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Binding of Type 1-Piliated *Escherichia coli* to Vaginal Mucus

MARIO F. VENEGAS,[1] ELENA L. NAVAS,[1] ROBERT A. GAFFNEY,[1] JAMES L. DUNCAN,[2] BYRON E. ANDERSON,[3]* AND ANTHONY J. SCHAEFFER[1]

Departments of Urology,[1] Microbiology-Immunology,[2] and Cell, Molecular and Structural Biology,[3] Northwestern University Medical School, Chicago, Illinois 60611

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To better understand the interactions involved in bacterial adherence and the role of mucus in the pathogenesis of urinary tract infections, we developed a system to study the binding of a recombinant *Escherichia coli* strain, HB101/pWRS1-17, expressing type 1 pili, to vaginal mucus collected from 28 women. Bacteria bound to differing extents to all specimens examined, and preincubation of bacteria with mannose inhibited binding by 50 to 89%. Additionally, all mucus samples showed reactivity with anti-mannose antibody, and the levels of reactivity correlated with the levels of bacterial binding, suggesting that the mannose-terminal saccharides present on these glycoproteins are the receptors for the binding of type 1-piliated bacteria. Mucus specimens collected over periods of 5 days and 12 weeks exhibited significant variation in bacterial binding, indicating temporal differences in the ability of vaginal mucus to act as a receptor for type 1-piliated *E. coli*. The results show that vaginal mucus can bind bacteria and may thus influence the initial attachment and subsequent colonization of the vaginal and urinary tract epithelium by *E. coli*.

Urinary tract infections (UTIs) are among the most common bacterial diseases, ranging from asymptomatic bacteriuria to severe kidney infection and occasional renal failure. Approximately 20% of women have frequent (three or more annually) recurrences of infection following the initial episode, producing additional morbidity. These women are considered to be infection prone. In about 80 to 90% of such cases, a single bacterial species, *Escherichia coli*, is the responsible pathogen (33).

Studies of women with recurrent UTIs have established that most episodes of infection are preceded by colonization of the patient's fecal flora, vaginal mucosa, and periurethral region by the same strain of *E. coli* later found in the patient's urine (6, 38). Adherence of bacteria, particularly *E. coli*, to carbohydrate structures of glycolipids and glycoproteins of uroepithelial cells is an important initial step in colonization and infection of the urinary tract. An individual's blood group profile and secretor status are important factors in determining the complex carbohydrate structures of epithelial cell membranes. Adherence is mediated by proteins (adhesins) on the surface of the bacterium, which are in most instances located on the distal tips of long filaments known as pili or fimbriae. Type 1 pili which mediate hemagglutination of guinea pig erythrocytes (3) are produced by most strains of *E. coli*. The reaction is inhibited by the addition of mannose and therefore termed mannose-sensitive hemagglutination (14, 24). In the urinary tract, studies from a number of investigators have documented the importance of mannose-sensitive adhesion mediated by type 1 pili in animal models of UTI (12). In humans, immunohistochemical studies have shown that type 1 pili alone can be found on *E. coli* adhering to voided uroepithelial cells of patients with UTIs (13), and type 1-piliated *E. coli* has been shown to adhere to the superficial cells of human ureteral mucosa (7). Apart from the urinary tract, type 1 pili may also contribute to the pathogenesis of *E. coli* sepsis and meningitis by promoting mucosal colonization prior to invasion of the bloodstream (8, 9). Type 1 pili have also been suggested to enhance the communicability of *E. coli* between individuals, perhaps by mediating oral-pharyngeal colonization (2).

Uroepithelial cells from infection-prone women bind bacteria more avidly than do cells from healthy women, and the cells from women with the nonsecretor phenotype are more receptive for *E. coli* than are cells from women with the secretor phenotype (17). Furthermore, the nonsecretor phenotype is overrepresented in women with recurrent UTIs (36). Thus, the nonsecretor status, increased cell receptivity for *E. coli*, and recurrent UTIs appear to be related.

The association of bacteria with epithelial cells has been studied extensively, but very few investigators have examined the role of specific mucus glycoproteins on bacterial adhesion. These investigations have been done mainly with regards to bacterial adherence to salivary glycoproteins (21), human intestinal mucins (5, 18), respiratory mucins (29, 31, 37, 41), and mucin specimens from the airways of patients with cystic fibrosis (39). *E. coli* adherence to rabbit, bovine, and human urinary bladder mucosa has been investigated in relation to recurrent UTIs (20, 27, 28), but no studies on *E. coli* binding to vaginal mucus have ever been reported.

To better understand the complex interactions involved in bacterial adherence and the role of mucus in the pathogenesis of UTIs, we developed an assay to study bacterial binding to vaginal mucus. We report here that recombinant *E. coli* strain HB101/pWRS1-17, which encodes type 1 pili, binds to vaginal mucus and the extent of binding correlates with amounts of reactive mannose in the mucus. Additionally, bacterial binding to mucus varies significantly with specimens collected from a variety of individuals and from the same individual on different days or weeks.

MATERIALS AND METHODS

Study population. Vaginal mucus specimens were obtained from 28 women, 14 of whom had a history of documented UTIs (three or more infections annually) and 14 who had no history of UTIs; 15 of the women were secretors and 13 were nonsecretors. The secretor status of each individual was determined by testing saliva samples with anti-Lewis a (Le^a) and anti-Lewis b (Le^b) antibodies (Chembiomed Ltd., Edmonton, Alberta, Canada) with an enzyme-linked fluorogenic immunoassay (15, 22).

Specimen collection. Vaginal mucus specimens were collected by gently scraping the vaginal mucosa with a wooden paddle. The paddle was immersed into 5

* Corresponding author.

ml of sterile phosphate-buffered saline (PBS; pH 7.3) and stored at −70°C. At the time of testing, the specimens were allowed to thaw at room temperature and centrifuged, and the mucus sample was obtained from the supernatant free of uroepithelial cells.

Bacteria. Binding experiments were carried out with *E. coli* HB101 transformed with the recombinant plasmid pWRS1-17, which encodes type 1 pili (35). As a negative control, *E. coli* HB101, transformed with the vector alone (pHSS22) and which does not express type 1 pili, was used. Bacteria were grown on agar plates supplemented with 40 μg of kanamycin (Sigma Chemical Co., St. Louis, Mo.) per ml. Bacterial type 1 expression was characterized by the D-mannose-sensitive agglutination of guinea pig erythrocytes (3).

Labeling of bacteria. Bacteria, both piliated and nonpiliated, were grown overnight at 37°C in shaking liquid Luria-Bertani broth (19) supplemented with 40 μg of kanamycin per ml and containing 5 μCi of [^{3}H]uridine (5, 6-^{3}H; ICN Biomedical Inc., Costa Mesa, Calif.) per ml. The bacteria were then collected by centrifugation at 1,000 × *g* for 15 min at room temperature, washed twice with PBS (pH 7.3), and resuspended in PBS. The range of radioactivity incorporated was 5×10^{-4} to 5×10^{-3} cpm per bacterium. Concentrations of bacteria were estimated by determining optical density values at 540 nm on a Coleman spectrophotometer (model 44); 10^9 bacteria per ml corresponded to an optical density reading of 0.65 to 0.70. The exact number of bacteria used in binding experiments was determined by plating serial dilutions on blood agar plates.

Bacterial binding to mucus. Protein concentrations of the vaginal mucus samples and control mucin glycoproteins were determined by the A_{215} and A_{225} (42). Bovine submaxillary mucin (BSM; type 1; Sigma) was used as a positive control, and AI, a mucin purified in our laboratories from ascitic fluid of an ovarian cancer patient, was used as a negative control (40). One hundred microliters of the vaginal mucus specimens and the control mucins at a concentration of 10 μg/ml of solution in 0.1 M $NaHCO_3$ (pH 9.0) were adsorbed to duplicate polystyrene microtiter wells (Removawell strips-Immulon 1; Dynatech Laboratories, Inc., Chantilly, Va.). After incubation overnight at 4°C, the wells were washed three times with PBS, and 50 μl of labeled bacteria was added to each well. After incubation for 2 h at 37°C, unbound bacteria were removed by gentle aspiration and the wells were washed five times with PBS. The wells were snapped apart and dissolved in 10 ml of Scintiverse E fluid (Fisher Scientific Co., Pittsburgh, Pa.). After overnight incubation at room temperature, the scintillation vials were vortexed and the counts per minute were determined in a scintillation spectrometer (Beckman model LS 1800).

The number of bacteria bound to BSM-positive controls varied slightly between experiments. Therefore, experimental data for bacterial binding to mucus specimens obtained at different times were normalized to BSM. The AI mucin exhibited low levels of bacterial binding and did not show reactivity with anti-mannose antibody. As shown in Results, prior incubation of bacteria with mannose inhibited the binding to AI mucin by only 10%. For this reason, the counts per minute values for the AI mucin were subtracted from all other values prior to comparative evaluation of the data.

Inhibition studies. ^{3}H-labeled *E. coli* bacteria were incubated with 1% mannose solution in PBS for 2 h at 37°C prior to addition to the mucus- or control mucin-coated polystyrene wells. The remaining steps of the binding assay were performed as described above.

Enzyme-linked fluorogenic immunoassay. Duplicate wells of polystyrene microfluor-B plates (Dynatech Laboratories, Alexandria, Va.) were coated with 2.5 μg of the BSM-positive control, the AI-negative control, or the vaginal mucus specimen per ml in 0.1 M $NaHCO_3$ (pH 9.0) and incubated for 2 to 3 h at room temperature. The wells were then backcoated with 380 μl of PBS containing 1% (wt/vol) of bovine serum albumin (BSA; Sigma) and 0.02% of sodium azide for 2 h at room temperature. The plates were then washed three times with PBS containing BSA and azide plus 0.1% (vol/vol) of Tween 20. Appropriate dilutions of the anti-mannose antibody (kindly provided by Clinton Ballou, University of California at Berkeley) were added to the wells, and then the wells were incubated overnight at 4°C. This antibody is a rabbit antiserum against α1→3- and α1→6-linked mannose on the surfaces of whole yeast cells which was purified by adsorption to mannan-Sepharose columns. The plates were subsequently washed three times, 100 μl of biotinylated goat anti-rabbit immunoglobulin G (IgG) reagent (Vector Laboratories Inc., Burlingame, Calif.) diluted 1:1,000 was added to each well, and the plates were incubated for 2 h at room temperature. After three washings with PBS-BSA-Tween 20 buffer, 100 μl of streptavidin–β-galactosidase (Gibco-BRL, Gaithersburg, Md.), diluted 1:1,000, was added, and the plates were incubated for 2 h at room temperature. The plates were washed again three times, and the assay was developed by adding 100 μl of the substrate 4-methylumbelliferyl-β-D-galactopyranoside (Sigma) prepared at 0.1 mg/ml in 10 mM sodium phosphate buffer (pH 7.5) containing 1 mM $MgCl_2$ and 0.1 M NaCl. After 30 min, the plates were read at 450 nm in a fluorescence concentration analyzer (Pandex FCA; Iddex Corporation, Portland, Maine), and the results were reported in relative fluorescence units (RFU). Negative controls included the omission of mucus samples for coating the microtiter plate wells and omission of the anti-mannose antibody. The reactivity of the anti-mannose serum reagent was verified by its binding to a preparation of zymosan A (Sigma), which is mainly a mannose polysaccharide, and bovine thyroglobulin (Sigma), which contains a high amount of mannose oligosaccharides. The BSM was used as a positive control because it was found by this test to contain mannose-terminated oligosaccharide chains and as a mucin may mimic the vaginal mucus specimens.

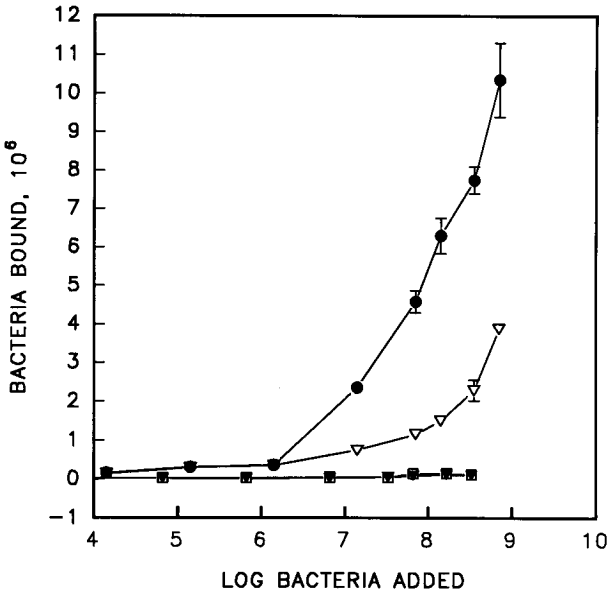

FIG. 1. Binding of *E. coli* HB101/pWRS1-17 to BSM (●) and AI (▽) and of HB101/pHSS22 to BSM (□) and AI (▼) mucins. Binding was determined with a polystyrene microtiter Removawell strip system assay as described in Materials and Methods. The results are presented as the $\log_{10}$ of the number of bacteria added (1.43×10^5 to 7.15×10^7 for the HB101/pWRS1-17 and 6.7×10^4 to 3.35×10^8 for HB101/pHS22) versus the number of bacteria bound. The error bars represent the standard errors of the means of duplicate samples (values lower than 0.0035 are not shown).

The small amount of reactivity to the AI mucin was not inhibited by the prior incubation of anti-mannose antibody reagent with 1 mM D-mannose nor with 1 mM methyl-D-mannoside solution and was considered to be nonspecific background binding. These sugars did inhibit the reactivity to BSM up to 50 to 60% and to vaginal mucus specimens to a range of 12 to 70%. Thus, the AI RFU value was subtracted from all other values for anti-mannose reactivity to mucus specimens and to BSM prior to evaluation of the data. The relationship between anti-mannose reactivity and bacterial binding to the mucus specimens was examined by using Kendall's rank correlation test (10, 11).

The same enzyme-linked fluorogenic immunoassay system was used to determine the secretor status of each individual, with anti-Le^a and anti-Le^b mouse monoclonal antibodies and a β-galactosidase-conjugated goat anti-mouse IgG, IgM, and IgA mixture (Zymed Laboratories, San Francisco, Calif.) as the secondary antibody. As positive controls for the corresponding Lewis epitopes, mucins that have high expression for either one were used. These mucins had been purified and characterized previously in our laboratories.

RESULTS

Model system of bacterial binding to mucin glycoproteins. To define the properties of bacterium-mucus interactions, we developed a binding assay using *E. coli* HB101/pWRS1-17 and mucin glycoproteins either obtained commercially or purified in our laboratories. BSM, among other mucin glycoproteins tested, was found to be a good positive control for binding type 1-piliated bacteria, whereas the AI mucin glycoprotein yielded low adherence and therefore was selected as a negative control. The number of bacteria bound to BSM increased in a nearly linear fashion as a function of the logarithm of bacteria added to the microtiter plate wells, whereas the number of bacteria bound to AI control wells was 20 to 35% of that for BSM, as shown in Fig. 1. Bacteria at concentrations higher than 10^9 bacteria per ml tended to aggregate, which may explain the apparent lack of saturation for BSM. The unpiliated strain, HB101/pHSS22, showed no binding to either mucin glycoprotein. As shown in Fig. 2, the number of bacteria bound increased only up to a concentration of 5.0 μg of BSM per ml used to coat the wells, whereas for AI, apparent saturation was

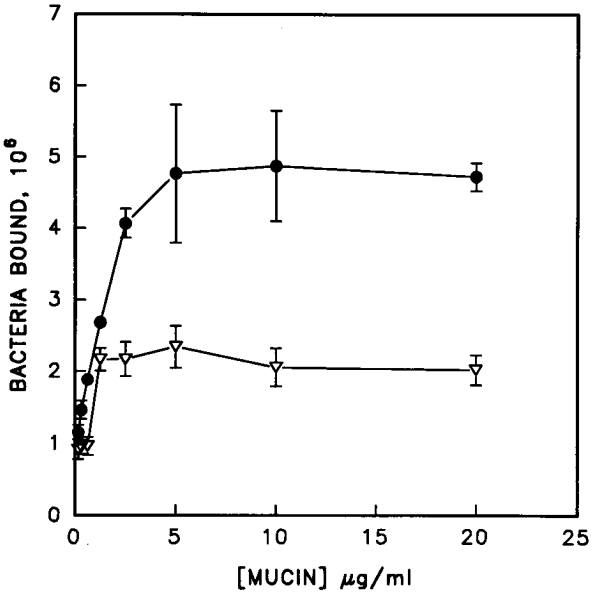

FIG. 2. Binding of *E. coli* HB101/pWRS1-17 to increasing concentrations of (●) and AI (▽). The number of bacteria added per well was 3.1×10^7 in 50 μl, and the data are plotted as the number of bacteria bound versus concentrations of mucins, in micrograms per milliliter, used for coating the microtiter plate wells. For an explanation of the error bars, see the legend to Fig. 1.

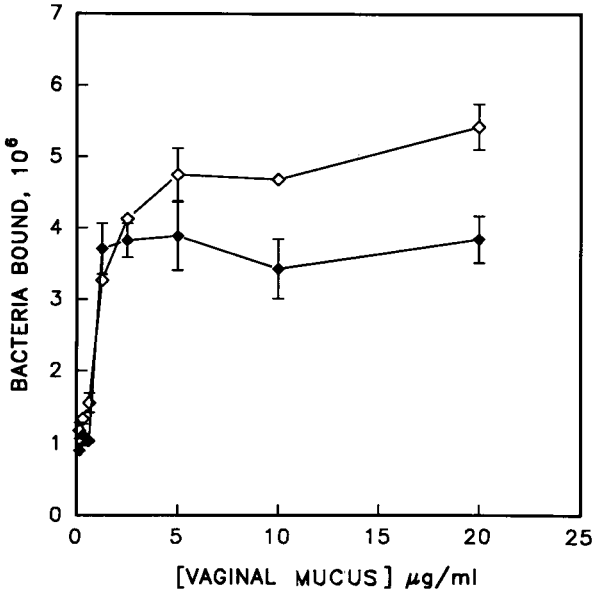

FIG. 3. Binding of *E. coli* HB101/pWRS1-17 to increasing concentrations of vaginal mucus specimens adsorbed to the microtiter plate wells collected from two women without a history of UTIs (a secretor [◇] and a nonsecretor [◆]). The number of bacteria added per well was 3.1×10^7, and the experimental conditions were the same as those described in the legends to Fig. 1 and 2. Results are presented in micrograms per milliliter for the vaginal mucus concentrations versus the number of bacteria bound. For an explanation of the error bars, see the legend to Fig. 1.

achieved at a concentration of 1.25 μg/ml. In subsequent experiments, the number of bacteria added ranged from 10^8 to 10^9 per ml.

Bacterial binding to vaginal mucus. To confirm the utility of the assay system developed with the control mucin glycoproteins, increasing concentrations of vaginal mucus specimens from two individuals (a secretor and a nonsecretor) were examined for bacterial binding. As shown in Fig. 3, a binding pattern similar to that obtained with BSM was observed, with saturation of bacterial binding at mucus coating concentrations of 2.5 to 5.0 μg/ml. For all subsequent binding experiments, 10 μg of mucus per ml was used to coat the wells.

Binding experiments of HB101/pWRS1-17 were performed with all 28 vaginal mucus specimens, and Fig. 4 shows the results for 14 of the samples analyzed. All specimens had receptors for binding of type 1-piliated *E. coli*, although the amounts of bacteria bound varied in a 15-fold range among the different mucus samples, from 0.15 to 2.20 times of that obtained with the BSM-positive control. The unpiliated bacterial strain HB101/pHSS22 did not show significant binding to any of the vaginal mucus specimens (data not shown).

Inhibition of bacterial binding to mucus. To demonstrate that bacterial binding was mannose dependent, 10 vaginal mucus specimens, as well as mucin glycoproteins BSM and AI, were tested for inhibition of bacterial binding by preincubation of the bacteria with a 1% D-mannose solution. As shown in Fig. 5, mannose inhibited bacterial binding to BSM by 77% but to AI by only 10%. Inhibition of bacterial binding to vaginal mucus specimens ranged from 50 to 89%. The highest levels of inhibition were observed for mucus specimens 7 (88.8%), 3 (80.7%), and 2 (76.0%), and the lowest levels were seen with specimens 9 (64.2%), 1 (61.7%), and 6 (49.5%). When the mannose-independent binding value to AI was subtracted from the other inhibition values, the inhibition by mannose approached 100% for all specimens examined. When preincubation of the bacteria was performed with a 1% solution of D-glucose and D-galactose, no inhibition was found neither for BSM or AI nor for the vaginal mucus specimens examined.

Anti-mannose reactivity of vaginal mucus. The presence of mannose receptors in the vaginal mucus specimens was immunochemically confirmed by their reactivity with anti-mannose

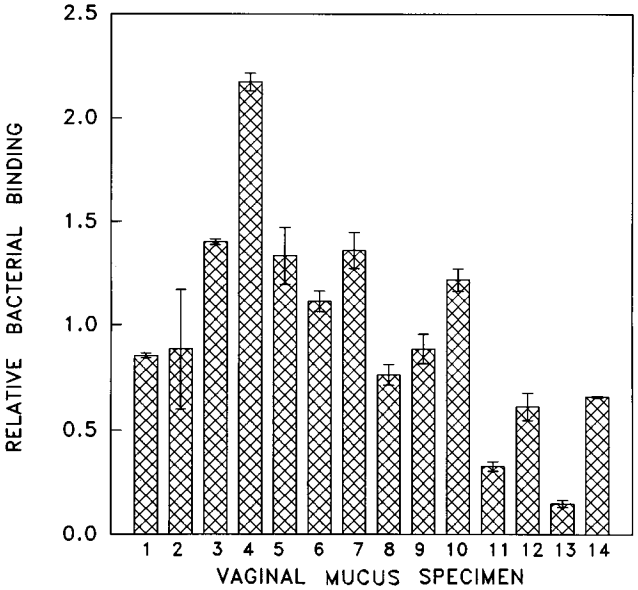

FIG. 4. Binding of *E. coli* HB101/pWRS1-17 to vaginal mucus specimens. The data are presented as the bacteria bound to the mucus specimens relative to bacteria bound to BSM, after subtraction of the values of bacteria binding to AI. The binding assays were performed by adding 1.1×10^7 to 3.6×10^7 ^{3}H-labeled bacteria to 10 μg of mucus per ml used to coat the microtiter plate wells. For an explanation of the error bars, see the legend to Fig. 1.

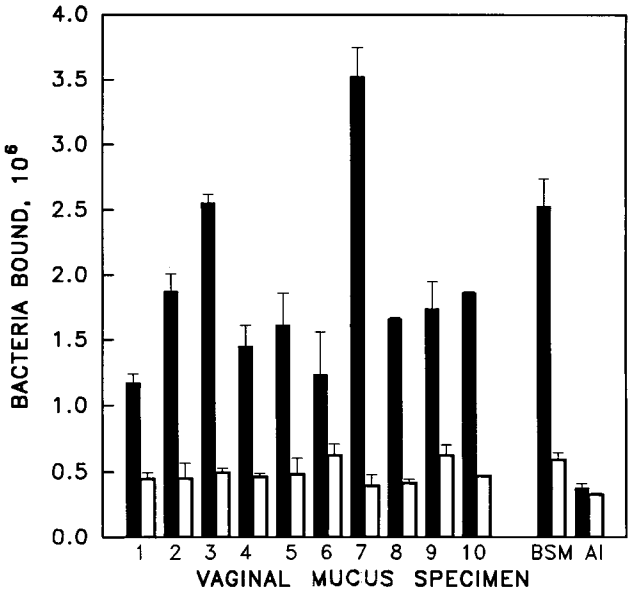

FIG. 5. Mannose inhibition of *E. coli* HB101/pWRS1-17 binding to vaginal mucus specimens. A total of 10 specimens were tested, together with BSM and AI mucins, without (filled bars) or with (open bars) 1% D-mannose solution. The number of bacteria added per well was 5.5×10^7, and the data are given in numbers of bacteria bound. For an explanation of the error bars, see the legend to Fig. 1.

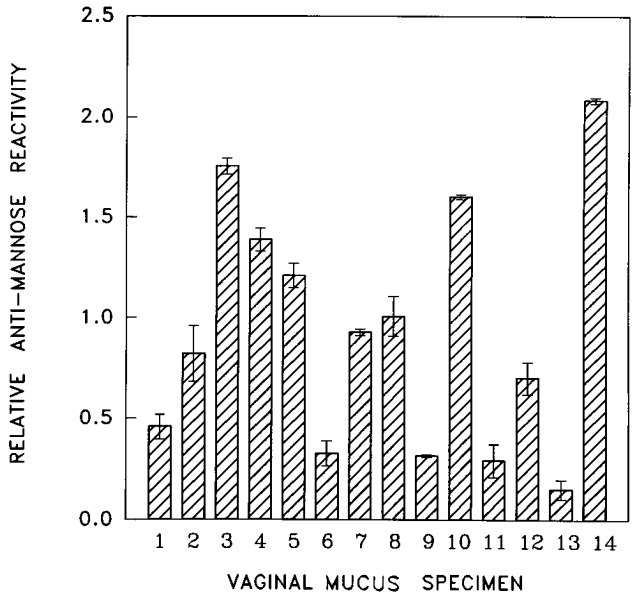

FIG. 6. Reactivity of anti-mannose polyclonal antibody to vaginal mucus specimens. The data shown are for the same individual specimens as those shown in Fig. 4. The data are presented as the RFU obtained for the mucus specimens relative to the RFU for BSM, after subtracting the RFU values for AI. For an explanation of the error bars, see the legend to Fig. 1.

antibody. The BSM-positive control showed significantly higher reactivity with the antibody (more than threefold) compared with that of the AI negative control (data not shown), a ratio similar to that of the bacterial binding to BSM and AI shown in Fig. 1. All of the mucus specimens tested (the same specimens analyzed for bacterial binding) exhibited positive reactivity with anti-mannose antibody to differing degrees. Figure 6 shows the reactivities of 14 of the 28 specimens examined (the same 14 shown for bacterial binding in Fig. 4). A nearly 14-fold range of reactivities of the anti-mannose antibody to the vaginal mucus specimens was observed (0.15 to 2.08 relative to the BSM control).

To compare the mannose-specific bacterial binding capacity and the anti-mannose reactivity of the mucus specimens, Kendall's rank correlation test was done to assess the relationship between both systems. The correlation coefficient for the total number of samples ($N = 28$) was 0.27 ($P = 0.046$), suggesting a significant relationship between the amount of mannose in the mucus specimens and the extent of bacterial binding to the mucus.

Bacterial binding to mucus specimens collected over time. (i) Samples collected over a 5-day period. To assess the possibility of variation in binding over time, vaginal mucus specimens from two secretor women (one with a history of UTIs and the other without) were examined for binding of HB101/pWRS1-17 bacteria. The results are shown in Fig. 7A and are normalized to the results with BSM. Each of the mucus specimens showed different amounts of bacteria bound for each day, with the greatest differences being 2.5- and 3-fold.

(ii) Samples collected over a 12-week period. Vaginal mucus specimens from one woman with a history of UTIs (a secretor) were collected weekly over 12 weeks and tested for bacterial binding. Results, normalized to the results with BSM, are shown in Fig. 7B. Bacterial binding to the specimen from each week differed to various extents, the greatest difference being observed between specimens collected on weeks 1 and 10 (17-fold). The coefficient of variation [(standard deviation/mean) $\times$ 100] was 40% for the bacterial binding to the specimens collected over these 12 weeks. This is 3.3-fold higher than the 12% coefficient of variation of the assay itself, as determined from duplicates of two different experiments performed on different days. These data indicate that the variations observed for the daily or weekly specimens reflect real differences of bacterial binding capacities of the mucus specimens.

DISCUSSION

Numerous in vitro systems have been developed to examine and assess the adhesive properties of bacteria, but many aspects of adhesion as it occurs in vivo are still unclear. Studies performed prior to this time have mainly examined the binding of bacteria to cell surface carbohydrate sequences; only recently has the role of mucus glycoconjugates been investigated in either promoting or inhibiting bacterial adhesion. These investigations have focused mainly on adherence to salivary glycoproteins by several strains of streptococci (21), adherence to human intestinal mucins by *Yersinia enterocolitica* (18) and *Helicobacter pylori* (5), and binding to respiratory mucins by *Pseudomonas aeroginosa* (30, 37, 41) and *Pseudomonas cepacia* (31). Several strains of staphylococci have been examined with respect to their binding to mucus of the upper respiratory tract in an in vivo ferret model (32) and to mucin specimens from the airways of patients with cystic fibrosis (39). Other studies have shown that of various type 1-piliated strains of *E. coli*, only one of them bound to rat intestinal mucins (29). Orskov et al. (26) compared two *E. coli* strains, one mannose sensitive (strain C1212) and one mannose resistant (strain C1214), for their capacity to adhere to human urinary epithelial cells and to mucus purified from urine, finding that strain C1214 adhered in great number to mucus and left the cells almost free of bacteria, whereas strain C1212 attached strongly to cells but not to urinary mucus. Babu et al. (1) reported that a 60-kDa glycoprotein isolated and purified from human saliva was

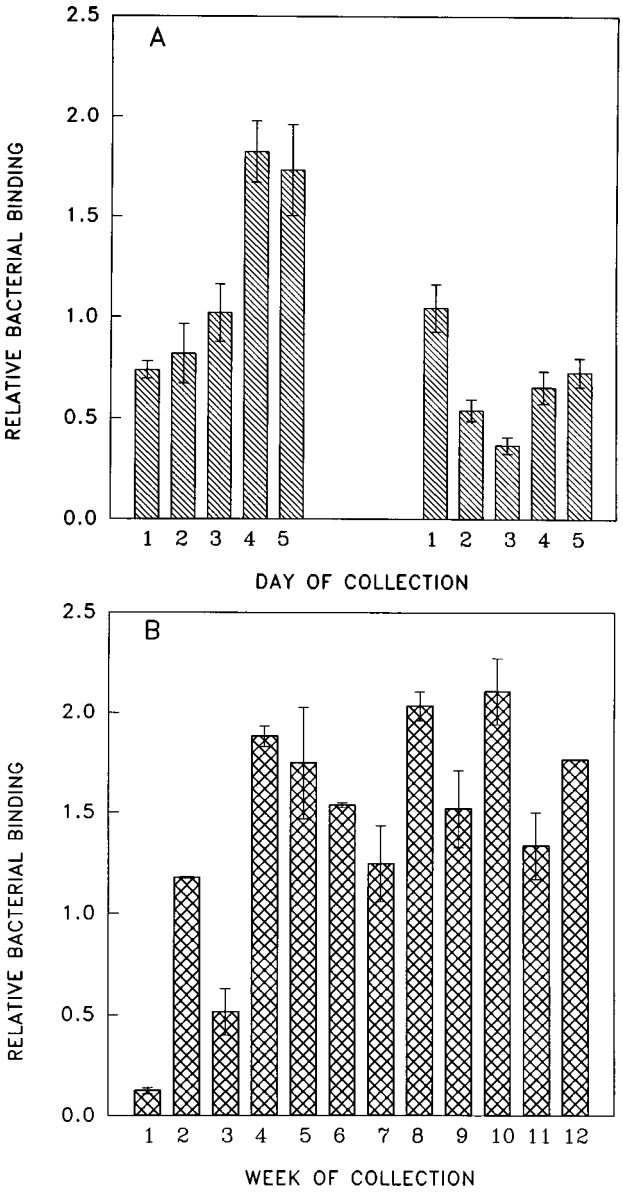

FIG. 7. Binding of *E. coli* HB101/pWRS1-17 to vaginal mucus specimens collected over time. (A) Bacterial binding to mucus from two women, one with a history of UTIs (bars 1 to 5 on left) and the other without a history of UTIs (bars 1 to 5 on right) on samples collected over a 5-day period; (B) bacterial binding to mucus specimens from one individual collected each week for a 12-week period. The assay was performed by adding 3×10^7 ^{3}H-labeled bacteria to 10 μg of mucus per ml used to coat the microtiter wells. The data are shown as bacteria bound to the mucus specimens relative to bacteria bound to BSM. For an explanation of the error bars, see the legend to Fig. 1.

found to interact with type 1 fimbriae and prevent adhesion of *E. coli* to human buccal epithelial cells in a mannose-sensitive manner, preventing colonization by these organisms of oropharyngeal mucosa and dental tissues. Furthermore, type 1-fimbriated *E. coli* (mannose sensitive) bound and was coated by Tamm-Horsfall glycoprotein, forming a pseudocapsule of host proteins which protected bacteria from phagocytosis and killing and therefore could possibly be more virulent (16). In addition, mucin glycoproteins from human milk have been reported to inhibit adhesion of S-fimbriated *E. coli* to buccal epithelial cells (34), and *E. coli* adherence to rabbit, bovine, and human bladder mucosa has been investigated in relation to recurrent UTIs (20, 27, 28).

Our study represents the first documented investigation of adhesion of bacteria to vaginal mucus, an important target relevant to ascending UTIs. We have developed a system to show that the type 1-piliated HB101/pWRS1-17 *E. coli* strain binds to all vaginal mucus specimens collected from a population of 28 women. The number of bacteria bound to solid phase-adsorbed vaginal mucus was a function of the number of bacteria added and was dependent on the concentration of mucus specimens used to coat the plate wells. In the different binding experiments, a positive mucin, BSM, was incorporated to control for variation in the number of bacteria bound, which may have been due to the different extents of piliation of bacteria in different experiments. The glycoprotein AI was used as a negative or background control since it lacked the mannose-terminated carbohydrate necessary for type 1-piliated *E. coli* binding. Thus, in all experiments, values for AI were subtracted before calculating the relative binding to the BSM control. Furthermore, the bacterial binding was mainly mannose dependent since 1% mannose added to the binding incubation mixture almost completely inhibited the adherence of bacteria to mucus.

An important observation in the development of the bacterial binding assay was that certain proteins, including BSA, ovalbumin, and casein, used for backcoating microtiter plate wells, resulted in substantial bacterial binding independent of mucus coating. Therefore, all binding experiments were performed with mucus-coated wells without backcoating. The apparent nonspecific binding of bacteria to backcoating proteins has been reported previously for the binding of *P. cepacia* and *P. aeruginosa* to human intestinal and respiratory mucins from patients with cystic fibrosis (30, 31) and in an investigation of binding of various staphylococci strains to mucus from the ferret respiratory tract (32). BSA has also been reported to slightly increase *E. coli* adherence to rabbit bladder mucin (27).

Clinical studies with animal models of UTIs have led to the suggestion that women with recurrent UTIs might have either more adhesin receptors for bacterial binding on their genitourinary mucosa, and therefore more binding sites for *E. coli*, or fewer soluble receptor compounds in their mucosal secretions (23, 33). Also, it has been reported that uroepithelial cells from women with the nonsecretor phenotype are more readily bound by bacteria as compared with cells from healthy women with the secretor phenotype (17) and that women with recurrent UTIs are more likely to be nonsecretors than women that are not infection prone (36). Calculations of the mean values for either the bacterial binding to mucus or the anti-mannose reactivities to mucus were similar for the groups of women with or without a history of UTIs or for the secretor or nonsecretor groups. Furthermore, there were 15-fold differences of bacterial binding and nearly 14-fold differences of anti-mannose reactivity between the various mucus specimens. We suggest that such wide variations between individual specimens preclude any meaningful comparison of groups of women based on a history of UTIs or secretor status. However, statistical analysis of the amount of mannose-terminal residues of the mucus specimens (by reactivity with the anti-mannose antibody) and the amount of type 1 bacteria bound showed a significant correlation ($P = 0.046$), indicating that accessibility and number of mannose residues may be the primary determinants for bacterial binding.

Two important considerations must be made when discussing the mechanisms of bacterial binding to vaginal mucus. First, mucin glycoproteins in solution are conformationally more flexible than they are when immobilized in microtiter

wells and, therefore, may coat bacteria more easily or make contact with bacterial binding sites more readily (31). Second, the variable expression of piliation by bacterial populations in urine has been reported, and bacterial populations from different sites within the urinary tract differ with respect to expression of type 1 pili (13). These observations suggest that type 1-piliated *E. coli* and vaginal mucus interactions may occur in vivo in a different manner than they do in vitro.

Another issue to be considered is that Tamm-Horsfall, a mannose-containing glycoprotein normally present in normal human urine and synthesized by the kidney, has been reported to have differential effects on the adherence of type 1-piliated *E. coli* to bladder epithelial cells by either inhibiting bacterial binding at high concentrations or enhancing it at low concentrations (4). Orskov et al. (25) have shown that type 1-fimbriated *E. coli* was trapped by precipitated Tamm-Horsfall and postulated that this trapping may serve as a host defense, allowing efficient clearing of bacteria from the urinary bladder. Also, Kuriyama et al. (16) have hypothesized that type 1-fimbriated *E. coli* coated with Tamm-Horsfall might be susceptible to phagocytosis and, hence, possibly could be more virulent.

We also observed that bacterial binding to vaginal mucus changes over time, with 3-fold differences observed in the five daily specimens and 17-fold differences between the lowest- and highest-binding specimens collected weekly for 12 weeks. The data clearly show temporal differences in the capacity of vaginal mucus to behave as a receptor for type 1-piliated bacterial binding. Thus, an individual may be more or less susceptible to the initial adherence of bacteria and then to colonization and UTI, depending on the amounts of glycoproteins and mannose receptors, which appeared to vary from day to day.

The data presented here showing the differences between an individual's vaginal mucus mannose receptors and bacterial binding to mucus suggest that such differences play a role in colonization of the vaginal mucosa and in the pathogenesis of UTIs. Furthermore, the results indicate that temporal differences of the receptors and bacterial binding, or an average over time of vaginal mucus characteristics, may be important factors in whether an individual is susceptible to the establishment of a UTI. Additional studies are in progress to assess vaginal mucus characteristics through the temporal course of UTIs.

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