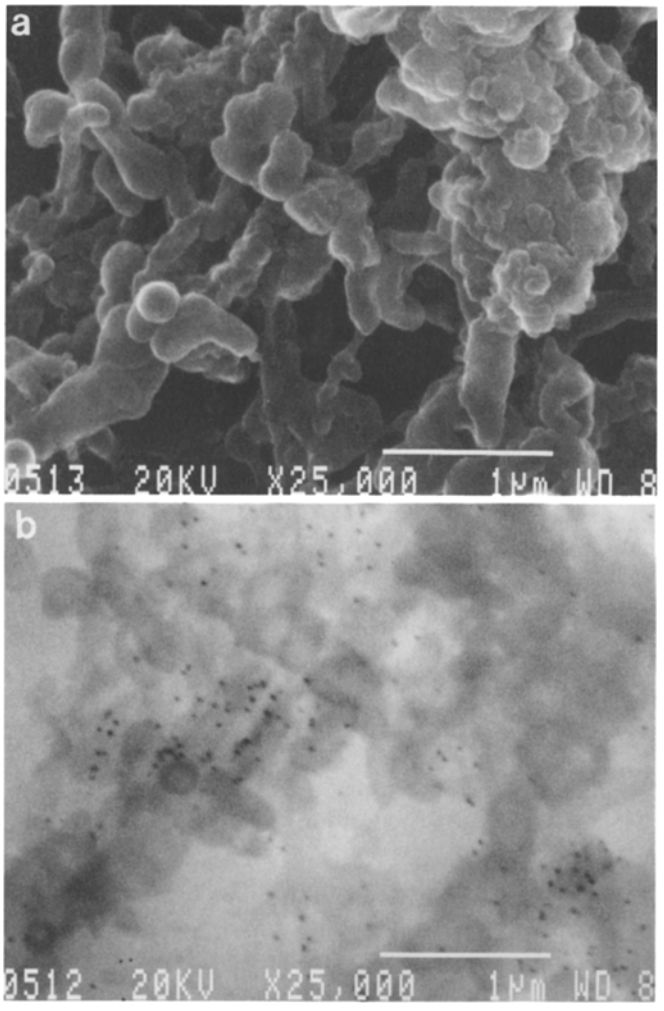

Fig. 1 a, b. Electron micrograph of untreated kidney epithelial cells. **a** Cell surface, **b** same cell in backscattering mode. The *dark dots* are immunogold indirect staining of THP on the cell surface

formed using a luminometer (Turner TD 20e, USA) to measure bacterial ATP as an indicator of cell viability in the two suspending liquids.

THP isolation

THP was isolated from pooled urine by repeated precipitation in 0.58 M NaCl. The precipitate was resuspended in 0.58 M NaCl and dialyzed against distilled water for 2 weeks. The THP was resuspended to give a concentration of 1.02 mg/ml PBS. The purity of the THP solution was determined by obtaining a single peak on a heparin-sepharose column using FPLC (Pharmacia, Uppsala, Sweden). Both the THP solution and PBS were pH-adjusted to 7.2, and this was maintained throughout the course of the experiments.

Cell adhesion assay

Human kidney cells were grown on acid-treated coverslips and upon reaching confluence were washed twice in PBS and exposed to one of the five uropathogens suspended in either PBS or THP solution for 1 h at 37°C. The cells were then washed (4×15 min) in 100 ml PBS with vigorous shaking. The cells were then fixed in 10% neutral buffered formalin and stained with crystal violet. The number of bacteria adhering to 50 cells was determined manually, using a Zeiss Axioskop under a 100× oil immersion objective. Mean results were calculated from triplicate data.

Immunogold localization of THP

Human kidney cells were grown and washed as described above before being exposed to murine anti-THP antibody (1 μg/ml, Cedarlane Labs, Milton, Ontario) for 2 h. Following two washes in PBS, the cells were incubated with goat anti-mouse IgG conjugated to 20 nm gold particles (10 μg/ml, Bioclin, Millipore). In order to rule out non-specific binding, the primary antibody was omitted from controls, which were incubated in the secondary antibody only. The secondary antibody was washed off and the cells fixed in 2.5% glutaraldehyde, dehydrated in an increasing graded series of ethanols, critical point dried and vapour carbon coated. The cells were then examined under a JOEL JSM 840 scanning electron microscope. Backscattering mode was employed to visualize the secondary immunogold label to THP. In order to determine whether THP was binding directly to bacteria, the five strains were washed three times in PBS and incubated for 1 h in either THP or PBS, at a concentration of 10^8 cells/ml at 37°C. The bacteria were then deposited on 0.22-μm nitrocellulose membranes (Millipore, Bedford, MA) and washed vigorously three times in PBS. The fixation and staining procedures for THP were as described above for the kidney cells.

Results

Immunogold localization of THP binding

The presence of THP being secreted by human renal epithelial cells is illustrated in the electron micrograph shown in Fig. 1, which was taken using the standard and backscattering modes of the scanning electron microscope. This displays the immunogold secondary label to THP. More than 20 epithelial cells were examined for each condition. The renal cells stained positive for THP, but there was no apparent difference between those incubated in THP or PBS, indicating that additional extraneous THP did not bind to the cells. The label that was observed was not due to nonspecific binding, as the cells incubated in the secondary antibody alone showed no immunogold stain. This indicates that the cells employed were capable of synthesizing THP.

Labelled gold particles were seen attached to bacterial cells and extracellular bacterial material. *E. coli* 2239 and Hu 734 each had two to five gold particles associated with their cells in THP, with zero particles associated in PBS. *E. coli* 917 had no gold particles associated with the bacteria in THP or PBS. *P. mirabilis* 28cii had two to three particles associated with the bacterial cell in THP, but not in PBS. *Ps. aeruginosa* SM1 was the most labelled, with particles localized around the mucoid periphery of the cells and attached to extracellular material.

Cell adhesion

All of the uropathogens adhered to the renal tubular cells (Table 1). Addition of extraneous THP reduced the adhesion of strains 2239 (Fig. 2), Hu 734 and 28cii by over

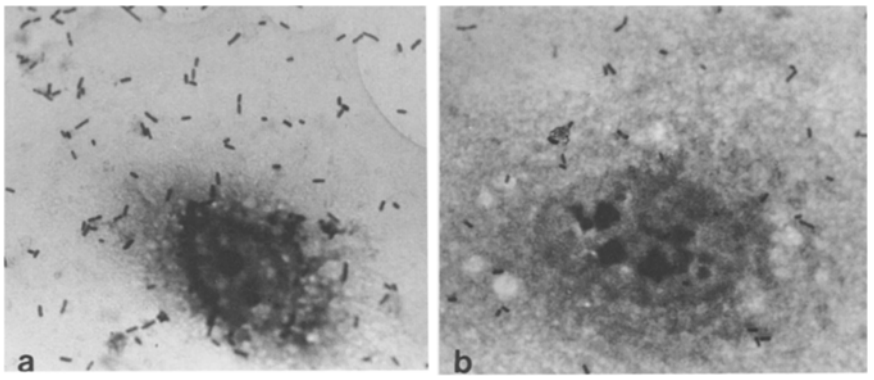

Fig. 2a, b. Cells incubated with *E. coli* 2239 in **a** PBS or **b** THP. Note the reduction in adherence with THP

Table 1. Mean number of bacteria adherent to human renal tubular cells ± SD. (*n*, 50 cells; SD standard deviation)

Bacterial species	Bacteria in PBS	Bacteria in THP
E. coli 2239	133.00 + 30.9	60.88 + 12.86
E. coli Hu734	141.32 ± 54.2	47.66 ± 17.1
P. mirabilis 28Cii	38.80 ± 12.9	15.68 ± 6.7
E. coli 917	96.80 ± 10.1	96.96 ± 11.0
Ps. aeruginosa SM1	> 150	> 150

THP, Tamm Horsfall with concentration 1.02 mg/ml

50%. No reduction was evident with P fimbriated *E. coli* 917 and *Ps. aeruginosa* SM1.

There was no statistical difference between the viability counts for the bacteria in PBS and THP.

Discussion

The present study has demonstrated that three species of uropathogens adhere to renal tubular cells which secrete Tamm Horsfall protein (THP). The addition of extraneous THP caused a reduction in the adherence of a type 1 fimbriated *E. coli*, indicating a competitive reaction for the binding of THP. This agreed with previous findings and verified the effectiveness of the assay. However, new conclusions can be drawn from the latest results.

Firstly, the addition of extraneous THP did not completely negate bacterial adhesion, indicating that this protein cannot remove all uropathogens from the uroepithelium. It had previously been hypothesized that THP binds to type 1 *E. coli* and could flush them out of the bladder [1, 8, 9]. Clinically this is difficult to imagine, as almost all urinary tract infection isolates of *E. coli* are type 1 fimbriated. Furthermore, the type 1 fimbriated *E. coli* 2239 was highly adherent to the renal tubular cells, supporting the virulence role for this strain in upper tract infection.

Secondly, a strain of *Proteus mirabilis* was found to bind to THP secreting renal tubular cells. This is a new finding, and the fact that extraneous THP interferes with this adhesion indicates some specificity in the adhesion process. The virulence of *Proteus* species has been well demonstrated in relation to urease production and struvite stone formation [6]. These latest data indicate that proteus colonization of renal tubular cells could be mediated by THP, again suggesting that this protein is not necessarily a host defense component.

Thirdly, *Ps. aeruginosa* bound avidly to the THP-coated cells, again a new finding that does not support a 100% defence role for THP in the urinary tract. The immunogold labelling studies showed a higher affinity for THP to these mucoid bacterial cells than to the other organisms. The fact that extraneous THP did not negate *Ps. aeruginosa* adhesion possibly shows that THP is not the only receptor for these organisms on the cells. Ramphal et al. [10] proposed that *Ps. aeruginosa* utilize an exopolysaccharide bridge to bind to mucin of tracheobronchial cells, thereby enhancing colonization. The possibility arises that similar exopolysaccharide-THP binding is involved in renal cell colonization.

Clearly, Tamm Horsfall protein secreted by renal tubular cells can bind to various uropathogens and has the potential to flush a proportion of them from the urinary tract. However, it is equally clear that uropathogens can colonize THP-coated renal cells and that any competitive exclusion by extraneous THP does not totally block bacterial colonization of the epithelial cells.

Acknowledgements. This work was supported by the Medical Research Council of Canada. We acknowledge the cooperation of Dr. M. Bulbul and the assistance of Dr. P. Van Nostrand for identifying the human cells.

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