INFECTION AND IMMUNITY, Dec. 1979, p. 837-840
0019-9567/79/12-0837/04$02.00/0

Vol. 26, No. 3

Attachment of *Escherichia coli* to Urinary Sediment Epithelial Cells from Urinary Tract Infection-Prone and Healthy Children

C. SVANBORG-EDÉN[1]* AND U. JODAL[2]

Departments of Clinical Immunology[1] and Pediatrics,[2] University of Göteborg, Göteborg, Sweden

Received for publication 10 July 1979

Escherichia coli isolated from patients with recurrent urinary tract infections were tested for ability to attach to human urinary sediment epithelial cells in vitro. Higher mean capacity to bind bacteria was found for epithelial cells of the patient from whom the *E. coli* strain had been isolated than for epithelial cells from subjects without a history of urinary tract infection. The two populations were age matched. No relation was found between the age of the cell donor (0 to 15 years) and the capacity for *E. coli* attachment.

Escherichia coli causing urinary tract infections (UTI) often originate in the stool (4, 19, 20). Via colonization of the vaginal introitus (12) and the periurethral area (2), bacteria ascend the urinary tract. The resulting bacteriuria may either be symptomatic, as in acute pyelonephritis or acute cystitis, or may only be detected at screening, as in asymptomatic bacteriuria.

E. coli causing symptomatic UTI differ in several ways from *E. coli* causing asymptomatic UTI (5). For example, *E. coli* isolated from the urine of patients with acute pyelonephritis and acute cystitis attach in larger numbers to human urinary tract epithelial cells in vitro than do *E. coli* from patients with asymptomatic bacteriuria or cells from the stools of non-bacteriuric children (16, 17).

The tendency to become colonized in the vaginal introitus and the periurethral area may be an important host factor determining the susceptibility to recurrent UTI. Thus, women with recurrent UTI show a higher incidence of vaginal colonization and a more prolonged carriage of enterobacteria in the vagina (11). Vaginal cells from UTI-prone women show greater ability to bind bacteria in vitro than do cells from healthy subjects (3), and more bacteria attached to periurethral cells from children with recurrent UTI than to cells from children without UTI (7). These findings were not reproduced when radioactivity instead of direct light microscopy was used to quantitate bacterial adhesion (10).

The present report compares the in vitro attachment of *E. coli* to urinary sediment epithelial cells from children with recurrent UTI and from controls without a history of UTI. The *E. coli* strains used were isolated from each patient during an earlier episode of UTI. An attempt is also made to analyze the recovery of urinary sediment epithelial cells in relation to age.

MATERIALS AND METHODS

Study population. Ninety-six children, 87 girls and 9 boys, 6 months to 16 years old (mean, 5 years), with recurrent UTI were included in the study. The children attended the UTI outpatient clinic at the Children's Hospital, Göteborg, either for monitoring after treatment of UTI or as part of a UTI follow-up program. The subjects without a history of UTI included 109 children, 68 girls and 41 boys, 6 months to 16 years of age (mean, 5 years).

Urinary sediment epithelial cells from one non-bacteriuric adult female donor, used in several previous studies, were used as reference cells (for a review, see reference 14). The cells were harvested from fresh morning urine and treated the same way as the patient and control cells.

Epithelial cells. About 30 ml of morning urine from each child was transported to the laboratory within 4 h of delivery. Sterility controls were done by inoculating one calibrated loopful (0.003 ml) of urine on blood agar plates. After centrifugation for 10 min at 250 × *g*, the urine was decanted. The number of epithelial cells in the remaining sediment was determined, and the cells were checked for presence of attached bacteria by direct light microscopy, using a Bürker chamber. The sedimented cells were then used for adhesion testing.

Bacteria. An *E. coli* strain had been isolated from the urine of each patient at each episode of *E. coli* bacteriuria. The criteria used to distinguish among acute pyelonephritis, acute cystitis, and asymptomatic bacteriuria have been described (9). The O and K antigens had been determined (6, 8), and the strains had been stored in deep agar. An *E. coli* strain isolated at an episode of symptomatic UTI was considered more likely to have adhesive properties and was preferred when a strain from each patient was chosen for adhesion testing. The *E. coli* strains were then trans-

ferred from deep-agar cultures to lactose-bromothymol blue agar plates, from which brain heart infusion broth cultures were prepared.

Adhesion testing. Adhesion testing was done as previously described (15). Bacteria were harvested from a 16-h broth culture by centrifugation, suspended in phosphate-buffered saline (PBS; pH 7.2, 300 mosmol/liter), and quantitated by direct light microscopy. To 10^8 bacteria were added 10^5 epithelial cells and PBS to a volume of 1 ml. After incubation during rotation (20 rpm, 37°C, 60 min), unattached bacteria were eliminated by repeated washing with PBS. Attachment was measured as the mean number of bacteria attached to 40 epithelial cells, using light microscopy. When only 10^4 to 10^5 epithelial cells were available from the urine of patients or controls, the same number of cells from the test person was added in order to avoid difference in attachment due to different concentrations of epithelial cells in the adhesion test.

The ability of epithelial cells from each UTI or non-UTI subject to bind bacteria was always tested simultaneously with that of cells from the reference, providing paired values. The cells from UTI patients were always tested against an *E. coli* strain isolated from the same patient at an earlier episode of bacteriuria. From the group of *E. coli* strains used to test the patient cells, a strain was randomly chosen to test against the epithelial cells of the non-UTI subjects.

Population analyses. The criteria used to select urine samples for adhesion testing were: no bacteriuria; $\geq 10^4$ epithelial cells recovered from 30 ml of urine; no bacteria on the epithelial cells; and transport to the laboratory within 4 h of delivery. Samples not fulfilling those criteria were excluded. In addition, samples were excluded because of crystalluria (Table 1). Forty-nine of the patient samples fulfilled these criteria; 47 were excluded. Of the 109 non-UTI subjects, 51 fulfilled the selection criteria and were included in the study. The reasons for exclusion of the remaining 58 are summarized in Table 1.

Statistical analysis. The χ^2 test was used for all statistical comparisons.

RESULTS

Yield of urinary sediment epithelial cells. The number of urine samples excluded because they contained $<10^4$ epithelial cells was similar for each age group of UTI and non-UTI subjects (Table 1). The yield of sedimented epithelial cells in each age group was also similar for both subject groups (Table 2). The youngest children (≤ 7 years) generally had fewer urinary sediment epithelial cells than did children >7 years old (Table 2). In the non-UTI subject group, 25 of 41 samples from boys (61%) and 26 of 68 samples from girls (38%) were suitable for use.

Attachment to the urinary sediment epithelial cells of UTI patients. Figure 1 shows the distribution of paired mean adhesion values for cells from UTI patients and the reference, using the patient's own infecting strain. Thirty-two of the 57 strains attached better to patient cells, and two strains attached better to the reference cells ($P < 0.01$) (Table 3). Seven strains not adhering to the reference cells attached to the patient cells. The remaining 23 strains lacked any adhesive ability.

Attachment to the urinary sediment epithelial cells of the non-UTI subjects. Figure 2 shows the distribution of paired mean adhesion values for epithelial cells from non-UTI subjects and the reference, using the same population of strains as shown in Fig. 1. No significant difference in ability to bind bacteria was found between the two types of cells. Eighteen of the 57 strains attached better to non-UTI subject cells (Table 3). No difference in ability to bind bacteria was found between epithelial cells from non-UTI boys and girls. No relationship was found between the age of the cell donor and the ability of cells to bind bacteria.

DISCUSSION

Many patients with recurrent UTI have no obstructions of the urine flow or other evident defects that would explain their increased susceptibility to UTI, whereas healthy individuals without UTI can carry fecal *E. coli* strains with

TABLE 1. *Analysis of urine samples from patients and control subjects included in or excluded from the study*

Subjects	Determination					
	No. included	No. excluded	No. with $<10^4$ epithelial cells	No. with bacteria on cells	No. with bacteriuria	No. with crystalluria
UTI						
<7[a]	29	34	27	2	2	3
8–11	14	6	4			2
≥12	6	7	3	4		
Non-UTI						
<7	31	44	25	6	1	12
8–11	13	7	3	1		3
≥12	7	7	3	1	1	2

[a] Age in years.

TABLE 2. *Recovery of epithelial cells from the sediment of UTI and non-UTI subjects*

Age (years)	No. of subjects with given number of epithelial cells					
	UTI			Non-UTI		
	$<10^4$	10^4-10^5	$\geq 10^5$	$<10^4$	10^4-10^5	$\geq 10^5$
<7	27 (43)[a]	31 (49)	5 (8)	26 (46)	24 (43)	6 (11)
8-11	4 (20)	12 (60)	4 (20)	1 (10)	6 (60)	3 (30)
≥12	3 (23)	4 (31)	6 (46)	4 (31)	6 (46)	3 (23)

[a] Numbers in parentheses are percentages.

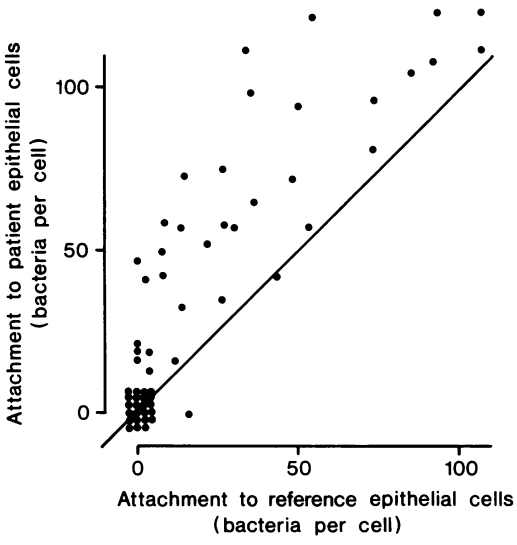

FIG. 1. *Attachment of E. coli to sedimented epithelial cells of patients with recurrent UTI and to cells from the reference person, without a history of UTI. The cells of each patient and of the reference person were tested pairwise for capacity to bind bacteria, using the patient's own strain isolated at an earlier episode of UTI. The line on the graph is a line of equivalence.*

TABLE 3. *Attachment to sedimented epithelial cells of study subjects relative to reference cells*

Subjects	Lower	Equal[a]	Greater
UTI	2	23	32[b]
Non-UTI	14	25	18

[a] Mean adhesion value within ±10% of the value for the reference cells.

[b] $\chi^2 = 10.9$ ($P < 0.01$).

several known virulence factors, which are then potential urinary tract pathogens. UTI patients seem to first become colonized by the *E. coli* strain in the vaginal introitus and the periurethral area and then develop bacteriuria. One host factor suggested to explain the increased frequency of vaginal and periurethral colonization by *E. coli* and subsequent UTI in susceptible patients is the receptivity of epithelial cells to bacterial attachment (3, 7, 11).

The present study partly confirms these findings, using urinary tract epithelial cells from the sediment of urine for adhesion testing. The ability of each patient's cells to bind attaching bacteria was tested with the *E. coli* strain isolated from that patient at an earlier episode of UTI. The attachment to the patient cells was consistently higher than to the reference cells. When the same group of *E. coli* strains was tested against epithelial cells from age-matched subjects without a history of UTI and the reference cells, no significant difference in attachment was found. The findings indicate that the capacity to bind bacteria is normally distributed in the population not prone to UTI. The children with recurrent UTI seem to represent a population selected partly because of high capacity of their epithelial cells to bind bacteria. It is therefore unlikely that a structure responsible for the binding of bacteria is present only on epithelial cell surfaces of UTI-prone patients. Rather, the density of the ligand may vary and explain the differences found both within the group of non-UTI subjects and, especially, between patients and the reference. The only structure so far isolated from human urinary tract epithelial cells with the potential to be a receptor is a glycolipid (H. Leffler and C. Svanborg-Edén, submitted for publication). Mannose-containing compounds or sialic acid may also bind certain *E. coli* causing UTI (T. Korhonen and C. Svan-

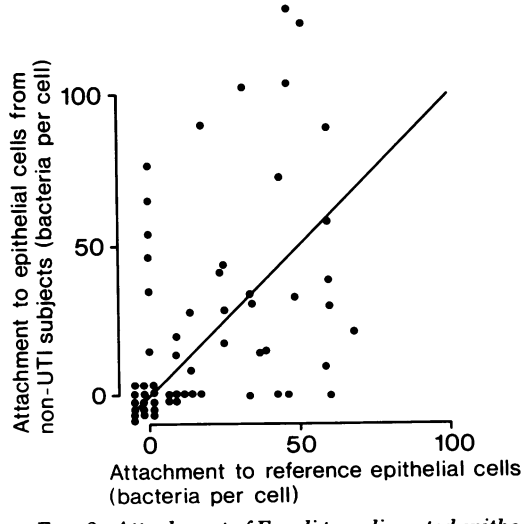

FIG. 2. *Attachment of E. coli to sedimented epithelial cells of subjects without a history of recurrent UTI and to cells from the reference person. The E. coli strains used are the same as those in Fig. 1. The line on the graph is a line of equivalence.*

borg-Edén, submitted for publication).

Some people may be prone to UTI partly because of their epithelial cell properties, but other host factors, such as local antibodies, are likely to influence vaginal colonization and subsequent UTI. More vaginal antibodies coating fecal *E. coli* were found in healthy women than vaginal antibodies coating the *E. coli* strain causing UTI in UTI-susceptible women (13). Recently, we reported that urinary antibodies of immunoglobulin G and secretory immunoglobulin A classes are capable of inhibiting the attachment of *E. coli* to human urinary sediment epithelial cells (18). Antibodies to the O antigen were efficient in this respect. Since UTI reinfections usually are caused by an *E. coli* strain of an O group different from that causing the preceding infection (1), it is possible that urinary antibodies, preventing colonization by inhibiting attachment, influence the selection of the reinfection strain. The protective value of antibodies against the bacterial structure mediating bacterial attachment to human mucosal epithelia remains to be demonstrated.

ACKNOWLEDGMENTS

The skillful technical assistance of A.-M. Månqvist and H. Lomberg is appreciated.

This study was supported by grants from the Swedish Medical Research Council (project no. 215), the University of Göteborg, and the Ellen, Walter and Lennart Hesselman Foundation for Scientific Research.

LITERATURE CITED

1. **Bergström, T., K. Lincoln, F. Ørskov, I. Ørskov, and J. Winberg.** 1967. Studies of urinary tract infection in infancy and childhood. VIII. Reinfection vs relapse in recurrent urinary tract infection. Evaluation by means of identification of infecting organisms. J. Pediatr. **71:** 13-20.
2. **Bollgren, I., and J. Winberg.** 1976. The periurethral aerobic flora in girls highly susceptible to urinary tract infections. Acta Paediatr. Scand. **65:**81-87.
3. **Fowler, J. E., and T. A. Stamey.** 1977. Studies of introital colonization in women with recurrent urinary tract infection. VII. The role of bacterial adherence. J. Urol. **117:**472-476.
4. **Grüneberg, R. N.** 1969. Relationship of infecting urinary organisms to the faecal flora in patients with symptomatic urinary infection. Lancet **ii:**766-768.
5. **Hanson, L. Å., S. Ahlstedt, A. Fasth, U. Jodal, B. Kaijser, A. Sohl Åkerlund, and C. Svanborg Edén.** 1977. Antigens of *E. coli* human immune response and the pathogenesis of urinary tract infections. J. Infect. Dis. **136** (Suppl.):S144-S149.
6. **Kaijser, B.** 1977. A simple method for typing of acidic polysaccharide K antigen of Escherichia coli. FEMS Microbiol Lett. **1:**285-288.
7. **Källenius, G., and J. Winberg.** 1978. Bacterial adherence to periurethral epithelial cells in girls prone to urinary tract infections. Lancet **ii:**540-543.
8. **Lidin Janson, G., E. Falsen, U. Jodal, B. Kaijser, and K. Lincoln.** 1977. Characteristics of antibiotic resistant Escherichia coli in the rectum of healthy school children. J. Med. Microbiol. **10:**299-308.
9. **Lindberg, U., L. Å. Hanson, U. Jodal, G. Lidin Janson, K. Lincoln, and S. Olling.** 1975. Asymptomatic bacteriuria in schoolgirls. II. Differences in *E. coli* causing asymptomatic bacteriuria. Acta Paediatr. Scand. **64:**432-436.
10. **Schaffer, A. J., S. K. Amundsen, and L. N. Schmidt.** 1979. Adherence of *Escherichia coli* to human urinary tract epithelial cells. Infect. Immun. **24:**753-759.
11. **Stamey, T. A., and C. C. Sexton.** 1975. The role of vaginal colonization with enterobacteriacea in recurrent urinary infections. J. Urol. **113:** 214-217.
12. **Stamey, T. A., M. Timothy, M. Miller, and G. Mihara.** 1971. Recurrent urinary infections in adult women. The role of introital enterobacteria. Calif. Med. **115:**1-19.
13. **Stamey, T. A., N. Wehner, G. Mihara, and M. Condy.** 1978. The immunologic basis of recurrent bacteriuria: role of cervicovaginal antibody in enterobacterial colonization of the introital mucosa. Med. **57:**47-56.
14. **Svanborg Edén, C.** 1978. Attachment of *Escherichia coli* to human urinary tract epithelial cells. Scand. J. Infect. Dis. **15**(Suppl.):1-70.
15. **Svanborg Edén, C., B. Eriksson, and L. Å. Hanson.** 1977. Adhesion of *Escherichia coli* to human uroepithelial cells in vitro. Infect. Immun. **18:**767-774.
16. **Svanborg Edén, C., B. Eriksson, L. Å. Hanson, U. Jodal, B. Kaijser, G. Lidin Janson, U. Lindberg, and S. Olling.** 1978. Adhesion to normal human uroepithelial cells of *Escherichia coli* from children with various forms of urinary tract infection. J. Pediatr. **93:** 398-403.
17. **Svanborg Edén, C., L. Å. Hanson, U. Jodal, U. Lindberg, and A. Sohl Åkerlund.** 1976. Variable adherence to normal human urinary tract epithelial cells of *Escherichia coli* strains associated with various forms of urinary tract infections. Lancet **ii:**490-492.
18. **Svanborg Edén, C., and A.-M. Svennerholm.** 1978. Secretory immunoglobulin A and G antibodies prevent adhesion of *Escherichia coli* to human urinary tract epithelial cells. Infect Immun. **22:**790-797.
19. **Turck, M., R. G. Petersdorf, and M. R. Fournier.** 1962. The epidemiology of non-enteric *Escherichia coli* infections: prevalence of serologic groups. J. Clin. Invest. **41:** 1760-1765.
20. **Vosti, K. L., L. M. Goldberg, A. S. Monto, and L. A. Rantz.** 1964. Host parasite interaction in patients with infections due to *E. coli* from intestinal and extraintestinal sources. J. Clin. Invest. **73:**2377-2385.