INFECTION AND IMMUNITY, Feb. 1996, p. 611–615 Vol. 64, No. 2
0019-9567/96/$04.00+0

Rapid Cyclic Changes in Density and Accessibility of Endometrial Ligands for *Escherichia coli* Dr Fimbriae

ANIL K. KAUL,[1]* DHRUV KUMAR,[2] MANUBAI NAGAMANI,[1] PAWEL GOLUSZKO,[1] STELLA NOWICKI,[1,3] AND BOGDAN J. NOWICKI[1,3]

Departments of Obstetrics and Gynecology,[1] Pathology,[2] and Microbiology,[3] University of Texas Medical Branch at Galveston, Galveston, Texas 77555-1062

Received 10 July 1995/Returned for modification 30 August 1995/Accepted 28 November 1995

The mechanisms of developing infection in young, noncompromised individuals are not well understood. Colonization is a prerequisite for the development of infection. In humans, ligands serving bacterial colonization belong to common antigens. Consequently, a majority of individuals should be sensitive to infection at all times. We hypothesize that the temporal patterns of some infections and sensitivity to them are associated with sudden changes in the density and accessibility of common receptors. Endometrial samples from women having normal menstrual cycles were examined for histological location, receptor density, and in situ hybridization of Dr (decay-accelerating factor) ligands for *Escherichia coli* Dr fimbriae. Significant up-regulation and luminal expression of Dr ligands occurred during the secretory phase, whereas receptors were expressed in the basement membrane and in smaller quantities during the proliferative phase. This observation agrees with our hypotheses that some ligands recognized by bacterial adhesins change their compartmentalization and, most importantly, that they up-regulate expression at specific times.

The mechanisms of acquiring infection in young, noncompromised individuals are not well understood. It is generally accepted that colonization is a prerequisite for the development of infection. Colonization occurs because of the anchoring of the microorganisms to the epithelial receptor(s) (1). Lack of the genetically encoded colonization-mediating receptor in some piglets prevented them from developing diarrhea (22). However, in humans, known ligands serving bacterial adhesins for attachment belong to common antigens that occur in the majority of individuals; consequently, a majority of individuals should be sensitive to infection at all times. Common, or public, antigens such as Dr (a^+) blood group antigens are recognized as receptors for *Escherichia coli* Dr fimbriae (6, 7, 11, 13, 19). Dr (a^+) antigen has been reported to be localized on a 70-kDa glycoprotein (24) that subsequently was identified by three groups of independent investigators (18, 21, 28) as decay-accelerating factor (DAF) (CD55).

We have reported that DAF is the natural ligand for the Dr fimbriae of uropathogenic *E. coli* in the human endometrium (9). Our recent structure-function study of *E. coli* Dr fimbriae-DAF interaction revealed that a point mutation in the short consensus repeat-3 (SCR-3) domain (Ser-165 to Leu) resulted in abolished Dr fimbriae binding to the ligand and supported evidence for the binding specificity of purified bacterial Dr adhesin (16). Dr fimbriae were reported to also bind to type IV collagen (30). However, DAF, but not type IV collagen, has been found to be the natural receptor for the Dr fimbriae in the endometrial glands (9). The use of purified bacterial Dr adhesin as a lectin-like reagent provides a good diagnostic method for detecting functional Dr ligands (20) in the endometrium (9). Interestingly, quantitative measurement of Dr ligands reveals substantial variation of the ligand density between tested human endometrial samples. Cyclic hormonal changes control the function of the female reproductive tract by effecting expression of several viable molecules in the endometrium (9). Hormones have also been postulated to affect the sensitivity to infection, although the underlying mechanisms are not clear.

We, therefore, hypothesize that the temporal patterns of some infections and sensitivity to them are associated with sudden changes in the density and accessibility of the common receptors. The discovery of even an infrequent example of a fluctuating receptor serving colonization would be valuable to our understanding of the pathogenesis of infection in noncompromised individuals.

In the present study, we localized and quantitated the expression of Dr ligand (DAF) in the human endometrium during the normal menstrual cycle.

MATERIALS AND METHODS

Specimen collection. We obtained 20 samples of endometria from 18 premenopausal women who were having normal menstrual cycles and undergoing hysterectomies for nonendocrine problems (cervical dysplasia, pelvic pain, or pelvic relaxation). Nine of the samples were obtained at the proliferative phase, and 11 were obtained at the secretory phase. None of the subjects received exogenous hormone treatment. With one subject, samples were obtained during two different visits: one at the proliferative phase and the other at the secretory phase. With one patient, two samples were collected during the same phase of two different menstrual cycles. An additional two samples were from two patients on exogenous progesterone treatment. Informed consent was obtained when samples were obtained at the time of endometrial biopsies. The protocol has been approved by the institutional review board of the University of Texas Medical Branch at Galveston.

The patients' ages ranged from 29 to 47 years, with a mean ± standard deviation age of 36 ± 5 years. All but one patient were multiparous, and their parities ranged from one to six. The ages and parities of patients in the proliferative and secretory phases of the cycle were similar. None of the patients had any evidence of pelvic infection, and cultures for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were negative.

Each endometrial tissue sample was obtained near the fundus of the uterus. Immediately after removal, each tissue sample was quick frozen in liquid nitrogen and stored at −80°C until processing. Six-micrometer frozen serial sections were obtained on poly-L-lysine-coated glass slides and fixed in ice-cold acetone. Following hematoxylin and eosin staining, sections from each biopsy were evaluated histologically and assigned to the proliferative or to the secretory phase by two independent pathologists.

Detection of Dr ligands, DAF, and DAF mRNA. A strain of *E. coli* IH11128 that expresses Dr fimbriae was isolated from a female patient with symptomatic urinary tract infection. A DNA fragment from the genome of *E. coli* IH11128

* Corresponding author. Present address: Minneapolis Medical Research Foundation D3, 914 S. 8th St., Minneapolis, MN 55404.

encoding the Dr adhesin was isolated, cloned, and plasmid transformed to a laboratory strain of *E. coli* K-12 (EC901), and the recombinant Dr adhesin was isolated from *E. coli* BN406(pBJN406) and purified as previously described (19).

Dr fimbria-binding sites were detected in the endometrium by exposing frozen tissue sections to purified Dr adhesin. Following five washing steps with phosphate-buffered saline, Dr binding sites were identified by anti-Dr fimbria immunoglobulin G (IgG) and were visualized by immunohistochemical staining with avidin-biotin complex. Similarly, the expression of DAF in the obtained parallel endometrial tissue sections was identified by immunohistochemical staining with anti-DAF/SCR-3 IgG and avidin-biotin complex procedures (29). The positive control for the specificity of purified Dr fimbriae included quantitation of fimbrial binding on Chinese hamster ovary cells transfected with human DAF cDNA (16). Because Dr fimbriae bind to the DAF/SCR-3 domain, parallel sections were stained with anti-DAF/SCR-3 IgG. The anti-DAF/SCR-3 IgG was also used to block Dr adhesin binding to the endometrial tissue ligand and in control experiments to bind to recombinant DAF (17). Negative controls included staining with replacement of Dr adhesin and each individual step by phosphate-buffered saline. In addition, parallel samples were stained with DAF-nonrelated antibody and anti-type IV collagen IgG to provide evidence that Dr adhesins and anti-DAF IgG stained endometrial glands specifically and did not contain type IV collagen binding sites (9).

Computer image analysis. The DAF expression and Dr adhesin binding sites were quantitated by measuring the integrated optical densities (ODs) of the stained tissue samples with a computer-based image analysis system (2). This method has been successfully used to analyze frozen tissue sections of breast carcinomas stained by immunohistochemical methods for estrogen receptors. Results obtained by the image analysis system have been proven to correlate well with results obtained by subjective nonautomated methods of quantitating stained sections or by biochemical analysis.

In the endometrium, a minimum of 20 glands were measured in stained and controlled parallel sections. The average OD per unit area of each stained section was measured. Slides were examined at various magnifications, ranging from ×4 to ×25. A magnification of ×20 was selected because it optimized resolution of individual structures and at the same time permitted rapid counting of the stained elements. Each microscope field was scanned twice, once at a low OD threshold to detect all elements stained and a second time at a high threshold to detect only strongly Dr-labeled elements. The ratio of labeled elements was then determined. Sections with large numbers of strongly stained elements had high mean normalized values for OD per unit area, while sections with intermediate and low levels of staining had low normalized values for OD per unit area. The relative increase in the OD value was calculated as the percentage of the increase in cumulative OD values. The baseline was measured in a parallel control section in which Dr fimbriae or anti-DAF IgG was replaced with an appropriate buffer. Variation in the staining intensity was estimated from the standard deviation of absorbance levels, which were reflected in the width of the peaks of the OD spectra. Peaks were broad in some samples, reflecting considerable heterogeneity of Dr receptor expression.

In situ hybridization with biotinylated cDNA probe (*Eco*RI-*Eco*RI 1.85-kb fragment) for human DAF was performed on corresponding parallel samples. The probe was biotin labeled with a Bionick labeling kit (GIBCO BRL, Grand Island, N.Y.). The tissue sections were fixed in freshly prepared 4% paraformaldehyde for 15 min at 4°C. Forty microliters of the hybridization mixture was applied to the tissue sections, which were then covered with paraffin film and incubated at 42°C for 16 h in a moist chamber. Following incubation, the tissue sections were washed. For detection of the hybridization signal, an in situ hybridization detection kit (DAKO Corporation, Carpenteria, Calif.) was used; DAF cDNA and anti-DAF IgG were kindly donated by D. Lublin (12). All samples were tested blindly, and the results were compared after the completion of histological staining, immunohistochemical staining, and in situ hybridization.

RESULTS

Purified *E. coli* adhesin and anti-DAF/SCR-3 IgG were used to quantitate Dr (DAF) binding sites in human endometria. During the proliferative phase of the menstrual cycle (days 1 to 14), Dr fimbria binding ligand was expressed predominantly in the basement membranes (Fig. 1). A computer image analysis of cumulative OD values of Dr fimbriae stained tissues was used to characterize possible changes in the receptor density. In the proliferative phase, the integrated OD values of all tested individuals varied from 1 to 30%, with a mean OD value of 10% (Fig. 2).

During the secretory phase (days 15 to 28) of the human menstrual cycle, Dr ligand was expressed predominantly in the apical sites in the luminal area of the endometrial glands (Fig. 1). The proportion of positively stained glands in individual samples increased from 30% in the proliferative phase to 80 to 100% towards the end of the secretory phase (days 20 to 25).

Computer image analysis of the mean integrated OD of Dr ligand showed significant increases (50%; $P \leq 0.001$) during the secretory phase compared with that during the proliferative phase (Fig. 2). For example, OD values for individual samples in the secretory phase varied from 40 to 80%. Individual positively stained glands in the secretory phase showed higher OD values than did glands in the proliferative phase. Additionally, the increase of cumulative OD values during the secretory phase resulted from both an increased expression and a larger number of stained structures. Therefore, there was not only a quantitative OD change in the expression of the Dr ligands but there was also a qualitative variation in the histological site of the expression of Dr receptors during different phases of the menstrual cycle.

Similarly to binding of recombinant Dr fimbriae, parallel samples were tested with monoclonal anti-DAF/SCR-3 IgG. Anti-SCR-3 IgG binds to the DAF epitope recognized by Dr fimbriae; anti-SCR-3 IgG blocks binding of Dr fimbriae in competitive assays. The binding patterns and the densities of binding sites for anti-SCR-3 IgG and Dr fimbriae were similar. Computer image analysis recorded OD values for the anti-SCR-3 IgG that did not differ from the OD values observed for the Dr fimbriae. At most, observed OD differences were at the level of 10%, and on average they were not greater than 5% OD. Because of the similarity that it would have to Fig. 1, an additional figure for anti-SCR-3 IgG OD values is not shown.

Endometrial samples were obtained from different individuals at different phases of the cycle. Retrospectively, some samples were found to originate from the same patient and were isolated at different visits. Of two samples from patient 1, one represented the secretory phase and the other represented the proliferative phase. While the secretory phase had a higher (60%) OD, the proliferative phase sample showed a very low OD value (12%), providing support for the temporal expression pattern occurring in the same individual. Two samples that originated from the same patient (patient 2) during the proliferative phase had OD values of 5 and 4%, indicating the reproducibility of the image analysis values.

Endometrial sections from two subjects treated with progesterone showed high values (92 and 96%) which were above the OD value for the highest reading (80%) in the nontreated secretory group. Fluctuation of Dr ligand density resembled the kinetics of progesterone levels during the cycle, with both reaching the highest values between days 21 and 25 (Fig. 2).

The difference in the expression of Dr ligands was further supported by the in situ hybridization with DAF cDNA as a probe (Fig. 1). During the secretory phase, a strong positive signal corresponding to DAF and Dr staining was detected in the endometrial glandular epithelium of the individual endometrial glands. During the proliferative phase, however, the positive but weak signal was associated with only a small number of glands and was located predominantly at the basolateral site of the glandular epithelium in direct proximity to the interstitial area and the basement membranes. Therefore, expression of Dr ligands during the menstrual cycle correlated with histological location in the DAF mRNA expression (Fig. 1).

DISCUSSION

This report provides evidence for rapid changes of the density and accessibility of the tissue ligand for *E. coli* colonization factor (Dr adhesin) in the human genital tract. This observation establishes a hypothetical basis for rapid, temporal increase in risk for the colonization and/or acquisition of infection in young, noncompromised individuals.

Despite extensive progress in the understanding of the struc-

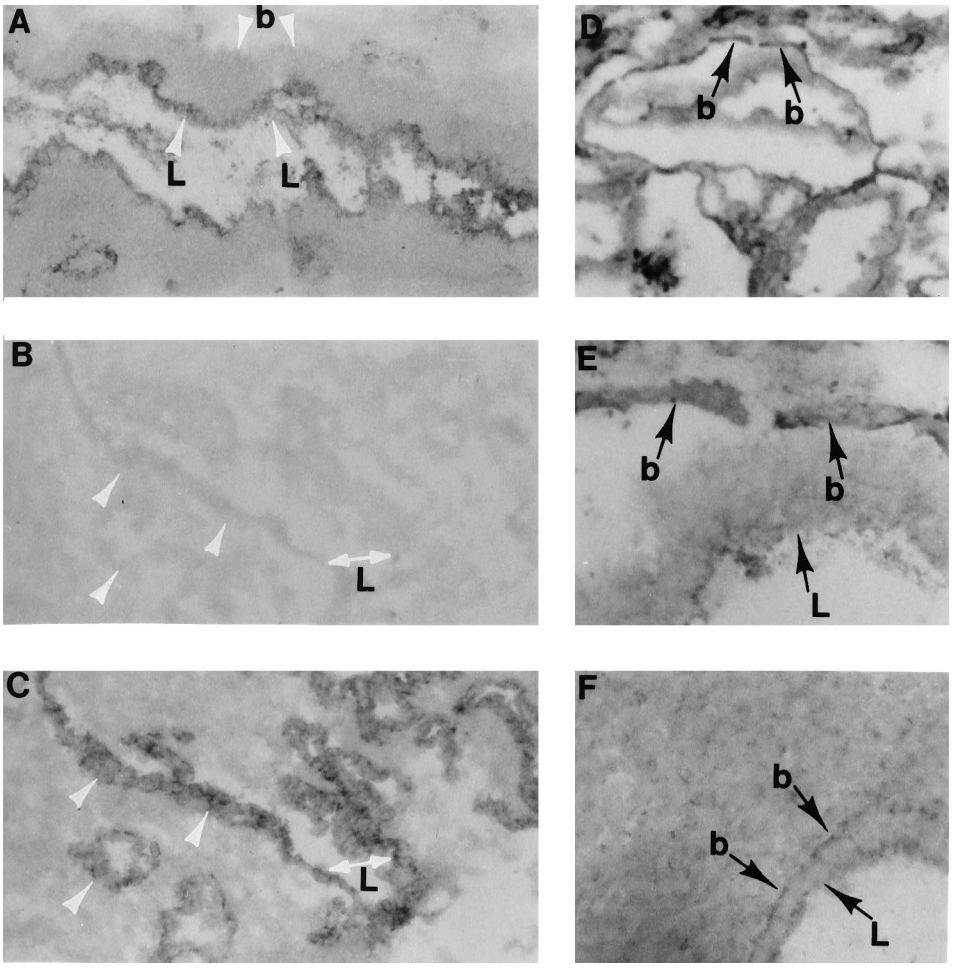

FIG. 1. Endometrial binding of recombinant Dr adhesin. (A) Secretory phase. Cryostat sections of endometrial tissue from a patient in the secretory phase demonstrate the characteristic staining pattern of apical sites in the endometrial gland. L, luminal site of the gland; b, nonstained region corresponding to the basement membrane. (For all other panels, b indicates the basement membrane.) (B) Negative control for in situ hybridization. A lack of signal of the cryostat section with unrelated DNA is shown. White arrows in panels B and C indicate corresponding sites in the parallel sections. (C) DAF cDNA in situ hybridization. Positive signal in the endometrial glands during secretory phase of the menstrual cycle is shown. (Panel C is a parallel section for panel B.) (D) Proliferative phase. Basement membrane binding of recombinant Dr adhesin is shown. b arrows indicate stained basement membranes at low magnification (×100). (E) Proliferative phase. Arrows show enlarged area corresponding to panel D. The enlarged photograph indicates a lack of Dr staining in the luminal portion of the gland and clear staining of the basement membrane. (F) DAF cDNA in situ hybridization in the proliferative phase. This section shows a weak signal in a basolateral site of the gland.

tural domains of bacterial adhesins and tissue ligands that allow colonization, it remains unknown why a young, noncompromised individual may have an increase or decrease in susceptibility to bacterial infection within a short time span. Rutter and colleagues provided the first evidence that lack of tissue ligand serving *E. coli* colonization prevented infection (22). This exciting finding concerned an experimental animal with a unique genetic background. In our present study, we proposed a hypothesis that would explain the variation in sensitivity to colonization for the majority of young, noncompromised individuals instead of that for unique subjects, who are missing a gene and its product.

Quantitative measurements with computer-based image analysis investigated several endometrial samples for the potential differences of *E. coli* Dr fimbria ligand density. Computer image analysis has been proven highly reliable and clinically useful for the measuring of estrogen receptor density in the diagnosis of breast cancers, and its results correlate well with results of the chemical quantitation of receptors. Our group was the first to adapt this technique to quantitate colonization receptors for microorganisms (9). With limitations of the availability of subsequent samples from the same individual, we found a temporal expression pattern in tested tissues. Significant up-regulation of Dr ligand in the endometrial glands was found to occur in the secretory phase. The Dr ligands were accessible in the luminal sites of the endometrial glandular epithelium, a compartment likely to be exposed to the microorganisms colonizing the urogenital tract. In contrast, proliferative-phase receptors were expressed in considerably smaller quantities and in the histologically unavailable substructures (basement membranes). The histological site of expression and up-regulation of Dr ligand (DAF) in the secretory phase correlated with the presence of DAF mRNA in the glandular epithelial cells detected by in situ hybridization. These observations agree with our hypotheses that some tissue ligands recognized by bacterial adhesins may change their compartmentalization and, most importantly, rapidly up-regulate expression at specific times.

A recently developed in vitro recombinant cell-cell interaction system allows the testing of host-pathogen (adhesin-li-

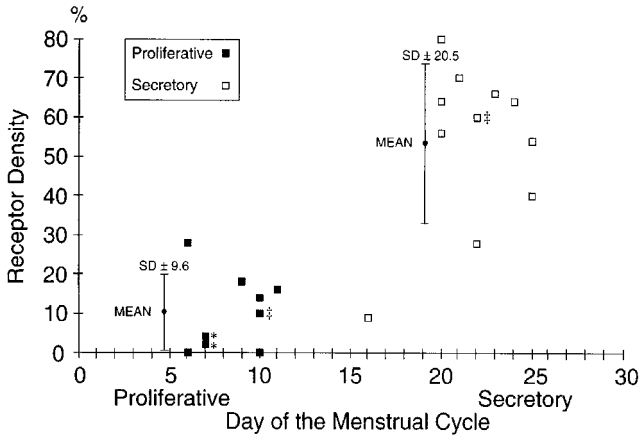

FIG. 2. Expression of endometrial Dr ligand during the menstrual cycle. Integrated OD values in respect to the day of the menstrual cycle are indicated as solid or open squares, representing proliferative and secretory phases, respectively. The differences between low mean OD values in the proliferative phase and the high OD in the secretory phase are statistically significant ($P < 0.001$). ‡, two samples from the same patient, one taken in the proliferative phase and the other taken in the secretory phase; ∗, two samples taken from the same patient in the proliferative phase.

gand) interaction (16). This model provided direct evidence that attachment of Dr fimbriae to DAF ligand is a dose-dependent phenomenon, with significant increases of binding to cells expressing increasing amounts of recombinant DAF (16). Therefore, *E. coli* bearing Dr adhesins recognizing Dr (DAF) ligand may more efficiently attach to the endometrial glands that express more binding sites. Association of this phenomenon with the menstrual cycle may increase or decrease the susceptibility of an individual to microbial colonization, depending on the phase of the cycle.

The human endometrium represents a unique tissue that is temporarily exposed to the blood-containing complement. Systems (such as DAF) that control complement activation (15) are essential for the protection of the uterus from the cytotoxic effect of the serum. Up-regulation of DAF toward the end of the cycle may represent several functions, including human adaptation to temporary physiological changes. The colonization mechanism developed by Dr fimbriae bearing *E. coli* in which an immunomodulatory molecule becomes a binding ligand may resemble the attachment strategy of human immunodeficiency virus to CD4 receptors on human lymphocytes.

Some indirect insights on the regulation of the temporal expression of DAF were accumulated because of retrospective analysis of the patients' histories. The temporal expression of Dr (DAF) ligands is likely to operate under the control of progesterone. This suggestion is based on association between high OD values for samples during days 21 to 25 of the cycle, the time period also characterized by the highest levels of progesterone. More support stems from Dr ligand OD values observed for two patients who were undergoing progesterone therapy and whose values were higher than those between days 21 and 25 in the nontreated population (8). Despite the complexity of the mechanisms operating the human menstrual cycle, this study demonstrates that human tissue ligands allowing bacterial attachment may undergo cyclic changes in relatively short time intervals.

The human genital and urinary tract serves as a striking example of tissue with variable sensitivities to infection. The onset of acute salpingitis shows a temporal pattern, with facultative aerobic and anaerobic infection occurring more frequently in the secretory phase and with gonococcal or chlamydial infection occurring in the proliferative phase (3, 5, 10, 14, 25, 27). The molecular pathogenesis of pelvic inflammatory disease and the involvement of bacterial virulence factors are, in general, unknown. *E. coli* accounts for 15 to 25% of all pelvic inflammatory disease cases, although reports suggesting evidence for lower (10%) as well as much higher (35%) frequencies exist (23, 26). Even a low frequency of *E. coli* in pelvic inflammatory disease does not exclude the necessity for up-regulation of specific ligands in the secretory phase for infection to occur.

Another temporal condition of increased risk for urogenital tract infection is pregnancy (4). Gestational pyelonephritis seems to be a patient condition with an increased Dr^+ *E. coli* infection rate in the third trimester of pregnancy (17). We recently found that an increased Dr^+ *E. coli* infection rate correlates with up-regulation of Dr receptors in the kidney in the third trimester (7a).

The temporal changes in receptor density may contribute to understanding of the pathogenesis of urogenital infections in individuals of reproductive age. The hypothetical explanation for the increased sensitivity of young noncompromised individuals to some of the infectious processes proposed in our report will be further investigated.

ACKNOWLEDGMENTS

We thank Samuel Baron and David H. Walker for their critical readings of the manuscript. We acknowledge Linda Morrow Smith and Robert G. McConnell and his staff of Publication, Grant, and Media Support for the Department of Ob/Gyn for their assistance in the preparation of the manuscript.

This project was funded in part by grants from NIH (RO1 DK42029) to B.J.N., the John Sealy Memorial Endowment Fund to B.J.N., and the McLaughlin Fund to A.K.K.

REFERENCES

1. **Beachey, E. H.** 1981. Bacterial adherence/adhesin receptor interactions mediating the attachment of bacteria of mucosal surfaces. J. Infect. Dis. **143:** 325–345.
2. **Franklin, W. A., M. Bibbo, M. I. Boria, and G. L. Weid.** 1986. Quantitation of estrogen receptor content and ki-67 staining in breast carcinoma by micro-TICAS image analysis system. Anal. Quant. Cytol. Histol. **9:**279.
3. **Gibbs, R. S.** 1987. Pelvic inflammatory disease in adolescents. Tex. Med. **83:** 5–6.
4. **Hart, A., T. Pham, S. Nowicki, E. B. Whorton, Jr., M. G. Martens, G. D. Anderson, and B. Nowicki.** Gestational pyelonephritis-associated *Escherichia coli* isolates represent nonrandom closely related population. Am. J. Obstet. Gynecol., in press.
5. **Johnson, D. W., K. K. Homes, P. A. Kvale, C. W. Halverson, and W. P. Hirsch.** 1969. An evaluation of gonorrhea case finding in the chronically infected female. Am. J. Epidemiol. **90:**438–484.
6. **Kallenius, G., R. Mollby, S. B. Svenson, J. Winberg, A. Lundblad, S. Svensson, et al.** 1980. The P antigen as receptor for the hemagglutinin of pyelonephritogenic *E. coli*. FEMS Microbiol. Lett. **7:**297–302.
7. **Karr, J., B. Nowicki, L. D. Truong, R. A. Hull, J. J. Moulds, and S. I. Hull.** 1990. Pap 2-encoded fimbriae adhere to the P blood group-related stage-specific embryonic antigen 4 in the human kidney. Infect. Immun. **58:**4055–4062.

7a. **Kaul, A., and B. Nowicki.** Unpublished data.

8. **Kaul, A. K., M. Nagamani, and B. Nowicki.** 1995. Decreased expression of endometrial decay accelerating factor (DAF), a complement regulatory protein, in patients with luteal phase defect. Am. J. Reprod. Immunol. **34:**236–240.
9. **Kaul, A. K., B. J. Nowicki, M. G. Martens, P. Goluszko, A. Hart, M. Nagamani, D. Kumar, T. Q. Pham, and S. Nowicki.** 1994. Decay-accelerating factor is expressed in the human endometrium and may serve as the attachment ligand for Dr pili of *Escherichia coli*. Am. J. Reprod. Immunol. **32:** 194–199.
10. **Koch, M. L.** 1947. A study of cervical cultures taken in cases of acute gonorrhea with special reference to the phases of the menstrual cycle. Am. J. Obstet. Gynecol. **54:**861–866.
11. **Leffler, H., and C. Svanberg-Edén.** 1980. Chemical identification of a glycosphingolipid receptor for *E. coli* attaching to human urinary tract epithelial cells and hemagglutinating erythrocytes. FEMS Microbiol. Lett. **8:**127–134.

12. **Lublin, D. M., and J. R. Atkinson.** 1989. Decay-accelerating factor: biochemistry, molecular biology and function. Annu. Rev. Immunol. **7:**35–58.
13. **Lund, B., B. I. Markhund, N. Strömberg, F. Lindberg, K. A. Karlsson, and S. Normark.** 1988. Uropathogenic *Escherichia coli* can express serologically identical pili of different receptor binding specificities. Mol. Microbiol. **2:**255.
14. **McCormack, W. M., and G. H. Reynolds.** 1982. Effect of menstrual cycle and method of contraception on recovery of *Neisseria gonorrhoeae*. JAMA **267:** 1292–1294.
15. **Nicholson-Weller, A., J. Burge, D. T. Fearon, P. F. Weller, and K. F. Austen.** 1982. Isolation of a human erythrocyte membrane glycoprotein with decay-accelerating activity for C3 convertase of the complement system. J. Immunol. **129:**184–189.
16. **Nowicki, B., A. Hart, K. E. Coyne, D. M. Lublin, and S. Nowicki.** 1993. Short consensus repeat-3 domain of recombinant decay-accelerating factor is recognized by *Escherichia coli* recombinant Dr adhesin in a model of a cell-cell interaction. J. Exp. Med. **178:**2115–2121.
17. **Nowicki, B., A. Hart, M. G. Martens, and S. Nowicki.** 1994. Gestational age-dependent distribution of *Escherichia coli* fimbriae in pregnant patients with pyelonephritis. Ann. N. Y. Acad. Sci. **730:**290–291.
18. **Nowicki, B., R. Hull, and J. Moulds.** 1988. Use of Dr hemagglutinin of uropathogenic *Escherichia coli* to differentiate normal from abnormal red cells in paroxysmal nocturnal hemoglobinuria. N. Engl. J. Med. **319:**1289.
19. **Nowicki, B., J. Moulds, R. Hull, and S. Hull.** 1988. A hemagglutinin of uropathogenic *Escherichia coli* recognizes the Dr blood group antigen. Infect. Immun. **56:**1057–1060.
20. **Nowicki, B., L. Truong, J. Moulds, and R. Hull.** 1988. Presence of the Dr receptor in normal human tissues and the possible role in pathogenesis of ascending urinary tract infection. Am. J. Pathol. **133:**11–14.
21. **Parson, S. F., F. A. Spring, A. H. Merry, G. Mallison, D. J. Anstee, V. Rawlingson, and M. Daha.** 1988. Evidence that Cromer-related blood group antigens are carried on decay-accelerating factor (DAF) suggests that Inab phenotype is a novel form of DAF deficiency, p. 116. *In* Proceedings of the XX Congress of the International Society for Blood Transfusion British Blood Transfusion Society, Plymouth Grove, Manchester, United Kingdom.
22. **Rutter, P. L., M. R. Burrows, R. Sellwood, and R. A. Gibbons.** A genetic basis for resistance to enteric disease caused by *E. coli*. Nature (London) **257:**135–136.
23. **Spitbart, H.** 1993. The cervical flora in pelvic inflammatory disease, p. 79. *In* E. R. Weissenbacher (ed.), Pelvic inflammatory disease (PID); diagnosis and therapy. Edition Material Media, Munich.
24. **Spring, F. A., P. A. Judson, G. L. Daniels, S. F. Parson, G. Mallison, and D. J. Anstee.** 1987. A human cell-surface glycoprotein that carries Cromer-related blood group antigens on erythrocytes and is also expressed on leucocytes and platelets. Immunology **62:**307.
25. **Sweet, R. L., M. B. Doyle, M. O. Robbie, and J. Schachter.** 1986. The occurrence of chlamydial and gonococcal salpingitis during the menstrual cycle. JAMA **255:**2062–2064.
26. **Sweet, R. L., D. L. Draper, J. Schachter, J. James, W. K. Hadley, and G. F. Brooks.** 1980. Microbiology and pathogenesis of acute salpingitis as determined by laparoscopy: what is the appropriate site to sample? Am. J. Obstet. Gynecol. **138:**985–989.
27. **Sweet, R. L., and R. S. Gibbs.** 1985. Infectious diseases of the female genital tract, p. 53–77. Williams & Wilkins, Baltimore.
28. **Telen, M. J., S. E. Hall, A. M. Green, J. J. Moulds, and W. F. Rosse.** 1988. Identification of human erythrocyte blood group antigens on decay-accelerating factor (DAF) and an erythrocyte phenotype negative for DAF. J. Exp. Med. **167:**1993.
29. **Troung, L. D., V. T. Phung, Y. Yoshikawa, and C. A. Mattioli.** 1988. Glycoconjugates in normal kidney; a histochemical study using biotinylated lectins. Histochemistry **90:**511–560.
30. **Westerlund, B., P. Kuusela, J. Risteli, L. Risteli, T. Vartio, H. Rauvala, R. Virkola, and T. K. Korhonen.** 1989. The 075X adhesin of uropathogenic *Escherichia coli* is a type IV collagen-binding protein. Mol. Microbiol. **3:**329–333.

Editor: B. I. Eisenstein