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Roles of Uropathogenic *Escherichia coli* Pili in Pathogenesis of Urinary Tract Infection

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Abstract: Uropathogenic *E. coli* (UPEC) strains account for 90% of all UTI and up to 50% of all nosocomial UTI. Infection is initiated when UPEC binds to the superficial epithelial cells by type 1 pili. In addition to attachment, the presence of type 1 pili can lead to bacterial invasion to bladder epithelial cells. However, P piliation of UPEC is characteristic of strains causing upper urinary tract infection as well as pyelonephritis leading to urosepsis. In this study we determine the roles of type 1 and P pili in interaction of UPEC with human polymorphonuclear leukocyts (PMN_S). Type 1 and P piliated and unpiliated strains of UPEC were used for determining the effects of these adhesins on migration of neutrophils towards bacteria in Boyden chamber. The lectinophagocytosis and intracellular killing of bacteria with purified human neutrophils were estimated by counting of the number of viable bacteria in 45 min. Type 1 piliated UPEC stimulated significantly greater chemotaxis than did P piliated, unpiliated bacteria and bacteria in which the piliation was suppressed. Phagocytosis of type 1 piliated UPEC occurred in the direct and opsonin-independent manner. In contrast, P piliated and unpiliated bacteria failed to bind to PMN_S. The results indicated that type 1 pili have a chemotatic effect and there was a positive correlation between type 1 piliation and bacterial killing by PMN_S. In contrast, PMN_S did not chemotaxis to UPEC with type P pili and unable to react with these bacteria. Therefore the expression of type P pili is critical to UPEC establishment in upper urinary tract.

Key words: Uropathogenic *E. coli*, neutrophil, type 1 pili, type P pili, phagocytosis, chemotaxis

INTRODUCTION

The interactions between the pathogen and host epithelial cells are usually mediated by adhesive structures on the surface of the bacteria that are responsible for recognizing and binding to specific receptor on host cells. The best known of bacterial adhesions are pili, which are protienaceous, filamentous organelles expressed on the surface of bacteria.

Urinary Tract Infections (UTI) are most commonly caused by Uropathogenic *E. coli* (UPEC). Adherence of bacteria is critical to prevent washout of the pathogen by the flow of urine (Sobel, 1997; Martinez *et al*., 2000). To effectively colonize and cause disease, UPEC express several classes of fimbrial adhesions that mediate attachment through specific binding to a variety of human cells (Sakarya *et al*., 2003; Hultgren *et al*., 1993). Type 1 and P pili have important roles in the pathogenesis of UTI_s. Infection is initiated when UPEC binds to the superficial epithelial cells by type 1 pili. This binding can activate complex signal transduction cascades in the host cell that can have diverse

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consequences including the activation of innate host defenses or the facilitating bacterial invasion (Hultgren *et al*., 1993). The P pili bind to receptors in the kidney epithelium and are critical for the development of pyelonephritis (Roberts *et al*., 1994; Wullt *et al*., 2000).

The presence of a large number of polymorphonuclear leukocyts (PMN_s) in the urine of patients with UTI, suggests a phagocytic activity of PMN_s constitute a crucial defense mechanism in bladder against bacterial infection (Svanborg *et al*., 1984). Type 1 pili mediate mannose-sensitive adherence to PMN_s and stimulate PMN oxidative metabolism and induce bacterial killing (Steadman *et al*., 1988; Gbarah *et al*., 1991; Sauter *et al*., 1993). In contrast, P pili do not interact with PMN (Svanborg *et al*., 1984; Wullt, 2003). Although the interaction of type 1 and P piliated UPEC with PMN_s were studied by details, the effect of these pili on chemotaxis of PMN_s has not been shown.

MATERIALS AND METHODS

Bacteria

The *E. coli* strains, ATCC35218 (type 1 pili), ATCC25922 (unpiliated), TMUP (P pili) and clinical Uropathogenic *E. coli* isolates (N1, N2 and P1, P2 which express type 1 and P pili respectively) were used in these experiments. Luria-Bertani (LB) and trypticase soy (TSB) broth medium were used for express of type 1 and P pili respectively and bacteria incubated at 35°C for 18-24 h. To repress the formation of pili, bacteria were grown at 20°C instead of 35°C in the same medium and for the same period of time (Hultgren *et al*., 1986).

Hemagglutination Assays

Slide hemagglutination (HA) assays were made by use of plate grown bacteria, 5% guinea pig erythrocytes suspension and 5% erythrocytes suspension of human P_1 erythrocytes in Phosphate-Buffered Saline (PBS), with or without mannose (Lomberg *et al*., 1986; Johnson *et al*., 1997).

Neutrophil Isolation

PMNs were obtained from heparin (20 U mL^{-1}) -anticoagulated venous blood of normal healthy volunteer. PMNs were isolated by Ficoll-Hypaque density-gradient centrifugation, dextran sedimentation of erythrocytes and selective lysis of residual erythrocytes with 0.84% ammonium chloride for 7 min.

Chemotaxis Assay

Chemotaxis of neutrophils towards chemoattractants was determined by using Boyden chamber (Neuro Probe AP48) in which PMNs are separated from the test substance by a membrane. The Boyden chamber were loaded triplicate with 26 μL of each piliated and unpiliated bacteria (1.5×10^8 cfu mL^{-1}), FMLP (N-formyl-L-methionyl-L-leucyl-L- phenylalanine, 10^{-8} ; Sigma) as the positive control and HBSS as the negative control(random migration).

A filter (Cellulose nitrate) was placed over the plate and 50 μL of a suspension that contained 10^6 PMN mL^{-1} was loaded onto the filter above each well. The chamber was incubated at 37°C with 5% CO_2 for 55 min. Excess cell were removed, after which filters were stained with Hematoxylin and cleared with xylene. Cells at the lower margin of the filter were counted under ×100 magnification. Twenty fields were examined at random by experienced observers. The result for each condition was calculated by averaging triplicate determinations. Migration under each condition was reported as the percentage of cells at the margin after exposure to FMLP.

Phagocytosis and Killing

Each of piliated and unpiliated bacteria was added separately to PMN suspension in HBSS (3×10^6 cells mL^{-1}) to yield a ratio of 10:1 and the tubes were incubated at 37°C on a gyratory shaker

at 150 rpm. Aliquots were removed at time 0, 15, 30 and 45 min and diluted in 1 mL distilled water to disrupt PMN. Then the number of viable bacteria was determined by colony counting method on blood agar.

Statistics

Student-t test was used for the generation of $p<0.05$ values.

RESULTS

Chemotaxis Assay

FMLP served as the internal laboratory reference and which all samples would be compared. Chemotaxis in response to *E. coli* ATCC35218 and two clinical isolates which posses type 1 pili was 56-73% of that observed with FMLP. As shown in Fig. 1, chemotaxis to HBSS (random migration) was 39.8% of that induced by FMLP. Differences among the type 1 piliated strains and HBSS were significant ($p>0.05$). In contrast, chemotaxis induced by *E. coli* ATCC25922 (unpiliated strain), type P piliated and bacteria in which the piliation was suppressed, ranged 35-40% of that induced by FMLP. A difference among the type P piliated, unpiliated strains and HBSS was not significant. There was significant difference between piliated strain (*E. coli* ATCC35218) and unpiliated strain (*E. coli* ATCC25922). After suppression of pili, chemotaxis induced by *E. coli* ATCC35218 and two clinical isolates(N1 and N2) was reduced significantly.

Bacterial Phagocytic Killing

Time-dependent killing of type 1 piliated strains occurred during incubation with PMN: at 30 min, ~ 45-64% of microorganisms were killed (Fig. 2). In contrast, the number of viable P piliated and unpiliated *E. coli* ATCC25922 not only did not reduce, but also increased. There were significant differences between type 1 piliated and P piliated bacteria. After suppression of pili, killing of *E. coli* ATCC35218 and two clinical isolates (N1 and N2) did not occurred under these conditions (Fig. 3). Differences between the numbers of viable bacteria in 30 min in type 1 piliated strains in compared to the strains in which the piliation was suppressed were significant ($p>0.05$).

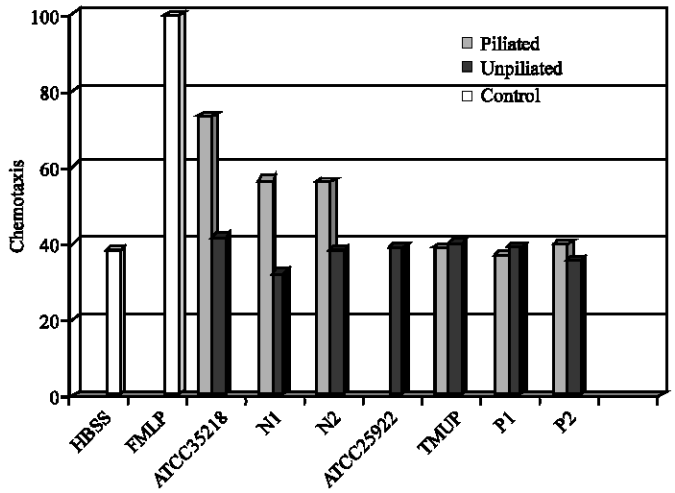

Fig. 1: Chemotaxis induced by piliated and unpiliated UPEC. Percentage of chemotaxis was stimulated by FLMP. HBSS (random migration), FMLP (chemotactic factor)

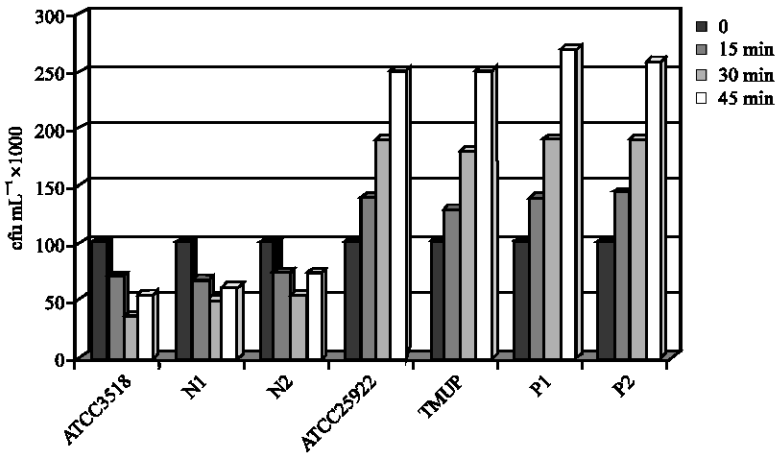

Fig. 2: PMN-mediated killing of piliated UPEC

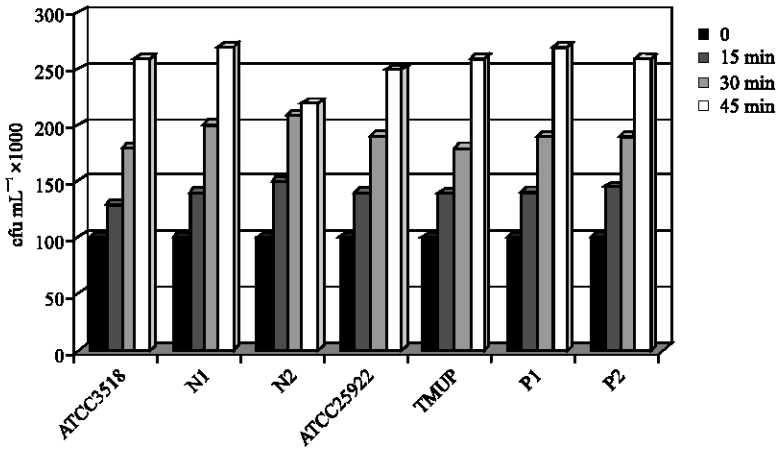

Fig. 3: PMN-mediated killing of the UPEC in which the piliation was suppressed

DISCUSSION

The pathogenesis of urinary tract infections caused by UPEC has been linked to encounter of microorganisms with host innate immune responses. Although it has been reported that type 1 pili have mediated adherence of UPEC to PMNs (Steadman *et al*., 1988; Gbarah *et al*., 1991; Sauter *et al*., 1993; Svanborg *et al*., 1984; Wullt, 2003), it was not known they also have chemotactic effects. We therefore studied the chemotaxis of UPEC to PMNs with only type 1 pili, only P pili and bacteria in which the piliation was suppressed. The hemagglutination assays were made to check of presence of pili. The results show a direct correlation between the presence of type 1 piliation and migration of PMNs (Fig. 1). When bacteria which had been grown at a pilus-restrictive temperature were used, it was found that bacteria in the unpiliated form did not stimulate of PMNs (Fig. 1). The direct effect of type 1 pili on chemotaxis of PMNs causes rapid recruitment of neutrophils into the site of infection, resulting in early clearance of bacteria. In additional of direct chemotactic effect, type 1 piliated UPECs

can attach to epithelial and mast cells, with resultant activation of target cells and production of high levels of certain cytokines, in particular TNF-α and IL-6 (Schiling *et al*., 2001; Schiling, 2006). These cytokines enhance the PMNs recruitment into the site of infection.

The ability of human neutrophils to kill UPEC *in vitro* and the roles of pili in this process were analyzed by two sets of experiment. There was a difference in susceptibility to killing between the type 1 piliated and P piliated bacteria (Fig. 2). We have determined that type 1 pili promote direct, opsonin-independent binding of UPEC to PMNs. In the absence of antibody and complement, phagocytes may recognize bacteria by lectin-carbohydrate interactions (Hultgren *et al*., 1986; Ofek and Sharon, 1988). The interaction of type 1 piliated UPEC with PMN_s involves mannose-containing structures and leukocyte integrins (CD11/18, CD66) act as major receptors (Gbarah *et al*., 1991; Sauter *et al*., 1993; Rosen, 2004). As shown in Fig. 2, about 45-64% of type 1 piliated microorganisms were killed at 30 min. The increase of the number of viable type 1 piliated bacteria in the last 15 min may be due to decrease in phagocytosis rate and multiplying the remained bacteria in suspension. In contrast to type 1 piliated bacteria, P piliated and unpiliated bacteria were not react with PMNs and resist to PMN-mediated killing. As has been previously shown (Hultgren *et al*., 1986; Sauter *et al*., 1993), there was a positive correlation between type 1 piliation and bacterial killing by PMNs. The histological studies on experimental UTI models have demonstrated that PMNs phagocytosis to take place in the epithelial lining or subepithelial tissue (Bryant *et al*., 1973; Rosen, 2004).

In general, activation of PMNs by type 1 pili would have adverse consequences for this microorganism. Bacterial killing is significantly greater for type 1 bearing bacteria. However, phase variation of type 1 pili has been proposed as a mechanism whereby a fraction of the bacterial population may avoid of interactions with PMNs (Eisenstein, 1981). Furthermore, type 1 mediated invasion to uroepithelial cells is the other mechanisms, that may provide a persistence advantage in urinary tract by allowing type 1 piliated UPEC to internalized and survival in intracellulary (Martinez *et al*., 2000; Mulvey *et al*., 2000). On the other hand, ability of the bacterium to adhere to epithelial cells in the human kidney by P pili has been shown to be critical for development of pyelonephritis (Roberts *et al*., 1994; Wullt *et al*., 2000).

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