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Role of Tamm-Horsfall protein in the binding and *in vivo* phagocytosis of type 1 fimbriated *Escherichia coli* by mouse peritoneal macrophages

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Abstract

Tamm-Horsfall glycoprotein (THP) contains manno-oligosaccharides that are recognized by type 1 fimbriae (F1) of *Escherichia coli*. In the present study, we examined the *in vivo* phagocytic activity of mouse peritoneal macrophages after treatment of bacteria with THP. At low THP concentrations (12.5 µg/ml and 50 µg/ml) no significant difference was observed in the phagocytosis of *E. coli* $F1^+$. However, at high THP concentrations (500 µg/ml and 1250 µg/ml) we obtained a reduction of bacterial phagocytosis by mouse peritoneal macrophages.

Key words
- Tamm-Horsfall protein
- Type 1 fimbriated *E. coli*
- Phagocytosis

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Introduction

Several members of the Enterobacteriaceae family, including some strains of *Escherichia coli*, have numerous filamentous fimbrial structures on their surface (1). Some strains of type 1 fimbriated *E. coli* adhere to uroepithelial cells and may cause lower urinary tract infections (cystitis) (2), but their relationship with upper urinary tract infections (pyelonephritis) is still not clear. These fimbriae facilitate the attachment of bacteria to mannose-containing receptors on a variety of host cells, as well as to a urinary tract glycoprotein known as Tamm-Horsfall protein (THP) (3-5).

Tamm-Horsfall glycoprotein, which is synthesized exclusively by the kidney (6), is present in large quantities (20-200 mg/ml) in human urine and has been implicated in a variety of renal diseases, although its physiological role is unknown (7). The attachment of type 1 fimbriated *E. coli* to THP suggests a protective role of THP in the defense against urinary tract colonization and infection by this strain of *E. coli* (3,5). On the other hand, an *in vitro* study (8) demonstrated that bacteria coated with THP are less susceptible to phagocytosis by polymorphonuclear leukocytes (PMNL), and this may allow the infection to persist. Type 1 fimbriae can also bind to mannose-containing receptors on the membranes of PMNL and mouse peritoneal macrophages (9).

The aim of the present study was to examine in an *in vivo* assay the effect of THP concentration on bacterial phagocytosis by mouse peritoneal cavity macrophages, a phenomenon that has only been described *in vitro* by others.

Material and Methods

Bacterial strains

E. coli strain ORN115, a recombinant with type 1 fimbriae (10), was obtained from Dr. Paul E. Orndorff (Department of Microbiology, Pathology, and Parasitology, North Carolina State University, Raleigh, NC, USA). Strains of non-fimbriated *E. coli* (K12 C600) were also used. The bacteria were cultured statically in trypticase soy broth for 18 h at 37ºC (11). The presence of type 1 fimbriae was confirmed by serum agglutination on glass slides with specific antiserum against $F1^+$ and by agglutination of guinea pig erythrocytes on slides.

Purification of THP

THP from human urine was purified by salt precipitation (12). After dialysis against deionized water and lyophilization, the material was dissolved in 8 M urea and chromatographed on a Sepharose CL-6B column (2 cm x 58.6 cm) equilibrated with 30 mM phosphate buffer, pH 6.8, containing 2 M urea at a flow rate of 2 ml/min. The elution profile was monitored at 280 nm. The active peak appeared as a single band (molecular weight ~94,000) on SDS-PAGE (13) after staining with silver nitrate (14). This band was positively identified as THP by Western blotting (15) following incubation with an anti-THP monoclonal antibody (Accurate Chemical and Scientific, Westbury, NY, USA). Protein concentrations during purification were determined using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA).

Preparation of anti-THP serum

Polyclonal anti-THP antiserum was produced in New Zealand rabbits as described by Dawnay et al. (16), with some modifications. The rabbits were immunized subcutaneously at various sites with 250 µg of THP in Freund's complete adjuvant. After 30 and 60 days, the animals received an additional injection of 100 µg of THP in Freund's incomplete adjuvant and were bled 10 days after the final dose.

Binding of *E. coli* to immobilized THP

The methodology developed by Karlsson et al. (17) was used. After SDS-PAGE (13), THP was transferred to a nitrocellulose membrane (15) and blocked by incubation with 5% non-fat dry milk in 20 mM PBS, pH 7.4, at room temperature for 1 h. The membrane was then washed twice in 20 mM PBS-Tween buffer, pH 7.4, and incubated in a vertical position in 10 ml of a bacterial suspension (10^8 cells/ml) of *E. coli* ORN115 and *E. coli* K12 C600 for 1 h at room temperature with no shaking. A control sample containing 20 mg/ml D-mannose was also run. After this period, the membranes were washed in 20 mM PBS-Tween buffer and placed protein side up on eosin blue methylene (EMB) agar plates and incubated at 37ºC for 18 h to allow colony formation.

Phagocytosis assay

Two hundred microliters of *E. coli* ORN115 suspension (10^8 cells/ml in 20 mM PBS, pH 7.4) was centrifuged at 17,700 *g* and resuspended in 20 mM PBS, pH 7.4, containing THP (2.5, 25, 100 and 250 µg/0.2 ml (8)) and incubated for 30 min at 37ºC.

The peritoneal cavities of 40 female 8-10-week-old BALB/c mice supplied by the University's central animal house (CEMIB/Unicamp) were stimulated by injecting a 10% peptone solution (18) (Difco Laboratories, Detroit, MI, USA) (0.5 ml/animal). Three days later, a control group of eight mice were injected intraperitoneally (*ip*) with 0.2 ml of *E. coli* (10^8 cells/ml in 20 mM PBS, pH 7.4 (19)) and four other groups received a 0.2-ml suspension of *E. coli* (10^8 cells/ml) con-

taining 2.5, 25, 100 and 250 µg of THP/0.2 ml, respectively. Thirty minutes after the *ip* injections (18), the mice were sacrificed by cervical dislocation, and their peritoneal cavities were washed with 3 ml of 20 mM PBS containing 5 IU heparin/ml. The macrophages collected were resuspended to 2 x 10^6 cells/ml in 20 mM PBS, pH 7.4. This suspension was cytocentrifuged and the cells were stained with Giemsa (8). The percentage of phagocytes was determined by counting phagocytic and non-phagocytic cells in a total of 100 cells. The numbers of macrophages were counted by light microscopy at a magnification of 100X. Differences between the experimental and control groups were compared by the Duncan multiple range test using a statistical software package (SAS Institute, 1986) (20).

Results

Characterization of THP

Chromatography of THP on Sepharose CL-6B in 8 M urea produced two main peaks, the first of which contained THP (data not shown). After applying SDS-PAGE and staining with silver nitrate (Figure 1), THP was detected as a single band of 94 kDa. Identical results were obtained by Western blotting (Figure 2).

Binding of *E. coli* to immobilized THP

Type 1 fimbriated *E. coli* bound to immobilized THP on nitrocellulose membranes (data not shown). This binding was observed after multiplication of *E. coli* around the 94-kDa THP band on EMB agar plates. No growth was observed in the presence of D-mannose (20 mg/ml) or with *E. coli* K12 C600.

Phagocytosis

Duncan's multiple comparisons test revealed significant differences ($P<0.0001$) in the phagocytosis of bacteria associated with THP. When the bacteria were preincubated with THP (2.5 µg or 10 µg/0.2 ml) there were no significant differences in phagocytosis compared to bacterial suspensions without

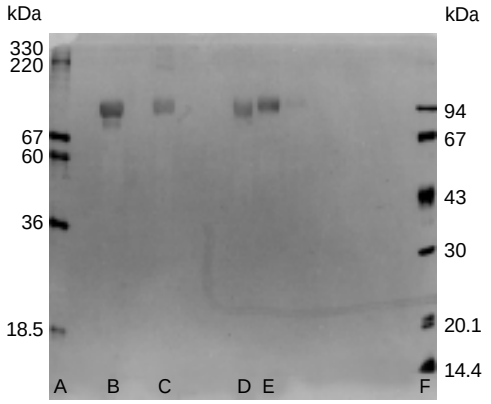

Figure 1. SDS-PAGE of purified Tamm-Horsfall protein (THP). Proteins were loaded onto 10% polyacrylamide gels in the presence of α-mercaptoethanol and stained with silver nitrate. *Lane A*, Molecular mass standards of thyroglobulin (330 kDa), ferritin-half unit (220 kDa), bovine albumin (67 kDa), catalase (60 kDa), lactate dehydrogenase (36 kDa), and ferritin (18.5 kDa); *lanes B-D*, THP purified by gel filtration chromatography on a Sepharose CL-6B column; *lane E*, standard THP; *lane F*, molecular mass standards of phosphorylase B (94 kDa), bovine albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin (20.1 kDa), and α-lactalbumin (14.4 kDa).

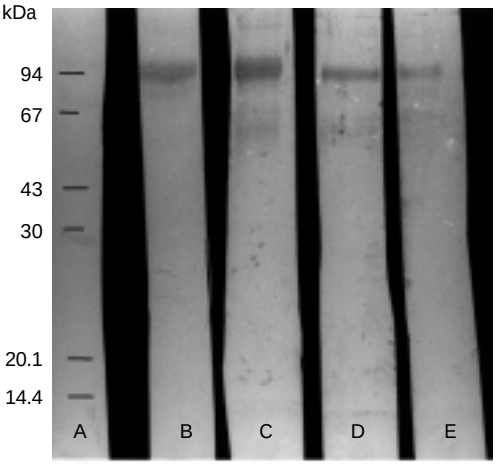

Figure 2. Western blotting of purified Tamm-Horsfall protein (THP). *Lane A*, Molecular mass standards (Pharmacia). *Lanes B* and *C*, THP purified by gel filtration and detected with mouse monoclonal anti-THP (Accurate Chemical and Scientific) and rabbit antiserum, respectively. *Lane D*, Standard THP detected with mouse monoclonal anti-THP, and *lane E*, standard THP detected with rabbit polyclonal anti-THP.

Table 1. Percentage of *E. coli* $F1^+$ phagocytosis by mouse peritoneal macrophages after incubation of bacteria with Tamm-Horsfall protein (THP).

THP (µg/0.2 ml)	Phagocytosis (%)
0	97.8
2.5	97.0
10	96.7
100	86.8
250	82.2

Differences between the experimental and control groups were compared by the Duncan multiple range test using a statistical software package (Ref. 20) ($P = 0.0001$; $F = 33.82$).

THP (Table 1). However, when the concentration of THP was increased to 100 µg and 250 µg/0.2 ml phagocytosis was significantly decreased.

Discussion

The ability of bacteria to bind to epithelial surfaces through fimbriae is correlated with their pathogenicity (1). Some strains of *E. coli* isolated from patients with lower urinary tract infections express type 1 fimbriae (2). These structures allow the bacteria to adhere to receptors containing mannose on the surface of host epithelial cells (3) and phagocytes (19). Glycoproteins present in urine can function as receptors for type 1 fimbriae and influence bacterial adhesion to the uroepithelium and to phagocytes (6,8).

As shown here, the urinary glycoprotein THP, which contains the sugar D-mannose, functions as a mannose-sensitive receptor for type 1 fimbriated *E. coli*. These results agree with the studies of Orskov (3) who suggested this binding to be mannose-sensitive. We observed this interaction at low THP concentrations (5 µg/ml), although others (21) have reported this effect only at THP concentrations up to 100 µg/ml. These same investigators observed that the receptor for $F1^+$ in urine was a low molecular weight substance. This contrasts with our results and previous reports (22) which characterized THP as a high molecular weight (94,000) glycoprotein.

THP may act as a competitive inhibitor of the binding of *E. coli* $F1^+$ to PMNL (8). PMNL are important cells in the initial stages of infection, before the formation of specific antibodies when serum opsonin levels are low.

We used mouse peritoneal macrophages to assess the effects of THP on the phagocytosis of *E. coli* $F1^+$. The phagocytosis of *E. coli* $F1^+$ varied with THP concentration. When *E. coli* $F1^+$ was preincubated with high THP concentrations (100 µg or 250 µg/0.2 ml) the phagocytosis of bacteria by macrophages decreased. In contrast, when *E. coli* $F1^+$ was preincubated with lower THP concentrations (2.5 µg and 10 µg/0.2 ml) there was no significant effect on phagocytosis.

Horton et al. (23) suggested that THP may stimulate phagocytosis since in aggregated form and at high concentrations (>500 µg THP/ml) it increased neutrophil phagocytosis and lysosome degranulation. However, these high concentrations exceed the physiological levels of THP in urine (20-200 µg/ml) and contradict results obtained by Reinhart et al. (24), who observed a decrease in the respiratory burst of PMNL after the ingestion of *E. coli* $F1^+$ bound to THP.

Our data agree with the results of Kuriyama and Silverblatt (8), who observed a decrease in bacterial phagocytosis by PMNL, even at THP concentrations <2.5 µg/ml. Nevertheless, THP did not totally inhibit bacterial binding and phagocytosis by macrophages. Multiple mannose-sensitive binding sites between $F1^+$ and THP have been suggested to contribute to bacterial binding (8). This could explain why THP only partially blocked the phagocytosis of *E. coli* $F1^+$ by mouse peritoneal macrophages since this glycoprotein may act as a competitive inhibitor of *E. coli* $F1^+$ binding to macrophage surface receptors.

These results confirm other reports (24) showing that THP inhibits binding between type 1 fimbriated *E. coli* $F1^+$ and PMN. Kuriyama and Silverblatt (8) also observed that type 1 fimbriated *E. coli*, which are usually efficiently phagocytosed by PMN, no longer become associated with these cells if they are coated with THP. This antiopsonic effect is similar to that observed when group A streptococci bind fibrinogen to their surface M protein (25).

The occurrence of THP on bladder or kidney mucosa suggests a mechanism similar to the interaction of mucus with tracheal and intestinal epithelium (26). There is still no conclusive evidence that THP occurs in

transitional epithelium *in vivo*.

Our results support the view that the soluble THP found in urine contains receptors which are recognized by type 1 fimbriae, thereby enhancing the urinary elimination of *E. coli.* However, in certain pathological states, this glycoprotein may accumulate on the renal parenchyma or perirenal tissue (27), and act as a binding site for *E. coli* $F1^+$, allowing bacterial colonization and possibly decreasing phagocytosis by macrophages.

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