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Blocking adherence of uropathogenic *Escherichia coli* isolate to HEP-2 cells and bladder of mice in the presence of antibody against p-fimbriae

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Abstract

Presence of fimbriae on *Escherichia coli* isolated from the urine of patients with urinary tract infection was related to the ability of the bacteria to attach to human uroepithelial cells. One of the 50 isolates that expresses high MRHA p-fimbriae, selected and antibody against p-fimbriae from it, showed blocking of attachment of bacteria to HEP-2 cell in 1:1024 titer. Also, 1:512 titer of this antiserum to blocking of attachment in bladder tissue of mice is significant.

Keywords: Blocking adherence; UPEC; p-Fimbriae; HEP-2 cell; Mice bladder

1. Introduction

The first step in successful colonization by many gram-negative pathogens is the adhesion to the host tissue receptors. Adhesion pili are specialized surface structures responsible for the successful recognition and binding of these bacteria to their host receptors which are at full force during the first stage of bacterial colonization. p-Fimbriae found on strains of *Escherichia coli* causing pyelonephritis (upper urinary tract infections that involve the kidneys) bind specifically to globoseries of glycolipids that are found on epithelial cells lining the urinary tract [17,22,11]. Several studies indicate a tie between adhesion and virulence of bacteria inducing infection in relation to mucous surfaces [1,6,10,32]. Approximately up to 90% of all strains isolated from urinary tract of children with acute pyelonephritis [14,31,35,12] support the concepts that p-fimbriae are involved in recurrent infections and that the organisms expressing these fimbriae could originate from the gastrointestinal tract. Urinary tract infections (UTIs) are one among the most frequent infections in both the general community and in hospital. Worldwide ca.150 million people contract UTI per year, costing approximately $6 billion. Women are more often affected than men. Depending on the location of infecting bacteria, distinct diseases can be defined, such as cystitis or pyelonephritis, with kidneys involved [13]. With *E. coli* isolated from the urine of patients with urinary tract infections, parallelism has been observed between the abilities to produce symptoms of urinary tract infection and to attach to human urinary tract epithelial cells in vitro [32,31,30]. The present study was carried out to show the relationship between piliation and adhesion of *E. coli* isolated from the urine of patients with UTI to HEP-2 cells and mice uroepithelial cells. Effect of antibody against p-fimbriae in attachment to HEP-2 cell and mice uroepithelial cell was shown.

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2. Materials and methods

2.1. Bacteria

A total 50 of *E. coli* strains were isolated from the urine of patients with urinary tract infection. Among these isolates, one was selected for the expression of p-fimbriae which had the highest expressed fimbriae.

2.2. Hemagglutination test

Mannose sensitive hemagglutination (MSHA) and Mannose resistance hemagglutination (MRHA) tests were precisely performed as described by Dozois et al. (1994). Isolates were tested for hemagglutination with human OP1 in the presence and absence of 2.5% D-mannose [4].

2.3. Culture condition

E. coli strain was subcultured to six consecutive passages at 37 °C for 24 h on tryptic soy agar (TSA) (Biolife Laboratories, Italy).

2.4. Purification of fimbriae

For maximal production of p-fimbriae and minimal production of type 1 fimbriae the MRHA isolate was subcultured on TSA. In order to enhance fimbrial production the isolate was initially passaged six times on TSA at 37 °C for 24 h. Bacterial cells were then harvested in Tris buffer (10 mM Tris–HCl, pH 7.4). Fimbriae were removed from the bacteria by heating at 56 °C for 20 min and by homogenization for 2 × 5 min on ice in sorvall omnimixer (Omni Corporative International, Waterbury, CT, USA) at 8000 rpm. The suspension was centrifuged at 8500 rpm at 4 °C for 20 min. The pellet was discarded and the fimbriae were purified from the supernatant by the method of Korhonen et al. [18]. Briefly, The fimbriae were precipitated overnight at 4 °C by adding 30% crystalline ammonium sulfate. After centrifugation at 12 000 rpm at 4 °C for 90 min. The pellet was suspended in Tris buffer and dialyzed exhaustively against Tris. They were treated with 0.25% sodium deoxycholate (DOC) for 3 days and the DOC insoluble material was removed by centrifugation. They were further purified by ultracentrifugation at 30 000 rpm at 4 °C for 2.5 h. After that, the pellet was fractionated on a sepharose 4B gel filtration column (Pharmacia, Uppsala, Sweden). The eluted fraction was concentrated and examined by TEM and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

2.5. Protein estimation

Protein was estimated through Bradford method by using bovine serum albumin as the standard.

2.6. Antisera

Polyclonal antiserum against the p-fimbriae of isolated *E. coli* was prepared in New Zealand white rabbit according to the standard procedures [33,8].

2.6.1. Absorbed antisera

To remove antibody to non-specific or common antigens, absorption was performed with homologous strain grown at 16 °C for 2 days on TSA, as described by Edwards and Ewing [8].

2.7. SDS-polyacrylamide gel electrophoresis

SDS-PAGE was performed as described by Laemmli [21]. Stacking and separating acrylamide gels of 5.0% and 12.5%, respectively, were prepared in mini-gel apparatus (Payapajoohesh Co., Mashhad, Iran).

2.8. Adherence assay to HEP-2

Adherence of UPEC to epithelial cells was studied by using cultures of human laryngeal epithelial cells-2 (HEP-2) cells grown on glass coverslips [2]. An inoculum of 10^6 CFU/ml was incubated with a monolayer of the cultured cells grown in complete Eagle's minimal essential medium (EMEM) (Biowhittaker, Walkersville, Maryland) (10% fetal calf serum, 20 mM L-glutamine, 100 mg of gentamicin per ml, 100 U penicillin G) and 0.5% D-mannose at 37 °C with 5% CO_2. *E. coli* $DH5_\alpha$ was used as a negative control in the adherence assay. At different time points of post-infection, the infected cultures were washed five times with phosphate buffered saline (PBS), fixed and prepared for hematoxylin and eosin (H&E) and Scanning Electron Microscope (SEM). To assess the effect of anti-fimbriae antibody on UPEC adherence, rabbit anti-fimbriae antiserum was added to the bacteria suspended in 1 ml of adherence medium and the bacterium–antiserum mixture was incubated at 37 °C for 30 min prior to infection of the HEP-2 cells.

2.9. SEM

Following the adherence assay, samples were fixed with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, post-fixed in 1% aqueous osmium tetroxide and dehydrated in 2,2-dimethoxypropane. Specimens were transferred to absolute ethanol, critical-point-dried using liquid carbon dioxide and were examined under a DSM940A, ZEISS Scanning Electron Microscope [27].

2.10. Murine model of ascending UTI

Female Razi mice (20–22 g, 6–8 weeks old) [Razi Vaccine & Research Institute] were inoculated transurethrally at 30 s interval with a suspension containing approximately 10^7–10^8 CFU/ml of the *E. coli* strain, using a 0.28-mm sterile polyethylene catheter connected to an infusion pump (Harvard Apparatus, Millis, Mass.) [23]. Urine samples were collected and diluted and then cultured on Plate Count Agar [24]. On day 7, mice were sacrificed and the bladders were removed. Bladder tissues were sectioned (5 μm thickness) and stained with H&E and modified gram stainings [16,3].