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Influence of Type 1 Piliation on Chemotaxis and Intracellular Killing of Uropathogenic *E. coli* by Human Polymorphonuclear Leukocyts

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Abstract: Uropathogenic *Escherichia coli* (UPEC) that cause urinary tract infections bind to target cells via several distinct paris of adhesins and receptors. In this study we determine the role of type 1 pili in interaction of UPEC with human neutrophils. Type 1 piliated and unpiliated strains (obtained by growth at a pilus-restrictive temperature) of UPEC were used for determining the effect of this adhesin 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. The results indicate that type 1 piliated UPEC stimulated significantly greater chemotaxis than did 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, unpiliated bacteria failed to bind to PMN.

Key words: UPEC, PMN, type 1 pili, phagocytosis, chemotaxis

INTRODUCTION

Urinary tract infections (UTI) are one of the most common infectious diseases in man with a prevalence strongly influenced by gender and age (Schaefer *et al*., 2001). Uropathogenic *E. coli* (UPEC) is the most common pathogen in UTI. Infection is initiated when UPEC binds to the superficial epithelial cells by type 1 pili. Adherence of bacteria is critical to prevent washout of the pathogen by the flow of urine. In addition to binding, type 1 pili may promote bacterial invasion and growth as a biofilm (Sakarya *et al*., 2003; Martinez *et al*., 2000). However, binding and invasion of bacteria also activates a cascade of innate host defenses (Mulvey *et al*., 2000). The presence of a large number of polymorphonuclear leukocyts (PMN) in the urine of patients with UTI, suggests a phagocytic activity of PMN constitutes a crucial defense mechanism in bladder against bacterial infection (Svanborg *et al*., 1984). Type 1 pili mediate attachment of UPEC to phagocytic cells by lection-carbohydrate interactions, resulting in lectinophagocytosis (Gbarah *et al*., 1991). Such interaction stimulates nonopsonic PMN oxidative metabolism and induces bacterial killing (Goetz and Silveblatt, 1987; Steadman *et al*., 1988). Although the interaction of type 1 piliated UPEC with PMN was studied by details, the effect of these pili on chemotaxis of PMN has not been shown.

MATERIALS AND METHODS

Bacterial strains: The standard strains of *E. coli*, ATCC 35218 which forms type 1 pili and ATCC 25922 which is unpiliated and three clinical Uropathogenic *E. coli* isolates (N1, N2, N3) which formed type 1 pili, based on hemagglutination (HA) assay, were used.

Hemagglutination assays: Formation of type 1 pili was identified by mannose-sensitive hemagglutination (MSHA) of 5% guinea pig erythrocytes suspension in phosphate-buffered saline (PBS) (Johnson *et al*., 1997; Steadman *et al*., 1988). The inhibition effect mannose on H was assessed by incubating the LB grown bacteria in PBS containing 1-2% mannose for 10 min at room temperature before HA reaction (Lomberg *et al*., 1986).

Induction and repression of piliation: For inducing the formation of type 1 pili, all strains were grown in LB broth

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at 37°C for 24 h. To repress the formation of pili, bacteria were grown at 20°C instead of 37°C in the same medium and for the same period of time (Hultgren *et al*., 1986).

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 like type 1 pili 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 LB grown piliated and unpiliated bacterial (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 strains 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^{-1} 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* ATCC 35218 and three clinical isolates which posses type 1pili was 46-73% 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 piliated strains and HBSS were significant ($p<0.05$). In contrast, chemotaxis induced by *E. coli* ATCC 25922 (unpiliated strain) and bacteria in which the piliation was suppressed, ranged 32-41% of that induced by FMLP. A difference among the unpiliated strains and HBSS was not significant. There was significant difference between piliated strain (*E. coli* ATCC 35218) and uppiliated strain (*E. coli* ATCC 25922). After suppression of pili, chemotaxis induced by *E. coli* ATCC 35218 and three clinical isolates was reduced significantly.

Bacterial phagocytic killing: Time-dependent killing of type 1 piliated strains occurred during incubation with PMN: at 30 min, ~40-70% of microorganisms were killed (Table 1). In contrast, the number of viable unpiliated *E. coli* ATCC 25922 not only did not reduce, but also increased. There were significant differences between piliated bacteria and unpiliated. After suppression of pili, killing of *E. coli* ATCC 35218 and three clinical isolates did not occurred under these conditions (Table 2). Differences between the numbers of viable bacteria in

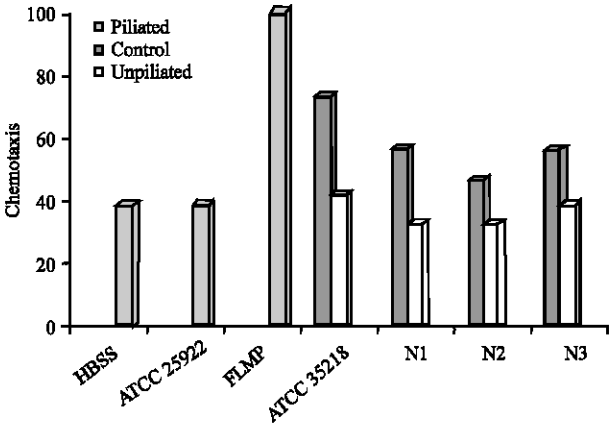

Fig. 1: Chemotaxis induced by piliated and unpiliated UPEC. Percentage of chemotaxis stimulated by FLMP

Table 1: PMN-mediated killing of type 1 piliated UPEC

	Time (min)			
Strain	0	15	30	45
ATCC 35218	10×10^4	7.2×10^4	3.6×10^4	5.5×10^4
ATCC 25922	10×10^4	14×10^4	19×10^4	25×10^4
N1	10×10^4	6.9×10^4	5.0×10^4	6.3×10^4
N2	10×10^4	8.6×10^4	6.8×10^4	8.8×10^4
N3	10×10^4	7.5×10^4	5.5×10^4	7.5×10^4

Table 2: PMN-mediated killing of unpiliated UPEC

	Time (min)			
Strain	0	15	30	45
ATCC 35218	10×10^4	13×10^4	18×10^4	26×10^4
ATCC 25922	10×10^4	14×10^4	19×10^4	25×10^4
N1	10×10^4	14×10^4	20×10^4	27×10^4
N2	10×10^4	13×10^4	17×10^4	23×10^4
N3	10×10^4	15×10^4	21×10^4	22×10^4

30 min in type 1 piliated strains in compared to the strains in which the piliation was suppressed were significant ($p<0.05$).

DISCUSSION

The innate immune response provides an early defense against bacterial infection, which serves to limit bacterial proliferation, localized the infection and also both activates and regulate the subsequent adaptive immune response. After bacterial challenge and the activation of uroepithelial cell, a chemotactic gradient of cytokines and inflammatory mediators is created in submucosa, which activates circulating neutrophils. The results of this study showed that the type 1 piliated UPEC could stimulate neutrophils directly. These data demonstrated that the migration of neutrophils towards type 1 piliated *E. coli* was significantly higher than unpiliated bacteria (Fig. 1). The observation that the blocking of type 1 pili affected significantly results of chemotaxis assay (~14-32%) indicated that the type 1 pili have a chemotatic effect.

The ability of human neutrophils to kill UPEC *in vitro* and the role of type 1 pili in this process were analyzed by three sets of experiment. There was a difference in susceptibility to killing between the type 1 piliated bacteria and unpilated bacteria. We have determined that type 1 pili promote direct, opsonin-independent binding of UPEC to PMN. In the absence of anitibody 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 PMNs involves mannose-containing structures and leukocyte integrins (CD11/18, CD66) act as major receptors (Gbarah *et al*., 1991; Rosen, 2004; Sauter *et al*., 1993). As shown in Table 1, about 40-70% of type 1 piliated micro organisms 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, unpiliated bacteria were not react with PMN 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 piliation and bacterial killing by PMN. The histological studies on experimental UTI models have demonstrated that PMN phagocytosis to take place in the epithelial lining or subepithelial tissue (Bryant *et al*., 1973; Connell *et al*., 2000). In the urine, the phagocytic function of PMN was suppressed by its extremes of osmolarity, pH and urea concentrations (Ofek and Sharon, 1988; Iwahi and Imada, 1988). Local production of IL-6 and IL-8 occurs in response to interaction of UPEC with uroepithelial cell, which facilitated and enhanced of migration of PMN to infection site (Wullt, 2003). Phagocytosis of bacteria results in the release of toxic molecules that kill and digest of bacteria. The extracellular release of toxic components may occur in this process that results in inflammation and damage to host tissues. The outflow of a large number of PMN in urine may be a response to prevent of these events.

REFERENCES

Bryant, R., M. Sutcliffe and Z. Megee, 1973. Human polymorphonuclear leukocyte function in urine. Yale. J. Biol. Med., 46: 113-124.

Connell, H., L. Poulsen and P. Klemm, 2000. Expression of type 1 and P fimbriae *in situ* and localization of uropathogenic *E. coli* strains in the murine bladder and kidney. Int. J. Med. Microbiol., 290: 587-597.

Gbarah, A., C.G. Gahmberg, I. Ofek, U. jacobi and N. Sharon, 1991. Identification of the leukocyte adhesion molecules CD11 and CD18 as receptors for type 1 fimbriated *E. coli*. Infect. Immun., 59: 4524-4530.

Goetz, M.B. and F.J. Silverblatt, 1987. Stimulation of human polymorphonuclear leukocyte oxidative metabolism by type 1 pili from *Esherichia coli*. Infec. Immun., 55: 534-540.

Hultgren, S., W. Schwan, A. Schaeffer and J. Duncan, 1986. Regulation of production of type 1 pili among urinary tract isolates of *Escherichia coli*. Infect. Immun., 54: 613-620.

Iwahi, T. and A. Imada, 1988. Interaction of *Escherichia coli* with polymorphonuclear leukocytes in pathogenesis of urinary tract infection in mice. Infect. Immun., 56: 947-953.

Johnson, J., J. Swanson, T. Barela and J. Brown, 1997. Receptor Specificities of variant Gal (α1-4) Gal-Binding PapG adhesins of uropathogenic *Escherichia coli* as assessed by hemagglutination phenotypes. J. Infect. Dis., 175: 373-381.

Lomberg, H., B. Cedergren, H. Leffler, B. Nilsson, A. Carlstio and C. Svanborg Eden, 1986. Influence of blood group on the availability of receptors for attachment of uropathogenic *Escherichia coli*. Infect. Immun., 51: 919-926.

Martinez, J.J., M.A. Mulvey and J.D. Schiling *et al*., 2000. Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. Embo. J., 19: 2803-2812.

Mulvey, M., J. Schilling, J. Martinez and S. Hultgren, 2000. Bad bugs and beleaguered bladders: Interplay between uropathogenic *Escherichia coli* and innate host defenses. Proc. Nat. Acad. Sci., 97: 8829-8835.

Ofek, I. and N. Sharon, 1988. Lectinophagocytosis a molecular mechanism of recognition between cell wall surface sugars and lectins in the phagocytosis of bacteria. Infect. Immunol., 56: 539-547.

Rosen, H., 2004. Bacterial response to neutrophil phagocytosis. Curr. Opin. Hematol., 11: 1-6.

Sakarya, S., G. Ertem, S. Oncu, I. Kocak, N. Erol and S. Oncu, 2003. *Escherichia coli* bind to urinary bladder epithelium through nonspecific Sialic acid mediated adherence. FEMS Immun. Med. Microbiol., 39: 45-50.

Sauter, S.L., S.M. Rutherfrd, C. Wagener, J.E. Shively and S.A. Hefta, 1993. Identification of the specific oligosaccharide sites recognized by type 1 fimbriae from *E. coli* on nonspecific cross-reacting antigen, a CD66 cluster granulocyte glycoprotein. J. Biol. Chem., 268: 15510-15516.

Schaefer, A.J., N. Rajan, Q. Cao, B.E. Andrson, D.L. Pruden, J. Sensibar and J.L. Duncan, 2001. Host pathogenesis in urinary tract infections. Int. J. Antimicrob Agents, 17: 245-251.

Steadman, R., N. Ropley, D.E. Jenner, M. Davies and J.D. Williams, 1988. Type 1 fimbriate *Esherichia coli* stimulate a unique pattern of degranulation by human polymorphonuclear leukocytes. Infec. Immun., 56: 815-822.

Svanborg, E.C., L. Bjursten, R. Hull, K. Magnusson, Z. Moldovano and H. Leffler, 1984. Influence of adhesins on the interaction of *Escherichia coli* with human phagocytes. Infect. Immun., 44: 672-680.

Wullt, B., 2003. Erratum to the role of P fimbriae for *E. coli* establishment and mucosal inflammation in the human urinary tract. Int. J. Antimicrobiol. Agents, 21: 605-621.