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Competitive Exclusion of Uropathogens from Human Uroepithelial Cells by *Lactobacillus* Whole Cells and Cell Wall Fragments

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Previous studies have shown that indigenous bacteria isolated from cervical, vaginal, and urethral surfaces of healthy women are able to adhere to human uroepithelial cells in vitro. Furthermore, these organisms were found to block the adherence of uropathogenic bacteria to uroepithelial cells from women with and without a history of urinary tract infections. In the present study, complete or partial inhibition of the adherence of gram-negative uropathogens was achieved by preincubating the uroepithelial cells with bacterial cell wall fragments isolated from a *Lactobacillus* strain. Competitive exclusion was most effective with whole viable cells and less effective with cell wall fragments obtained by sonication, extraction with sodium dodecyl sulfate, and treatment with sodium dodecyl sulfate and acid. Analysis of the *Lactobacillus* cell wall preparations suggested that lipoteichoic acid was responsible for the adherence of the *Lactobacillus* cells to uroepithelial cells but that steric hindrance was the major factor in preventing the adherence of uropathogens. This conclusion was also supported by blockage studies with reconstituted lipoteichoic acid-peptidoglycan, which was more effective at blocking adherence than lipoteichoic acid or peptidoglycan alone. The results suggest that the normal flora of the urinary tract may be used to protect against the attachment of uropathogens to the surfaces of uroepithelial cells. The long-term implications of these findings may lead to alternative methods for the management and prevention of recurrent urinary tract infections in females.

Urinary tract infections (UTI) are a common cause of illness in females. The causative organisms are known to include *Escherichia coli*, *Proteus* sp., *Klebsiella* sp., and *Pseudomonas aeruginosa* strains (11). The adherence of uropathogenic organisms to the vesical epithelium is considered to be a prerequisite for the onset of UTI (20) and appears to be influenced by both bacterial and host cell properties (5, 15). Recently, receptor sites for *E. coli* strains which express P fimbriae have been identified on human uroepithelial cells (10, 12). In addition, attempts to inhibit bacterial colonization of the bladders and kidneys of mice have been made with glycolipid compounds, which block the attachment receptor sites (21).

Studies in our laboratories have shown that the normal urethral, vaginal, and cervical flora of healthy females can competitively block the in vitro attachment of uropathogenic bacteria to the surfaces of uroepithelial cells from women with and without a history of UTI (6). In the present study, we used cell wall fragments from a *Lactobacillus* isolate to competitively exclude uropathogens from uroepithelial cell surfaces. In addition, we investigated the mechanism by which the normal flora adhere to the host cells and block the attachment of pathogens. An in vitro radiometric adherence assay was used for these studies, and results were confirmed by both light and electron microscopy.

MATERIALS AND METHODS

Bacteria. Six uropathogens were isolated from the urine of patients with chronic cystitis, maintained on brain heart infusion (BBL Microbiology Systems) agar slants, and stored at −70°C (6). These included an *E. coli* strain possessing a mannose-sensitive (MS) hemagglutinin (adhesin), an *E. coli* strain possessing a mannose-resistant (MR) hemagglutinin (adhesin), an *E. coli* strain expressing a capsule, a *Klebsiella pneumoniae* strain, a mucoid strain of *P. aeruginosa*, and a nonmucoid strain of *P. aeruginosa*. A *Lactobacillus* strain was isolated from a swab specimen of the distal urethra of a healthy woman and identified by standard diagnostic procedures (6). All organisms were transferred to urine before use in adherence assays or electron microscopy. All the strains attached well to uroepithelial cells after growth in urine, as previously reported (5, 6).

Growth and labeling of bacteria. The bacteria were grown overnight at 37°C in filter-sterilized urine, inoculated into 2.0 ml of fresh, filter-sterilized urine supplemented with 20 μCi of [^{3}H]uridine per ml, and incubated at 100 rpm for 18 h. The bacteria were harvested by centrifugation and suspended to a concentration of 10^8 CFU/ml in phosphate-buffered saline (PBS) (pH 6.7). The ratio of CFU to disintegrations per minute was determined by viable plate counting and by collecting 0.2-ml aliquots of labeled bacteria on a 0.2-μm-pore-sized polycarbonate membrane filter (Nucleopore Corp.), determining the counts per minute associated with the filter by scintillation counting, and subsequently determining the disintegrations per minute (6).

Uroepithelial cells. Uroepithelial cells were obtained from freshly voided midstream urine samples from four healthy, premenopausal women (20 to 36 years old) with no past history of UTI, as their cells had been shown to be as receptive to bacterial attachment as cells from women with a past history of UTI (6). The urine samples were collected on day 10 of the menstrual cycle, as previous studies had established that the receptivity of uroepithelial cells to bacterial attachment was optimum on this day (before ovu-

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lation) (6, 15). Uroepithelial cells were harvested by centrifugation, washed once with PBS, and suspended in PBS to a concentration of 10^5 cells per ml (counted with hemacytometer).

In vitro adhesion assay. The adhesion assay has been described in detail elsewhere (3, 5). Briefly, aliquots of labeled bacteria and uroepithelial cells were combined, incubated at 37°C for 30 min, and then filtered through 5.0-μm-pore-sized filters, which were then washed with PBS, air dried, placed in a scintillation vial containing 15 ml of scintillation fluid, and counted in an LKB 1211 Minibeta Counter. The number of bacteria adherent to uroepithelial cells retained on the filters was determined by light microscopy before scintillation counting. The number of adherent bacteria was determined by subtracting the control counts (uroepithelial cells only and bacterial cells only) from the disintegrations of the mixtures on the 5.0-μm filters per minute. Mean attachment figures were calculated by dividing the number of adherent bacteria by the number of uroepithelial cells. For competitive-exclusion experiments, the assay was performed by preincubating (37°C, 30 min) pooled uroepithelial cells with nonlabeled whole cells or cell wall fragments, obtained from the *Lactobacillus* strain, before challenge with radiolabeled uropathogens. *Lactobacillus* whole cells and cell wall fragments did not aggregate uroepithelial cells under the assay conditions, as verified by light microscopy. The inhibition of uropathogen adherence was quantitated by measuring the number of labeled uropathogens which adhered to uroepithelial cells, with and without the addition of the whole cells and cell wall fragments.

Preparation of *Lactobacillus* cell wall fragments. A distal urethral *Lactobacillus* isolate was grown overnight at 37°C in 750 ml of filter-sterilized urine supplemented with 0.5% glucose and 0.5% lactose. The cells were harvested by centrifugation at 15,000 × *g* for 10 min at 4°C, washed, and suspended in PBS. The culture was divided into aliquots, and the cells were then subjected to various treatments. For fraction A, the bacterial cells were sonicated by 10 30-s pulses, with cooling in between, centrifuged at 12,000 × *g* for 10 min, and suspended in 20 mM Tris (pH 7.4)–8 mM $MgCl_2$–1 μg of RNase A (Sigma Chemical Co.) per ml–1 μg of DNase I (Sigma) per ml. The sample was incubated at 37°C for 3 h, washed twice, and suspended in PBS. For fraction B, the fraction A sample was heated to 100°C in PBS with 1.0% (wt/vol) sodium dodecyl sulfate (SDS) for 4 h, centrifuged three times at 12,000 × *g* for 10 min (each time), dialyzed against 1 liter of 1% bovine serum albumin in distilled water, and resuspended in PBS. For fraction C, the cell wall fragments (fraction A) were treated with SDS as described for fraction B, treated with 10 ml of 0.1 N H_2SO_4, neutralized with sodium hydroxide, washed twice with distilled water, and dialyzed for 72 h in 4 liters of water. Fraction C (peptidoglycan) did not contain any chloroform-methanol-extractable phospholipid, did not incorporate any [^{32}P]PO_4, and was not found to have any associated proteins by SDS-polyacrylamide gel electrophoresis. The wet and dry weights of each fraction were determined.

Formalin-treated and acid-treated whole cells. Whole cells of the *Lactobacillus* culture were fixed with 10% Formalin in PBS at room temperature for 1 h, washed three times in PBS, and resuspended in PBS. Acid-treated whole cells were prepared by incubating the cells in 0.1 N H_2SO_4 for 3 min, neutralizing them with NaOH, washing them three times with distilled water, and suspending them in PBS.

Studies of LTA. For studies of lipoteichoic acid (LTA), the bacteria were grown overnight in filter-sterilized urine supplemented with 10 μCi of [^{32}P]PO_4 per ml. The cells were harvested, and the LTA was extracted by boiling the cells for 1 h in 100% phenol. The LTA-containing cell walls were then washed in PBS by centrifugation, and suspended in PBS, and dialyzed overnight against water. A sample of the LTA was incubated in 0.1 N ammonium hydroxide overnight to deacylate the LTA; the sample was then neutralized and suspended in water. The wet and dry weights of each sample were then determined. The LTA was found to be protein free and did not contain any chloroform-methanol-extractable phospholipid.

For the adherence assays, a dry weight equivalent to 10^8 *Lactobacillus* whole cells for each fraction was added to 10^5 uroepithelial cells. For fractions A, B, and C and for the Formalin- or acid-treated whole cells, the property of competitive exclusion was measured as the percent inhibition of the adherence of uropathogens; in LTA studies, the amount of ^{32}P which bound to uroepithelial cells was determined by scintillation counting.

Reconstitution of LTA-peptidoglycan cell wall fragments. Excess amounts of purified, nonlabeled LTA were incubated with peptidoglycan (fraction C) at 37°C for 1 h. The excess of LTA was then removed from the LTA-peptidoglycan mixture by centrifugation, and these reconstituted cell wall fragments were dialyzed and suspended in PBS before being used for competitive-exclusion studies. The binding of LTA to peptidoglycan was assessed by running a parallel experiment with ^{32}P-labeled LTA.

Transmission electron microscopy. For examination of bacterial adherence to uroepithelial cells, the assay specimens were fixed in 5% (vol/vol) glutaraldehyde in cacodylate buffer (0.1 M; pH 7.2) containing 0.15% (vol/vol) ruthenium red for 2 h at room temperature. The specimens were then washed five times in buffer, postfixed in 2% osmium tetroxide in buffer, washed five times in buffer, and dehydrated through a graded acetone series. All the solutions used after primary fixation up to 70% acetone were made to contain 0.05% (wt/vol) ruthenium red. Ruthenium red was omitted from the 90 and 100% acetone because of limited solubility in these solutions. After further dehydration in propylene oxide, the specimens were embedded in Vestopal (J. B. EM Service Inc.), sectioned, and stained with uranyl acetate and lead citrate. The sections were examined with a Hitachi 500 electron microscope operating at an accelerating voltage of 60 kV.

Statistical analysis. The adherence results were analyzed with the Kruskall-Wallis test applied to a two-way lay out. This is a test for which there is no distribution assumption (13).

RESULTS

The *Lactobacillus* isolate obtained from the distal urethra of a healthy woman was found to adhere readily in vitro to pooled uroepithelial cells, confirming previous findings (6). The *Lactobacillus* isolate appeared to attach to the surfaces of uroepithelial cells by means of either its cell wall or its glycocalyx (Fig. 1).

Adherent *Lactobacillus* cells substantially inhibited the adherence of a number of uropathogens to uroepithelial cells, with the following percentages of inhibition of adherence: MS *E. coli*, 75.4%; MR *E. coli*, 74.1%; encapsulated *E. coli*, 70.6%; *K. pneumoniae*, 76.9%; nonmucoid *P. aeruginosa*, 69.6%; and mucoid *P. aeruginosa*, 61.9% (Table 1). Formalin-fixed *Lactobacillus* whole cells were less effective

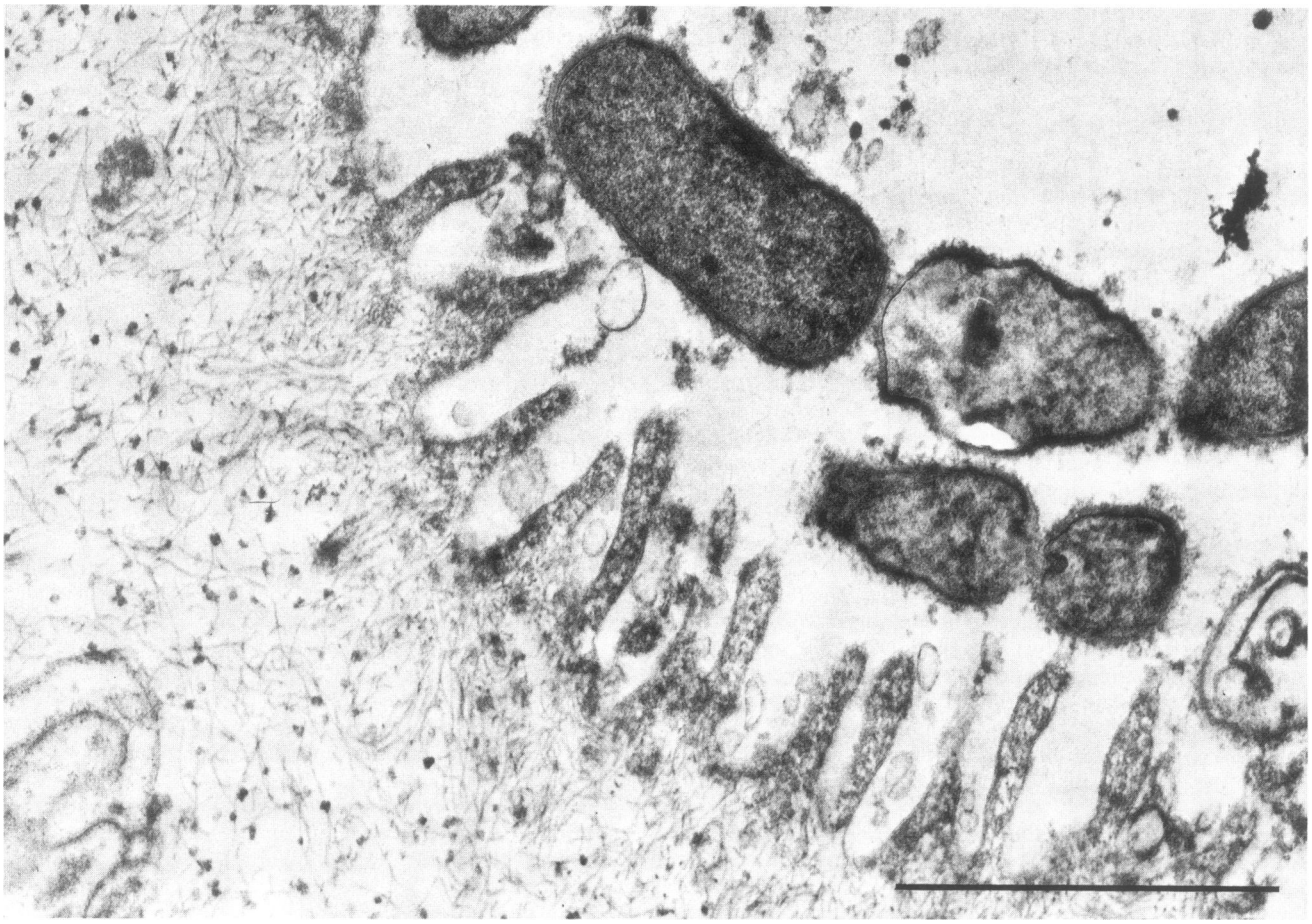

FIG. 1. Transmission electron micrograph of a thin section of a ruthenium red-stained *Lactobacillus*-uroepithelial cell mixture, showing bacteria adhering to the uroepithelial cell surface. The adherence appears to be mediated by the bacterial glycocalyx or cell wall or both. Bar, 1.0 μm.

than untreated whole cells in inhibiting uropathogen adherence to uroepithelial cells, as confirmed statistically with the Kruskall-Wallis test ($P < 0.025$), with the exception of the MR *E. coli* strain (Table 1). Acid-treated *Lactobacillus* whole cells were considerably less effective than Formalin-fixed cells ($P < 0.005$) or viable cells ($P < 0.001$) in blockinguropathogen adherence (Table 1).

TABLE 1. Inhibition of uropathogens adherence by *Lactobacillus* whole cells[a]

Uropathogen	% Inhibition (mean ± SD) of adherence to uroepithelial cells caused by:		
	Whole cells	Formalin-treated whole cells	Acid-treated whole cells
E. coli (MS)	75.4 ± 34.7	42.5 ± 23.4	31.2 ± 16.4
E. coli (MR)	74.1 ± 36.6	71.9 ± 13.6	17.3 ± 9.8
E. coli (encapsulated)	70.6 ± 41.2	38.1 ± 16.3	12.3 ± 6.8
K. pneumoniae	76.9 ± 32.6	43.0 ± 19.8	24.7 ± 13.0
P. aeruginosa (nonmucoid)	69.6 ± 34.9	41.2 ± 16.8	29.7 ± 18.7
P. aeruginosa (mucoid)	61.9 ± 36.9	38.1 ± 16.3	4.8 ± 0.7

[a] Uroepithelial cells were incubated first with *Lactobacillus* whole cells and then with uropathogenic bacteria to test for competitive exclusion.

Sonicated cell wall fragments inhibited the attachment of the encapsulated *E. coli* strain by 74.7%, of the MS and MR *E. coli* strains by 52.1 and 50.9%, respectively, of the nonmucoid and mucoid *P. aeruginosa* strains by 46.4 and 42%, respectively, and of *K. pneumoniae* by only 33.8%

TABLE 2. Inhibition of uropathogens adherence by various *Lactobacillus* cell wall fragments[a]

Uropathogen	% Inhibition (mean ± SD) of adherence to uroepithelial cells caused by:		
	Sonicated cell fragments (fraction A)	SDS-extracted cell fragments (fraction B)	SDS-extracted, acid-treated cell fragments (fraction C)
E. coli (MS)	52.1 ± 5.4	36.8 ± 19.0	3.9 ± 2.8
E. coli (MR)	50.9 ± 20.1	27.9 ± 12.3	4.2 ± 3.5
E. coli (encapsulated)	74.7 ± 6.8	52.4 ± 18.1	4.8 ± 2.6
K. pneumoniae	33.8 ± 6.1	22.7 ± 16.7	4.6 ± 3.8
P. aeruginosa (nonmucoid)	46.4 ± 8.2	47.5 ± 8.8	2.5 ± 1.3
P. aeruginosa (mucoid)	42.0 ± 8.6	30.3 ± 0.14	6.3 ± 7.4

[a] Uroepithelial cells were preincubated with *Lactobacillus* cell wall fragments (dry weight equal to the dry weight of whole cells) before being incubated with uropathogenic bacteria to test for competitive exclusion.

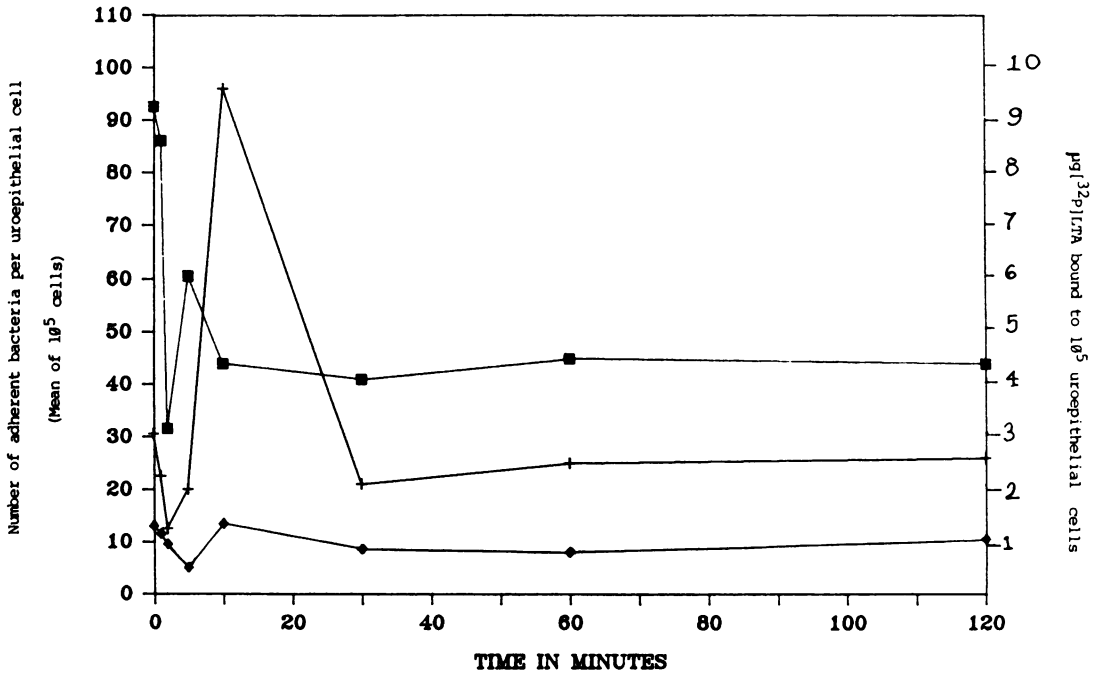

FIG. 2. Graphic representation of the binding kinetics of *Lactobacillus* cells (■), [^{32}P]LTA (+), and deacylated [^{32}P]LTA (◆) to uroepithelial cells.

(Table 2). SDS-extracted cell wall fragments were somewhat less effective than sonicated cell wall fragments ($P < 0.25$) or whole cells ($P < 0.005$) in inhibiting uropathogen attachment, but a considerable level of inhibition still occurred (Table 2). SDS-extracted, acid-treated cell wall fragments were essentially noneffective in blocking uropathogen attachment (Table 2).

LTA has been reported to mediate the attachment of gram-positive organisms to buccal cells, polymorphonuclear leukocytes, and human plasma fibronectin (4, 7, 8). LTA was purified from *Lactobacillus* cells to determine whether this compound was involved in the inhibition of uropathogen adherence. Purified LTA was found to bind to uroepithelial cells, and the kinetics of LTA binding were very similar to those observed for *Lactobacillus* whole cells (Fig. 2). Deacylated LTA did not bind effectively to uroepithelial cells.

LTA inhibited the adherence of the MS, MR, and encapsulated *E. coli* strains by 23.6, 28.8, and 27.4%, respectively, of *K. pneumoniae* by 24.4% and of the nonmucoid *P. aeruginosa* strain by 24.8%, but the adherence of the mucoid *P. aeruginosa* strain was only inhibited 8.7% by LTA (Table 3). Blockage by LTA was not as effective as blockage by whole cells ($P < 0.005$). Deacylated LTA did not effectively block the adherence of uropathogenic bacteria (Table 3). The adherence of *Lactobacillus* cells to uroepithelial cells was inhibited by LTA, and the mean inhibition value obtained was 62%.

Purified LTA and cell wall fraction C (SDS-extracted, acid-treated cell wall fragments) from *Lactobacillus* cells readily reassociated unless the LTA was deacylated. The reconstituted LTA-cell wall complex inhibited the attachment of the MS, MR, and encapsulated *E. coli* strains to uroepithelial cells by 48.6, 48.8, and 69.5%, respectively, and of the *K. pneumoniae* and nonmucoid and mucoid *P. aeruginosa* strains by 40.2, 44.0, and 31.2%, respectively (Table 4). The blocking effect conferred by the reconstituted LTA-peptidoglycan was significantly greater than that conferred by LTA alone ($P < 0.005$) and was similar to that

TABLE 3. Inhibition of uropathogens adherence by LTA isolated from *Lactobacillus* cells[a]

Uropathogen	% Inhibition (mean ± SD) of adherence to uroepithelial cells caused by:	
	LTA	Deacylated LTA
E. coli (MS)	23.6 ± 4.2	8.7 ± 1.4
E. coli (MR)	28.8 ± 8.7	14.6 ± 3.1
E. coli (encapsulated)	27.4 ± 6.0	12.4 ± 4.2
K. pneumoniae	24.4 ± 5.2	6.3 ± 0.4
P. aeruginosa (nonmucoid)	24.8 ± 6.0	4.8 ± 2.0
P. aeruginosa (mucoid)	8.7 ± 1.4	0.0

[a] Uroepithelial cells were preincubated with *Lactobacillus* LTA or deacylated LTA (dry weight equal to the dry weight of whole cells) before being incubated with uropathogenic bacteria to test for competitive exclusion.

TABLE 4. Inhibition of uropathogens adherence by reconstituted *Lactobacillus* cell wall fragments (LTA-peptidoglycan [fraction C])[a]

Uropathogen	% Inhibition (mean ± SD) of adherence to uroepithelial cells caused by reconstituted LTA-peptidoglycan
E. coli (MS)	48.6 ± 5.2
E. coli (MR)	48.8 ± 6.4
E. coli (encapsulated)	69.5 ± 13.1
K. pneumoniae	40.2 ± 8.8
P. aeruginosa (nonmucoid)	44.0 ± 6.6
P. aeruginosa (mucoid)	31.2 ± 2.8

[a] Uroepithelial cells were preincubated with reconstituted LTA-peptidoglycan (fraction C) (dry weight equal to the dry weight of whole cells) before being incubated with uropathogenic bacteria to test for competitive exclusion.

conferred by the cell wall fragments fraction A ($P < 0.75$) and fraction B ($P < 0.25$).

DISCUSSION

It is now accepted that an important step in the onset of UTI in females is the capacity of pathogenic bacteria to attach to uroepithelial cells (16). Although previous studies have shown that nonpathogenic bacteria also adhere to uroepithelial cells, the role of these organisms has not been fully established (9). It was recently demonstrated that indigenous bacteria could inhibit the adherence of uropathogens to uroepithelial cells (6). The present investigation is an extension of earlier studies and examines the mechanism of blockage of pathogen adherence to uroepithelial cells by a "normal" indigenous bacterial strain.

The *Lactobacillus* isolate used in this study readily attached to uroepithelial cells (Fig. 1), although the entire surface of uroepithelial cells was never completely occupied by the *Lactobacillus* cells. The attachment of *Lactobacillus* cells to uroepithelial cells appeared to be mediated by the bacterial glycocalyx or cell wall. Uropathogenic bacteria also adhered in large numbers to uroepithelial cells in previous studies (3, 5, 6).

Attached *Lactobacillus* whole cells were fairly effective in blocking the attachment of MS, MR, and encapsulated *E. coli* strains and of *K. pneumoniae* and mucoid and nonmucoid *P. aeruginosa* strains (Table 1). The extent of the blockage of uropathogen adherence was somewhat variable for all the isolates examined and may have been due to the inability of the *Lactobacillus* cells to consistently occupy the same uroepithelial cell surface area under these experimental conditions. Previous studies also demonstrated a blocking effect which varied depending on the strains of normal flora and uropathogenic bacteria used in the assay; occasionally, uropathogen adherence was completely inhibited by *Lactobacillus* whole cells (6). These earlier studies also showed that the inhibition of uropathogen adherence to uroepithelial cells was less effective when *Lactobacillus* cells were incubated with various uropathogen-uroepithelial cell mixtures (6). In the present study, the ability of *Lactobacillus* cells to block uropathogen adherence was reduced by Formalin fixation (except in the case of the MR *E. coli* strain) and considerably reduced by acid treatment (Table 1), as confirmed by statistical analysis (13).

To examine the nature of the blockage of uropathogen adherence, we assessed the ability of various *Lactobacillus* cell wall preparations and components to block uropathogen adherence. *Lactobacillus* sonicated cell wall fragments inhibited uropathogen adherence to uroepithelial cells, although to a lesser degree than did whole cells, with the exception of the encapsulated *E. coli* strain (Table 2). There was considerably less variation in the ability of cell wall fragments to block adherence than in that of *Lactobacillus* whole cells to do so. This suggests that the binding of *Lactobacillus* whole cells to uroepithelial cells was more variable than that of cell wall fragments, possibly due to steric limitations. *Lactobacillus* SDS-extracted cell wall fragments inhibited uropathogen adherence, although not to as great an extent as nonextracted cell wall fragments (Table 2), indicating that the blockage of uropathogen adherence was probably not due to protein(s). *Lactobacillus* SDS-extracted acid-treated cell wall fragments were largely noneffective at blocking adherence (Table 2), thus implicating an acid-labile component of the *Lactobacillus* cell wall, such as teichoic acid, as the critical component for blocking adherence.

Lactobacillus [^{32}P]-labeled LTA readily bound to the surfaces of uroepithelial cells, with kinetics similar to those observed for whole cells (Fig. 2). Deacylated [^{32}P]-labeled LTA did not bind well to uroepithelial cell surfaces (Fig. 2), nor did it inhibit uropathogen adherence (Table 3). The addition of LTA to uroepithelial cells was found to inhibit the adherence of *Lactobacillus* whole cells. The *Lactobacillus* LTA which bound to the surfaces of uroepithelial cells was effective in blocking uropathogen adherence, but to a lesser degree than the SDS-extracted cell wall fragments (fraction B), which are basically peptidoglycan and LTA ($P < 0.1$; cf. Tables 2 and 3). It is therefore unlikely that there is direct competition between indigenous bacteria, such as *Lactobacillus* spp., and uropathogens for receptor sites on uroepithelial cell surfaces. The reconstitution study with SDS-extracted, acid-treated cell wall fragments (peptidoglycan) and LTA indicated that the reconstituted LTA-peptidoglycan was more effective in blocking uropathogen adherence than the sum of the individual components ($P < 0.025$) and similar in effectiveness to sonicated cell wall fragments ($P < 0.75$; cf. Table 4 with Tables 2 and 3). This suggests that steric hindrance of receptor sites is important in the blockage of uropathogen adherence, particularly as the various uropathogens are likely to have separate and distinct uroepithelial cell surface receptor sites.

Previous studies have shown that LTA from *Streptococcus pyogenes* binds to human polymorphonuclear leukocytes and inhibits the attachment of avirulent streptococci and that the LTA-binding sites are different from those which bind *E. coli* (7). Human plasma fibronectin has been found to contain at least one population of high-affinity binding sites for LTA (8). In addition, epithelial cells from the oropharyngeal cavity have been shown to vary in their receptivity for *S. pyrogenes* and gram-negative bacteria (1), and staphylococcal LTA has been found to reduce the adherence of *Staphylococcus aureus* to buccal cells (4). Our results suggest that LTA mediates the adherence of *Lactobacillus* cells to the surfaces of uroepithelial cells via receptors which differ from those utilized by uropathogens.

The importance of bacterial interference in preventing respiratory tract infections has been examined in various ways by a number of investigators (2, 17–19). With regard to the urinary tract, nonpathogenic bacteria have been found to coexist with uropathogenic bacteria in the introitus of women with and without a history of UTI (9), whereas another study has shown that the urethras of healthy women with no history of UTI are colonized only by indigenous bacteria (14). The results of this latter study may indicate that in some cases indigenous bacteria are important in competitively excluding uropathogens from the uroepithelium in vivo, as suggested by our in vitro results.

The mechanism of competitive exclusion appears to be due to steric hindrance rather than to a specific blockage of receptor sites. The concept that indigenous bacteria can sterically or otherwise prevent the colonization of the uroepithelial mucosa is in need of further investigation. In recent studies in our laboratory, *Lactobacillus* cells were used successfully to exclude uropathogenic colonization of the urinary tract and to prevent the onset of UTI in female rats (G. Reid, R. C. Y. Chan, A. W. Bruce, and J. W. Costerton, manuscript in preparation). The demonstration that at least one indigenous, nonpathogenic bacterium can substantially inhibit the adherence of a number of different uropathogens suggests that the normal flora may eventually provide an alternative strategy for the management of recurrent UTI.

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