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Enhancement of *Escherichia coli* Adherence to Epithelial Cells Derived from Estrogen-Stimulated Rats

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The effect of exogenous estrogen administered to male and oophorectomized female rats was investigated with regard to in vitro adherence of eight uropathogenic strains of *Escherichia coli* to exfoliated bladder and vaginal epithelial cells. Uroepithelial cells obtained from estrogenized male and estrogenized oophorectomized female rats and vaginal cells obtained from estrogenized oophorectomized female rats demonstrated significantly enhanced ($P < 0.005$) host cell avidity for *E. coli* attachment, irrespective of bacterial adhesin expressed, when compared with such cells from nonestrogenized male and female oophorectomized rats. These animal studies suggest that female reproductive hormones may contribute to urinary-tract infection in premenopausal females by enhancing susceptibility to *E. coli* colonization of uroepithelial cells.

Microbial adherence to mucosal epithelial cells is now recognized as a prerequisite for successful colonization of the lower genitourinary tract (23). Bacteria unable to attach to bladder cells are likely to be swept away by the dynamic forces of micturition.

Much of the increase in our knowledge of bacterial adherence is derived from studying bacterial adhesins-ligands, and relatively little is known about host factors that influence epithelial-cell avidity for bacterial adherence (23). Several investigators, however, concluded that uroepithelial and vaginal cells obtained from children and adult females prone to recurrent urinary-tract infections were more susceptible to in vitro adherence of *Escherichia coli* (7, 9, 15–17, 24). Moreover, other investigators observed variable adherence to vaginal and uroepithelial cells obtained at different times during the menstrual cycle and suggested that hormonal factors might influence host epithelial-cell receptivity for bacterial attachment (1–5, 14, 16, 21, 25). In particular, adherence appeared maximal in cells obtained during the peak estrogen in vivo stimulation (3, 4, 14). We have further explored the relationship between in vivo estrogen stimulation and in vitro *E. coli* adherence to rat bladder and vaginal epithelial cells.

MATERIALS AND METHODS

Bacteria. Eight strains of *E. coli* were selected for the study (Tables 1 and 2). All strains were clinical isolates obtained from urine of patients with symptomatic urinary-tract infections. All strains were stored in deep agar tubes at 4°C. For designation of the adhesin status of test bacteria, hemagglutination assays with erythrocytes from guinea pigs and human group A blood were performed in the presence or absence of 0.14 M D-(+)-mannose as previously described (20). Confirmation of mannose sensitivity (MS) or resistance (MR) was achieved by yeast agglutination assays utilizing *Candida albicans* and *Saccharomyces cerevisiae* as described by Mirelman et al. (10). To encourage the expression of the MS adhesin, strains were cultured in 10 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) for 48 h at 37°C, while expression of the MR adhesin was encouraged by culture on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) for 18 h at 37°C. Bacteria were centrifuged at 570 × *g* for 15 min at 4°C. The bacterial pellet was suspended in 20 ml of sterile phosphate-buffered saline (PBS), washed, and finally resuspended in 3.0 ml of PBS.

A spectrophotometer (Coleman Instruments, Inc., Maywood, Ill.) was used to standardize the bacterial suspensions turbidimetrically to the desired concentration and to correlate the suspensions with viable counts made by plating 10-fold dilutions of the bacterial suspensions. The *E. coli* suspension in 1.0 ml of PBS was added to glass vials for use in adherence experiments.

Rat bladder and vaginal epithelial cells. Thirty-two female oophorectomized Sprague-Dawley rats weighing 175 to 200 g were studied. Pseudoestrus was induced in 16 of the oophorectomized rats by the administration of 0.5 mg of estradiol valerate (E. R. Squibb & Sons, Princeton, N.J.) in sesame oil by subcutaneous injection in the abdominal wall and was maintained by weekly injection of an identical dose of estrogen. At weekly intervals, commencing 1 week after the first injection of estradiol valerate, four oophorectomized estrogenized and four oophorectomized nonestrogenized rats were sacrificed, and the bladders and vaginas of the rats were surgically removed and immediately opened. Thereafter, the mucosal surfaces were gently scraped with a scalpel, and the superficial epithelial cells were collected by washing the mucosal surface with sterile PBS (pH 7.2). Bladder and vaginal epithelial cells so obtained were washed three times in PBS and adjusted to 5×10^4 cells per ml by using a hemacytometer counting chamber. The test bacteria were added to the epithelial cells as described below. Adherence experiments were performed after 1, 2, 3, and 4 weeks of estrogen administration.

Sixteen male Sprague-Dawley rats were similarly studied. In eight male rats, 0.5 mg of estradiol valerate was administered weekly for the duration of the experiment, and the remaining rats served as nonestrogenized control animals. Thereafter, at 4 and 6 weeks, four estrogenized and four nonestrogenized rats were sacrificed, their bladders were

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TABLE 1. Adherence of four strains of *E. coli* to female rat vaginal and bladder epithelial cells obtained at weekly intervals from estrogenized and estrogen-deprived rats

Duration of estrogen (wk)	*E. coli* strain (adhesin)	Adherence value (mean ± SE) in rats[a]			
		Estrogen-deprived		Estrogenized	
		VEC	BEC	VEC	BEC
1	431 (MR)	1.5 ± 0.2	2.3 ± 0.3	10.3 ± 2.2	2.3 ± 0.2
	C1212-77 (MR)	34.0 ± 3.2	9.7 ± 3.4	45.1 ± 3.9	19.0 ± 3.2
	73 (MR/MS)	72.9 ± 7.5	17.5 ± 3.6	94.2 ± 6.1	42.1 ± 9.0
	C1214-77 (MS)	28.9 ± 7.5	14.4 ± 2.7	44.0 ± 3.9	21.6 ± 2.6
2	431	2.9 ± 0.5	0.8 ± 0.6	14.1 ± 2.9	1.4 ± 0.2
	C1212-77	5.2 ± 0.9	1.8 ± 0.3	10.3 ± 1.1	2.8 ± 0.4
	73	1.9 ± 0.5	0.9 ± 0.2	9.0 ± 1.3	1.8 ± 0.1
	C1214-77	3.7 ± 0.4	1.5 ± 0.3	16.2 ± 3.0	2.7 ± 0.3
3	431	0.4 ± 0.1	0.4 ± 0.1	36.2 ± 3.1	1.9 ± 0.4
	C1212-77	19.0 ± 1.8	9.6 ± 2.3	54.3 ± 4.2	23.6 ± 3.1
	73	30.8 ± 4.9	13.2 ± 3.3	100.0 ± 6.8	60.2 ± 6.0
	C1214-77	13.6 ± 2.1	7.5 ± 1.3	50.2 ± 4.7	19.3 ± 3.1
4	431	5.8 ± 1.0	3.5 ± 0.5	10.6 ± 0.9	7.9 ± 0.7
	C1212-77	31.7 ± 4.3	17.4 ± 1.7	64.0 ± 4.3	30.8 ± 3.2
	73	16.7 ± 2.2	3.6 ± 0.5	27.9 ± 2.2	5.2 ± 1.2
	C1214-77	27.9 ± 3.5	12.9 ± 1.8	37.8 ± 2.6	20.0 ± 1.9

[a] VEC, Vaginal epithelial cells; BEC, bladder epithelial cells.

excised and dissected, and bladder epithelial cells were obtained and processed for in vitro adherence studies.

Adherence assay. Glass vials containing 1 ml each of *E. coli* and epithelial cells with a bacterial-to-epithelial cell ratio of 1,000:1 were capped, gently vortexed, and incubated in a water bath with gentle agitation for 1 h at 37°C. After incubation, 3.0 ml of ice-cold PBS containing 0.1% gelatin was added to each vial. The vials were recapped, gently vortexed, and centrifuged for 5 min at 250 × *g* to accomplish sedimentation of the epithelial cells and the adherent *E. coli* cells. The low-speed centrifugation left the unattached *E. coli* cells suspended in the slightly viscous gelatinized buffer, and these nonadherent *E. coli* cells in the supernatant were aspirated by gentle suction. Only the epithelial-cell pellet and approximately 100 μl of buffer were left after aspiration of the supernatant, and the procedure was repeated three times, each time with the addition of 3.0 ml of the 0.1% gelatin PBS. After final aspiration of the supernatant, small samples (approximately 20 μl) of epithelial cells in PBS were removed via micropipette, applied to glass slides, and allowed to air dry. The slides were stained with methylene blue and examined microscopically under oil immersion (×1,000). For each specimen, 50 epithelial cells were randomly selected, the number of *E. coli* cells adherent to each cell were counted, and a mean value was determined. All slides were coded and read blindly by a single investigator. Triplicate replicates of bacteria and epithelial-cell suspensions were used in all experiments.

Statistical analysis was performed by using paired and unpaired *t* tests.

RESULTS

Effect of in vivo estrogen administration on bacterial adherence to female rat bladder and vaginal epithelial cells. The results of experiments comparing the in vitro adherence of four strains of *E. coli* to rat bladder and vaginal epithelial cells obtained from estrogen-depleted and estrogen-treated female rats are shown in Table 1. Adherence to vaginal epithelial cells in both groups of rats was significantly greater than attachment to bladder epithelial cells ($P < 0.001$). By *t*-test analysis, all four strains of *E. coli* attached in significantly greater numbers to both vaginal and bladder epithelial cells obtained from estrogenized rats than to cells obtained from nonestrogenized rats ($P < 0.005$), irrespective of the duration of estrogen therapy. Comparison of adherence experiments performed at weekly intervals failed to demonstrate significant changes in adherence with continuation of estrogenization ($P < 0.5$). Adherence results revealed considerable variation among bacterial strains tested.

Effect of in vivo estrogen administration on bacterial adherence to male rat bladder epithelial cells. The results obtained with in vitro experiments with male rats are shown in Table 2. By *t*-test analysis, adherence of all four strains of *E. coli* to bladder epithelial cells was significantly greater ($P < 0.0005$) in estrogen-treated rats than in nonestrogenized male rats. With male rats, as with female rats, there was considerable variation among bacterial strains of *E. coli* studied. The highest levels of adherence were consistently obtained with *E. coli* 100, which caused MS hemagglutination of guinea pig erythrocytes.

TABLE 2. Adherence of four strains of *E. coli* to male rat bladder epithelial cells obtained at weekly intervals after estrogen administration

E. coli strain	Adhesin	Duration of estrogen (wk)	Adherence value (mean ± SE) in rats	
			Estrogen-deprived	Estrogenized
24	MR	4	1.9 ± 0.1	3.1 ± 0.4
		6	2.1 ± 0.2	3.4 ± 0.2
3155	MR	4	8.2 ± 0.4	12.6 ± 1.0
		6	11.7 ± 0.8	14.3 ± 1.2
100	MS	4	19.4 ± 2.1	24.9 ± 2.1
		6	16.4 ± 0.9	19.7 ± 1.9
1163	MS	4	4.8 ± 0.3	5.7 ± 0.8
		6	2.9 ± 0.5	4.6 ± 0.7

DISCUSSION

Several authors have suggested that ovarian hormones are implicated in the course of genital infections caused by different pathogenic microorganisms. Clinical examples include vaginitis caused by *C. albicans* (19), pelvic inflammatory disease induced by *Neisseria gonorrhoeae* (5, 6), and experimental endometritis caused by *E. coli* (12). Predisposition to infection by different organisms appears to be affected by differing hormonal influences. Experimental candidal vaginitis and *E. coli* endometritis are strongly dependent on the presence of estrogen and the induction of pseudoestrus (12, 19). Cessation of estrogen administration is accompanied by prompt remission of genital-tract infection.

Additional supportive evidence suggesting a role for estrogens in enhancing bacterial adherence was provided by Sugarman and Epps (22), who observed that estrogen increased the receptivity of HeLa cells to adherence by a variety of bacteria. Not all cell lines studied were influenced by estrogen administration. Similarly, in human studies, Sobel and Muller (18) found reduced adherence of several *E. coli* strains to exfoliated vaginal epithelial cells in elderly postmenopausal women, and Forslin demonstrated that estrogen therapy increased adherence of bacteria in postmenopausal women (6, 18). In a recent study of *E. coli* adherence to uroepithelial cells, the highest levels of adherence were consistently found in young premenopausal females, and the lowest levels were observed in young adult males (J. D. Sobel, G. Muller, and D. Kaye, Clin. Res. 32:228, 1984). Finally, numerous investigations have demonstrated cyclical changes in both vaginal- and uroepithelial-cell receptivity for *E. coli* during the menstrual cycle (3, 4, 14, 21, 25). Although there were inconsistencies in results obtained, the general conclusion was that endogenous estrogen enhanced adherence and that the highest adherence of *E. coli* to vaginal and uroepithelial cells corresponded with peak levels of estrogen in serum and urine (3, 4, 14).

In the present study, the administration of exogenous estrogen to oophorectomized female rats in concentrations sufficient to induce pseudoestrus was associated with significantly increased in vitro attachment of *E. coli* strains to both bladder and vaginal epithelial cells. Similarly, in male rats, the adherence of *E. coli* cells to bladder epithelial cells increased after estrogen administration. Although the exact mechanism for increased attachment remains as yet undetermined, the experimental design was such that an increased receptivity of epithelial cells for bacterial adhesins or ligands appears responsible. In vivo administration of estrogens may have several effects on the urinary tract, including changing the quality and quantity of the mucopolysaccharide layer lining the bladder and urethra and increasing lower-urinary-tract visceral smooth-muscle tone and contractility (11). However, our studies suggest that a direct effect on bacterial receptor sites may also occur, the specificity of which, however, requires further study. Estrogens also have an important influence on vaginal and periurethral bacterial flora, as indicated by the dramatic quantitative and qualitative changes that follow menopause (13).

Although it is widely known that uterine and vaginal epithelial cells contain estrogen receptors, it is of interest that several authors have recently demonstrated the presence of high-affinity estradiol receptors in both the cytosol and nuclear fractions of urethral cells as well as in the nuclear fractions of bladder cells (8). The present animal studies together with clinical observations and the presence of estrogen receptors support the possible role of estrogens in directly influencing the bacterial receptivity of genitourinary mucosal cells. Additional in vivo experiments in estrogenized and estrogen-deprived rats are required to confirm these preliminary observations and to corroborate the clinical significance of estrogens in contributing to the pathogenesis of urinary-tract infections.

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