INFECTION AND IMMUNITY, Apr. 1981, p. 194-197
0019-9567/81/040194-04$02.00/0

Vol. 32, No. 1

Adherence of Bacteria to Vaginal Epithelial Cells at Various Times in the Menstrual Cycle

JACK D. SOBEL,[1] JAN SCHNEIDER,[2] DONALD KAYE,[1] AND MATTHEW E. LEVISON[1]*

Departments of Medicine[1] and Obstetrics and Gynecology[2], The Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129

Adherence of vaginal isolates of *Escherichia coli*, *Lactobacillus* species, group B streptococci, *Gardnerella vaginalis* and *Neisseria gonorrhoeae* to exfoliated vaginal epithelial cells was studied in 10 healthy, sexually active medical students. Studies were done pre- and postmenstrually and at midcycle for two consecutive menstrual cycles. The mean number of adherent bacteria per vaginal epithelial cell (range) was 3.4 (0 to 14) for *E. coli*, 60.5 (12 to 152) for *Lactobacillus* species, 54.8 (21 to 76) for group B streptococci, 67.4 (15 to 161) for *G. vaginalis*, and 58.9 (15 to 186) diplococci for *N. gonorrhoeae*. Adherence of *G. vaginalis* increased with increasing acidity of the test medium (pH 4 to 8). There were no significant differences in adherence to vaginal epithelial cells obtained at the various times in the menstrual cycle for any of the organisms ($p > 0.05$). The pattern and extent of adherence among the women was similar for each organism. In this in vitro model, adherence characteristics did not vary with the menstrual cycle.

Microbial adherence to epithelial cells is necessary for successful colonization both by members of the normal flora and bacterial pathogens (4, 8, 9, 18). Selectivity of adherence may explain tropism of pathogens for certain tissues (8).

Studies of the bacterial microflora of the vagina and cervix have shown variations during the menstrual cycle, pregnancy, labor, and puerperium (3, 10, 12, 17, 19). The vaginal mucosa is known to be influenced by reproductive hormones, which cause cyclical shedding and proliferation of mucosal cells (15). The observed variation in the colonization pattern might be due to differences in bacterial adherence to vaginal epithelial cells (VEC) morphologically and functionally altered by the changing hormonal milieu. This study was undertaken to investigate bacterial adherence to VEC obtained at different phases of the normal menstrual cycle.

MATERIALS AND METHODS

Subjects. Ten healthy, nonpregnant, volunteer female medical students with a mean age 25 years (range, 22 to 29 years) were studied. All the volunteers were asymptomatic and sexually active with regular menses. None of the subjects used vaginal douches or medication, nor did any use oral hormonal contraceptives; instead each used a diaphragm and sperimicidal cream.

Gynecological examination of all volunteers was normal. Quantitative aerobic and anaerobic culture of vaginal contents revealed a predominance of aerobic and anaerobic *Lactobacillus* sp., *Gardnerella vaginalis*, *Neisseria gonorrhoeae*, group B streptococci, and *Enterobacteriaceae* were not isolated in any of the subjects.

Cell sampling. Cells were obtained from the mucosal surface of the midvaginal wall by gentle scraping with an Ayre's spatula and immediately transferred to minimum essential medium, pH 7.2 (Gibco). Adherence experiments began within 30 min of sampling.

Postmenstrual samples were obtained within 48 h of the cessation of menstrual flow, midcycle specimens were sampled 14 days before the expected date of menses, and premenstrual samples were obtained within 72 h of the expected onset of menses. Midcycle and pre- and postmenstrual VEC samples were obtained from each volunteer for two consecutive cycles.

Bacteria. The bacteria studied were all isolated from vaginal secretions of patients or volunteers. The following species were studied: *E. coli*, two strains of *Lactobacillus* species, group B streptococci, *G. vaginalis*, and *N. gonorrhoeae*; the latter was a fresh isolate taken from a patient with symptomatic gonorrhoea. Samples of each isolate were stored at −20°C in appropriate broth. For adherence testing, a frozen sample of each bacterium was thawed 24 h before the experiment, transferred to sheep blood agar plates (Difco) (*E. coli*, group B streptococci, *Lactobacillus* sp.), Casman agar plates (*G. vaginalis*), or chocolate agar plates (*N. gonorrhoeae*), and incubated at 37°C in 5% CO_2 for 24 h. In additional experiments, both a vaginal and a fecal isolate of *E. coli* were thawed and incubated in nutrient broth at 37°C for 48 h under stationary conditions to encourage the formation of type 1 pili. Only the fecal isolate demonstrated pili formation, as confirmed by transmission electron microscopy.

Adherence test. The method used for the adherence test was similar to that described by Mardh and Weström (13). VEC were washed free of adherent vaginal bacteria by differential centrifugation three

times in 20 ml of minimum essential medium at 800 rpm for 10 min. The cells were resuspended in 10 ml of minimum essential medium and subjected to filtration through a membrane filter with a pore size of 8 μm (Millipore Corp.), which retains cells but allows free bacteria to pass. The cells were resuspended in minimum essential medium and adjusted to a final concentration of 2×10^4 washed epithelial cells per ml, as determined in a hemacytometer counting chamber.

The bacteria were scraped from the agar, suspended in phosphate-buffered saline, pH 7.2, and washed three times in 20 ml of phosphate-buffered saline by centrifugation at 2,500 rpm. After resuspension of bacteria in phosphate-buffered saline, the bacterial suspensions were passed through a 25-gauge needle to obtain single organisms, as determined by Gram stain. The final suspension of bacteria was adjusted to 10^8/ml, using a spectrophotometer, after initial quantitative cultures had confirmed the bacterial density.

A 1-ml sample of bacterial suspension was mixed with 1 ml of the epithelial cell suspension (5×10^3 bacteria to one epithelial cell) and incubated aerobically on a rocker at 37°C for 30 min (pH 7.2). Control tubes contained epithelial cells suspended in minimum essential medium at pH 7.2, diluted 1:2 in phosphate-buffered saline. All experimental conditions were studied in duplicate. Epithelial cells were then washed free of nonadherent bacteria by differential centrifugation at 800 rpm and filtration through a 8-μm membrane filter. To transfer the cells to slides, the membranes were gently pressed against the glass slides, which had previously been coated with a thin layer of albumin. After this procedure, less than 1% of the epithelial cells remained on the filter, as detected by microscopic examination of Gram-stained filters. Cells attached to the slide were fixed in 95% ethanol for 10 min, air dried, and Gram stained. The number of bacteria adherent to epithelial cells was counted under a light microscope (×1,000). In each experiment, 50 epithelial cells were studied, and duplicate determinations were performed.

In additional experiments, the piliated fecal *E. coli* was incubated with and without 25 mg of D(+)-mannose (Sigma) per ml for 60 min before incubation with epithelial cells.

Reproducibility of the results on any one day and from day to day in the same individual was studied in additional experiments by measuring adherence in quadruplicate for each experimental condition and by repeating experiments daily for 4 successive days.

The effect of pH upon *G. vaginalis* adherence was performed in phosphate-buffered saline at pH 4 to 8. Statistical results were assessed with the Student *t* test and with an analysis of the variance of bacterial adherence evaluating the variables of subjects, cycles, stages, bacterial species, and replicate variation.

RESULTS

Differences in adherence among various bacterial species. The results of adherence of the five bacterial species studied are shown in Table 1.

Adherence of *Lactobacillus* sp., group B streptococci, *G. vaginalis*, and *N. gonorrhoeae* was not significantly different ($p > 0.05$), although these four organisms adhered significantly better than *E. coli* ($p < 0.01$). The vaginal *E. coli* isolate, which failed to piliate when grown on agar or in nutrient broth, revealed a consistently low adherence of 3.4 ± 0.9 and 4.8 ± 1.1 bacteria per VEC, respectively, whereas the piliated fecal strain adhered relatively better (28.9 ± 6.1 bacteria per VEC). Preincubation of piliated *E. coli* with mannose reduced adherence by 90%.

TABLE 1. *Adherence of bacterial species to VEC*[a]

Species	Adherent bacteria per cell		Difference between adherence of species and vaginal *E. coli* (p)
	Mean ± S.E.	Range	
E. coli (vaginal)	3.4 ± 0.9	0–14	
Lactobacillus sp.	60.5 ± 9.2	12–152	< 0.01
Group B streptococci	54.8 ± 3.9	21–76	< 0.01
G. vaginalis	67.4 ± 5.7	15–161	< 0.01
N. gonorrhoeae	58.9 ± 7.8	15–186	< 0.01

[a] Results represent mean adherence ± standard error (S.E.) of pooled data for 50 cells, done in duplicate, at the premenstrual, postmenstrual, and midcycle phases of two cycles for all volunteers.

No difference in quantitative adherence characteristics was encountered among the ten women. In each volunteer, overall adherence of *E. coli* was extremely poor when compared with that of the other four species ($p < 0.001$). In further experiments, adherence of another strain of *Lactobacillus* sp. isolated from one of the volunteers was compared with the *Lactobacillus* sp. used in the remainder of the experiments. Bacterial adherence was similar for both strains of *Lactobacillus* sp. ($p > 0.05$).

Differences in bacterial adherence obtained at different phases of menstrual cycle. Day-to-day variability of adherence was assessed by determinations on 4 consecutive days in one volunteer. Bacterial adherence to 50 cells was measured in quadruplicate (Table 2). There was little day-to-day variation in adherence for the given bacteria under the experimental conditions, allowing for valid comparison of adherence at selected phases in menstrual cycle. No significant difference in bacterial adherence to VEC occurred among the 10 volunteers or among the different phases of the menstrual cycle when tested by a three-way analysis of variance ($p > 0.05$).

Effect of pH on bacterial adherence of *G. vaginalis*. Adherence of *G. vaginalis* to epithelial cells increased with increasing acidity of the incubation medium (Fig. 1). Adherence observed at pH 4.0 was significantly greater than that at pH 6, 7, or 8 ($p < 0.01$).

TABLE 2. *Mean adherence of G. vaginalis and E. coli to VEC*[a]

Bacterium	Mean bacteria per VEC ± S.E. at day:				*p* Value
	1	2	3	4	
G. vaginalis	63.3 ± 4.8	63.7 ± 5.1	54.1 ± 7.1	59.0 ± 4.4	>0.05
E. coli	1.0 ± 0.1	1.1 ± 0.2	1.2 ± 0.2	1.0 ± 0.1	>0.05

[a] One subject was measured daily for 4 consecutive days. The *p* value, determined by the Student *t* test, reflects the significance of difference of replicate bacterial adherence to VEC on consecutive days. S.E., Standard error.

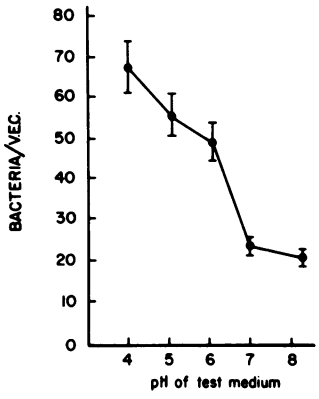

FIG. 1. *Influence of pH of the test medium on adherence of G. vaginalis to VEC. Bars indicate mean ± standard error.*

DISCUSSION

Levison et al. (12), in studying the quantitative aerobic and anaerobic bacterial flora of the vagina, observed that the total facultative and *G. vaginalis* count were significantly higher and that obligate anaerobic bacterial counts were lower during pregnancy. Additional studies have shown variations in vaginal and cervical microflora during the menstrual cycle, pregnancy, labor, and puerperium (3, 10, 19). Certain female genital tract infections have been correlated with a critical phase of the menstrual cycle. Candida vaginitis tends to become clinically evident premenstrually and during pregnancy (14, 16). Trichomonas infection and symptomatic genital gonococcal disease and dissemination are exacerbated during menstruation (2, 6, 16). Furthermore, Baker and Plotkin (1) demonstrated enhancement of vaginal infection in mice by herpes simplex virus type 2 with progesterone.

Cyclical changes in pH, secretions, and glycogen content as well as a shedding and renewal of superficial VEC under the influence of the female reproductive hormones occur in the microenvironment of the lower genital tract. It is conceivable that cyclic changes in bacterial flora and propensity to clinical infection may be related to cyclical differences in bacterial adherence. Indeed, Botta reported that adhesion of group B streptococci to VEC was significantly lower in the first week of the menstrual cycle in a study of 10 volunteers (5).

In this in vitro study with washed exfoliated VEC, no differences in bacterial adherence were observed for any of the bacterial species studied over two consecutive cycles. Epithelial cell specimens obtained in the immediate postmenstrual phase differed somewhat morphologically, with poorer total cell yields, a greater degree of cell clumping, and more cuboidal parabasal cells as compared with the higher yield and squamous cell type in specimens obtained premenstrually; nevertheless, quantitative adherence was the same. This result does not exclude in vivo differences in adherence during the cycle in that, under in vitro experimental conditions, the bacteria and cells were not exposed to hormones or vaginal mucus. In addition, hormonal influences upon cell membrane receptors might have been affected during the cell preparation procedure. Furthermore, most of the experiments were carried out at pH 7.2, whereas the vaginal pH is usually lower (11).

The finding of marked adherence of pathogens *N. gonorrhoeae* and group B streptococci is similar to that of Mardh and Weström (13). However, in contrast to their observation of low quantitative adherence of *Lactobacillus* sp., we found equally good adherence for *Lactobacillus* sp. This is not surprising, considering that *Lactobacillus* constitutes the dominant microorganism normally present in the vagina (12). This difference may reflect variations in adherence of different strains of *Lactobacillus*. *G. vaginalis* is the subject of considerable dispute concerning its role as a pathogen in nonspecific vaginitis (12). In this study, *G. vaginalis* demonstrated good adherence in all volunteers, a finding similarly noted by Mardh and Weström (13), who thus concluded that the adherence pattern supported the hypothesis that *G. vaginalis* is a true vaginal pathogen. However, the impressive adherence of *Lactobacillus*, a nonpathogen in the vagina, supports the concept that, whereas adherence to a mucosal surface is an essential characteristic of most mucosal pathogens, nonpathogenic colonizing bacteria likewise adhere well. Adherence patterns per se cannot distinguish pathogens from nonpathogens.

The significance of the striking increase in *G. vaginalis* adherence to epithelial cells with increasing acidity of the test medium is unclear. Similar findings have been observed with gonococci (13). The pH of the vagina is normally acid but varies little under physiological conditions and even in the presence of vaginitis (7). It is conceivable that the increased attachment of *G. vaginalis* and gonococci to vaginal mucosal cells in an acid environment is another factor in the tissue tropism of these organisms. The results of these experiments suggest that piliation is important for adherence of *E. coli*. The almost total inhibition of adherence of piliated *E. coli* to epithelial cells by mannose is highly suggestive of the presence of functional mannose-like receptors on the vaginal cells.

ACKNOWLEDGMENT

We thank Pauline Gashi Myers for her excellent technical assistance.

LITERATURE CITED

1. **Baker, D. A., and S. A. Plotkin.** 1978. Enhancement of vaginal infection in mice by Herpes Simplex virus type II with progesterone. Proc. Soc. Exp. Biol. Med. **158:** 131-134.
2. **Bard, D. S.** 1974. Urogenital trichomoniasis, p. 206-221. *In* G. R. G. Monif (ed.), Infectious diseases in obstetrics and gynecology. Harper & Row Publishers, Inc., New York.
3. **Barlett, J. G., A. B. Onderdonk, E. Drude, C. Goldstein. M. Anderka, S. Alpert, and W. M. McCormack.** 1977. Quantitative bacteriology of the vaginal flora. J. Infect. Dis. **136:**271-277.
4. **Beachey, E. H., and I. Ofek.** 1976. Epithelial cell binding of Group A streptococci by lipoteichoic acid on fimbriae denuded of M protein. J. Exp. Med. **143:**759-771.
5. **Botta, G. A.** 1979. Hormonal and type-dependent adhesion of group B streptococci to human vaginal cells. Infect. Immun. **25:**1084-1086.
6. **Braude, A. I., L. B. Corbeil, S. Levine, J. Ito, and J. A. McCutchan.** 1978. Possible influence of cyclic menstrual changes on resistance to the gonococcus, p. 328-337. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
7. **Cohen, C.** 1969. Influence of pH on vaginal discharge. Br. J. Vener. Dis. **45:**241-246.
8. **Gibbons, R. J., D. M. Spirell, and Z. Skobe.** 1976. Selective adherence as a determinant of the host tropisms of certain indigenous and pathogenic bacteria. Infect. Immun. **13:**238-246.
9. **Gibbons, R. J., and J. Van Houte.** 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. **29:**19-45.
10. **Goplerud, C. P., M. J. Ohm, and R. P. Galask.** 1976. Aerobic and anaerobic flora of the cervix during pregnancy and the puerperium. Am. J. Obstet. Gynec. **126:** 858-868.
11. **Karnaky, K. J.** 1959. Comparison of battery pH readings and electronic pH recordings of the vagina in *Trichomonas vaginalis*. Am. J. Obstet. Gynec. 77:149-154.
12. **Levison, M. E., L. C. Corman, E. R. Carrington, and D. Kaye.** 1977. Quantitative microflora of the vagina. Am. J. Obstet. Gynec. **127:**80-85.
13. **Mardh, P. A., and L. Weström.** 1976. Adherence of bacteria to vaginal epithelial cells. Infect. Immun. **13:** 661-666.
14. **Mead, P. B.** 1974. *Candida albicans*, p. 242-267. *In* G. R. G. Monif (ed.), Infectious diseases in obstetrics and gynecology. Harper & Row Publishers, Inc., New York.
15. **Papanicolaou, G. N.** 1933. The sexual cycle in human female as revealed by vaginal smears. Am. J. Anat. **52:** 519-532.
16. **Penza, J. F.** 1973. Moniliasis and trichomoniasis, p. 209-224. *In* M. Finland and D. Charles (ed.), Obstetric and prenatal infections. Lea & Febiger, Philadelphia.
17. **Saigh, J. H., C. C. Sanders, and W. E. Sanders, Jr.** 1978. Inhibition of *Neisseria gonorrhoeae* by aerobic and facultative anaerobic components of the endocervical flora: evidence for a protective effect against infection. Infect. Immun. **19:**704-710.
18. **Swenson, J.** 1973. Studies on gonococcus infection IV. Pili: their role in attachment of gonococci to tissue culture cells. J. Exp. Med. **137:**571-589.
19. **Thadepalli, H., W. H. Chan, J. E. Maidman, and E. C. Davidson. Jr.** 1978. Microflora of the cervix during normal labor and puerperium. J. Infect. Dis. **137:**568-572.