Adherence of Type 1–Fimbriated Escherichia coli to Uroepithelial Cells

More in diabetic women than in control subjects

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OBJECTIVE — Women with diabetes have bacteriuria more often than women without diabetes. Because Escherichia coli adhere better to vaginal cells of nondiabetic patients with recurrent urinary tract infections (UTIs) than to those obtained from healthy control subjects, it was hypothesized that E. coli adhere more to the uroepithelial cells of diabetic women, either because of substances excreted in the urine (e.g., albumin, glucose, and Tamm Horsfall protein) or because of a difference in the uroepithelial cells.

RESEARCH DESIGN AND METHODS — A T24 bladder cell line and uroepithelial cells of 25 diabetic women and 19 control subjects were incubated with three different E. coli strains.

RESULTS — The mean numbers of type 1–fimbriated E. coli that adhered to diabetic and control cells were 12.9 and 6.1 (P = 0.001), respectively, whereas those of P-fimbriated E. coli were 8.8 and 8.1 (P = 0.8), and those of nonfimbriated E. coli were 2.7 and 3.4 (P = 0.4). The addition of various substances did not influence the adherence of E. coli to a T24 bladder cell line.

CONCLUSIONS — Type 1–fimbriated E. coli adhere more to diabetic than to control uroepithelial cells.

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Diabetic women have bacteriuria more often than nondiabetic women (1–3). The cause of this increased prevalence, however, is not yet clear. One factor may be microbial adherence, because the adherence of microorganisms to host cells is an important step in the pathogenesis of many infections. The adherence of Escherichia coli (the most common causative microorganism in bacteriuria) to uroepithelial cells, for example, is the first step in the pathogenesis of urinary tract infections (UTIs) (4). In vitro studies have shown that E. coli adhere better to vaginal cells obtained from nondiabetic patients with recurrent UTIs than to those obtained from healthy control subjects (5). Other investigators have shown that Candida albicans adheres better to buccal cells isolated from patients with diabetes than to those isolated from nondiabetic control subjects (6).

Therefore, we hypothesized that E. coli might adhere better to uroepithelial cells isolated from diabetic women than to cells from nondiabetic control subjects.

Because various substances (e.g., glucose and albumin) are present in the urine of diabetic patients, we were interested in investigating whether these substances influence the adherence of E. coli to uroepithelial cells. An important substance is the Tamm Horsfall protein (THP), a large glycosylated polypeptide produced by renal tubular cells and excreted via the urine in daily amounts of 20–200 mg (7). Studies have shown that the binding of THP to type 1 fimbriae of E. coli results in a decreased binding of E. coli to uroepithelial cells (8,9). Elderly men and women, a group of patients prone to develop bacteriuria, have decreased secretion of THP in their urine (10). Diabetic patients can also have decreased THP production. Moreover, their THP might have a different chemical composition (a higher glucose content) (11–13). The latter might lead to an altered binding of THP to E. coli and, consequently, to decreased or increased adherence of E. coli to uroepithelial cells.

The aims of the present study were 1) to investigate the adherence of E. coli to the uroepithelial cells isolated from women with diabetes and to compare it to the adherence of E. coli to cells isolated from women without diabetes and 2) to investigate the influence of THP and a few other substances on the adherence of E. coli to a T24 bladder cell line.

RESEARCH DESIGN AND METHODS

Study population

Diabetic women were recruited from the outpatient clinic of our hospital, whereas the nondiabetic women (control subjects) were laboratory employees. None of the women had a history of recurrent UTIs, had used antimicrobial therapy in the 14 days period before recruitment, or was pregnant at the moment of urine collec-
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The uroepithelial cells, obtained from one or two diabetic women and one control subject, were simultaneously investigated during each experiment.

Collection of uroepithelial cells

Uroepithelial cells were obtained from the urine (collected over a 24-h period) by centrifugation (250g, 10 min), stored at 4°C in Isocove’s modified Dulbecco’s medium (IMDM) (Life Technologies, Breda, the Netherlands), supplemented with 5% FCS (Gibco) and 0.01 mg/ml gentamicin and used within 24 h of collection. The cells were recovered by centrifugation (250g, 10 min) before the experiment and resuspended in IMDM. This centrifugation/resuspension process was repeated four times. The number of cells was calculated by direct light microscopy using a Bürker chamber.

Bacterial strains

Three E. coli strains (all clinical isolates) were used: MS− MR+ (O18K H−), genotype- and phenotype-positive for P fimbriae and G-II adhesin, genotype-positive and phenotype-negative for type I fimbriae, MS+, MR− (O8K H-nontypeable, genotype- and phenotype-positive for type I fimbriae, genotype- and phenotype-negative for P fimbriae), and MS− MR− (O17K H−, genotype- and phenotype-negative for afimbrial adhesin and type 1, S, and P fimbriae). The phylogenetic expression of type 1 fimbriae was measured by the occurrence of mannose-sensitive hemagglutination (MSHA), whereas that of P fimbriae was measured by mannose-resistant hemagglutination (MRHA) (14). Suspensions of 10% human erythrocytes and 10% guinea pig erythrocytes in PBS were used for MRHA and MSHA, respectively. Strains were cultured overnight either in Luria broth (MS+, MR− and MS− MR−) or on blood agar plates (MS+, MR+) to maximize the expression of type 1 and P fimbriae, respectively. Bacteria were then centrifuged (2,510g, 10 min) and resuspended in PBS. The initial inoculum (2–5 × 10⁹/ml) for the experiment was measured by optical density (the Dr. Lange Photometer, Berlin, Germany).

Purification of P fimbriae

E. coli expressing P fimbriae (MS− MR+) were grown overnight on blood agar plates at 37°C and suspended in PBS. The bacteria were collected by centrifuging for 10 min at 2,510g and washing twice with PBS. The bacterial pellet was then suspended in PBS/4 mol/l ureum, placed in a 60°C water bath for 30 min, and left to cool gradually. Next, the suspension was ultracentrifuged for 20 min at 20,000 rpm at 4°C (Centrifor T-2000; Beun-de Ronde, Abcoude, the Netherlands) to mechanically remove the fimbriae from the bacteria. The supernatant was then ultracentrifuged overnight at 40,000 rpm at 4°C. The pellets were suspended in PBS/0.4% SDS and incubated for 30 min at 40°C, followed by centrifugation overnight at 40,000 rpm at 16°C. The pellets were subsequently resuspended in PBS, 4% PEG6000, and 0.5 mol/l NaCl. This suspension was incubated on ice for 1 h and centrifuged for 10 min at 9,500g, after which the pellet was suspended in PBS and kept at −20°C until further use. The purity of the fimbriae was confirmed by SDS-PAGE analysis.

Adherence assay uroepithelial cells

For each experiment, 2 × 10⁵ uroepithelial cells were incubated with either 1 ml of 10⁹/ml bacterial suspension or 1 ml PBS (negative control), with shaking 350 motilities/min for 1 h at 37°C. After incubation, the suspension was washed four times (250g, 10 min) with PBS to remove any unattached bacteria. A portion of the final cell suspension was then dried, fixed on a microscope slide, and Diff-Quik stained (Dade Diagnostika, Duesseldorf, Germany). Cell suspensions with adherent bacteria were examined with oil-immersion light microscopy (×400). The number of E. coli adhering to each of the first 50 uroepithelial cells was counted. The investigator (E.C.v.L.) was blinded with regard to the patient group from which the cells were isolated. Epithelial cells that overlapped other cells were excluded from evaluation. The mean number of E. coli per cell was calculated. All experiments were performed in duplicate, and the cell slides with the different substances were tested at the same time.

Isolation of THP

THP was isolated according to Tamm and Horsfall (16) from the urine of diabetic and nondiabetic women. As stated above, the urine had been collected during a 24-h period. The isolated protein was tested for purity by SDS-PAGE using a 10% polyacrylamide gel and found to have a molecular weight of 92 kDa, as described in the literature (7). Using BCA protein assay (Pierce, Rockford, IL), the following THP concentrations were made: 1, 3, 10, 30, and 100 μg/ml.

Secretor status

The secretor status of the women was determined using the method described by Mollison et al. (17). A blood sample was
taken from eight diabetic and nine nondiabetic women who wanted to participate and whose urine was used for the isolation of uroepithelial cells. These women were classified according to their Lewis blood group type. Those with the red cell phenotype Le(a+b−) were classified as nonsecretors of ABH substance and those with the red cell phenotype Le(a−b+) as secretors. The secretor status of women with the recessive phenotype Le(a−b−) was not further determined.

Statistics
Because the distribution was normal, differences in the adherence of \(E. coli\) to the uroepithelial cells from diabetic women and control subjects, as well as differences in THP isolated from diabetic and control subjects, were tested using the unpaired t test. The paired t test was used to calculate the differences in adherence before and after the addition of various substances (glucose, albumin, and THP) to the T24 cell line. Linear regression analysis was used to calculate the correlations between adherence and age, duration of the diabetes, GHb, and creatinine. \(P < 0.05\) was considered statistically significant.

RESULTS

Study population
A total of 27 women with and 20 women without diabetes were eligible for the study. The uroepithelial cells from one control and two diabetic women, however, were not acceptable for the experiments because many adherent bacteria (>10/cell) were present before the experiment had been started. Therefore, we used uroepithelial cells from 25 women with diabetes (age 55.5 ± 3.0 years, GHb 7.7 ± 0.3%, duration of diabetes 17.8 ± 4.2 years, mean ± SE; of these women, 40% had type 1 diabetes, 40% had retinopathy, 20% had neuropathy, 24% had microalbuminuria) and 19 women without diabetes (age 38.2 ± 3.3 years). THP was isolated from the urine of eight women with diabetes (aged 56.5 ± 1.8 years, GHb 7.7 ± 0.1%) and seven women without diabetes (aged 42.6 ± 1.7 years).

Adherence of \(E. coli\) to uroepithelial cells
The number of type 1–fimbriated \(E. coli\) (MS⁺,MR⁺) adhering to the uroepithelial cells isolated from diabetic women was twice as high as the number in the control subjects (Fig. 1). The mean number of \(E. coli\) (MS⁺,MR⁻) per diabetic cell was 12.9 ± 1.8, compared with 6.1 ± 0.8 per control cell (\(P = 0.001\)). The level of adherence ranged from 2 to 33 (median 12.5) bacteria in diabetic patients and from 2 to 17 (median 5) bacteria in the control subjects. This difference in adherence was not found for the strain expressing P fimbiae or the afimbrial strain (Fig. 1).

We also tested whether the increased adherence of type 1–fimbriated \(E. coli\) could be diminished by adding isolated P fimbiae. We measured the adherence of MS⁺,MR⁻ to the uroepithelial cells of two diabetic patients after the addition of different concentrations of isolated P fimbiae (1, 10, and 50 \(\mu g/ml\)): no decrease in adherence was found (data not shown).

The degree of bacterial adherence of type 1–fimbriated \(E. coli\) was correlated with GHb (\(P = 0.009\)) (Fig. 2). In other words, well-controlled diabetic patients (low GHb) showed less adherence of type 1–fimbriated \(E. coli\) to the uroepithelial cells than poorly controlled diabetic patients. No correlations were found between the adherence of the \(E. coli\) strains and age, postmenopausal status, or the duration or presence of secondary diabetes complications (e.g., retinopathy, neuropathy, and microalbuminuria). Also, no differences in adherence were noted between women with type 1 or type 2 diabetes.

Adherence to T-24 bladder cell line
The mean number of MS⁺,MR⁺ (geno- and phenotypically P fimbiae positive) was 267 ± 33 per 100 cells, the number of MS⁺,MR⁻ (geno- and phenotypically type 1 fimbiae positive) was 168 ± 22 per 100 cells, and the number of MS⁻,MR⁻ (no fimbiae) was 74 ± 16 per 100 cells before the addition of various substances. No significant differences were found for either type 1– or P-fimbriated \(E. coli\) after the addition of the different concentrations of glucose or albumin (all \(P > 0.3\)). The adherence of MS⁺,MR⁻ and MS⁺,MR⁺ measured after the addition of 0.1, 0.5, 1, 5, 10, and 50 mg/ml mannose was used as a positive control (18). As expected, adherence decreased in a dose-response relationship from 82 to 15 bacteria per 100 cells for MS⁺,MR⁻ (phenotype: type 1 fimbiae positive) and remained unchanged for MS⁻,MR⁺ (phenotype: P fimbiae positive).

Physiological concentrations of THP (1, 3, 10, 30, and 100 \(\mu g/ml\)) isolated from either diabetic or control women did not inhibit the mean adherence of MS⁺,MR⁻ to the T24 cell line (all \(P > 0.2\)). No differences could be demonstrated between the THP isolated from diabetic women and that from nondiabetic women (all \(P > 0.2\)).

Secretor status
All of the diabetic women tested were secretors. Of the nine control women
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Figure 2—The GHb (%) vs. x-axis was correlated with the degree of bacterial adherence of type 1-fimbriated E. coli (mean number per cell, y-axis) (P = 0.09, regression coefficient 0.5).

tested, six were secretors. The remaining three had the recessive phenotype Le(a−b−) and were not further classified as secretor or nonsecretor.

CONCLUSIONS  The aim of this study was to determine the adherence of E. coli to uroepithelial cells isolated from women with and without diabetes and the influence of glucose, albumin, and THP on E. coli adherence to a T24 bladder cell line. We found that type 1-fimbriated E. coli adhered to the uroepithelial cells from diabetic women twice as well as to those from nondiabetic control subjects. We also demonstrated that different concentrations of glucose, albumin, or THP did not inhibit the adherence of either type 1- or P-fimbriated E. coli to a T24 bladder cell line. Furthermore, no differences in adherence could be detected after the addition of THP isolated from either diabetic or control women.

Relationships between diseases and the adherence of microorganisms to patient cells have been investigated before (6,19,20). In general, it was found that microorganisms adhere more to (buccal) epithelial cells isolated from patients (children, girls, and women) with recurrent UTIs (5,21,22). This increased adherence of uropathogens to the cells of patients has been considered to be an important factor in the pathogenesis of recurrent UTIs. The increased adherence of type 1-fimbriated E. coli to diabetic uroepithelial cells found in the present study might also be a good explanation for the increased prevalence of asymptomatic bacteruria in women with diabetes, since these fimbriae are the most prevalent virulence factor of E. coli (86% genotypically and 59% phenotypically positive for type 1 fimbriae) isolated from nondiabetic and diabetic patients with asymptomatic bacteriuria (14,23). To evaluate the biological relevance of our finding, we analyzed the data of our previous study (1): among 636 women with diabetes, 115 (20%) of the women developed a symptomatic UTI during 18 months of follow-up. In the subgroup of 478 women without a history of a symptomatic UTI in the year before the study, 69 women (14%) developed a UTI (data not published). Seven of the women with diabetes in the present study also participated in the study mentioned above. Two of them (29%) developed a symptomatic UTI during follow-up.

Because the uroepithelial cells were used in solution, and no other substances (albumin, P fimbriae, glucose, and THP) inhibited the adherence of E. coli with type 1 fimbriae, our results suggest that the increased binding of type 1-fimbriated E. coli to diabetic uroepithelial cells is caused by a difference between the type 1 fimbriae receptor on diabetic and nondiabetic uroepithelial cells. This is supported by Weinmeister and Dal Nogare (24), who showed that the receptors on the buccal cells of severely ill (mechanically ventilated, intensive care unit) patients have a decreased amount of sialic acid and galactose compared with those of healthy control subjects. Those authors suggested that the altered receptor on the epithelial cells of the upper respiratory tract might explain the high prevalence of gram-negative bacterial colonization and pneumonia in the critically ill patients. At this moment, however, we do not know what the difference is between the receptors for type 1 fimbriae: are the receptors on diabetic uroepithelial cells present in a higher density, or do they have a different composition than those on nondiabetic uroepithelial cells? In the present study, we did find a correlation between GHb and the adherence of type 1 fimbriae. Because receptors for the type 1 fimbriae of E. coli are glycoproteins (uroplakins that line the bladder mucosa) (25), we hypothesize that diabetic uroepithelial cells have a different glycosylation of the receptor on their cells, which results in a higher adherence capacity. To test whether the uroepithelial cell receptor for type 1 fimbriae of E. coli does indeed have a different chemical composition in diabetic patients compared with control subjects, we are currently isolating and comparing the receptors for type 1-fimbriated E. coli that are present on these two types of uroepithelial cells.

In concordance with other adherence studies (5,21,26), we found no relationship between the ability of a female subject’s cells to bind bacteria and her age. It has been suggested that bacterial adherence may also be affected by the blood group antigens, which are found on the surface of uroepithelial cells. Individuals with the Lewis blood group phenotype Le(a−b+) secrete Le^b and A, B, or H substances in their saliva and plasma and are called “secretors,” whereas “nonsecretors” with the Le(a+b−) phenotype do have Le^a antigens in their secretions but not A, B, or H substances. Several studies have shown a correlation between the Lewis blood group phenotypes and recurrent
UTIs in adult women (27, 28): nonsecretors have a higher risk of recurrent UTIs. When comparing diabetic and nondiabetic individuals, other authors found similar numbers of individuals who secreted blood group substances (6). We determined the secretor status of a randomly chosen sample of diabetic and control women whose uroepithelial cells had been isolated for the adherence part of the study. Because all diabetic women tested were secretors, the increased adherence we found is not likely caused by an increased frequency of the Lewis blood group nonsecretor phenotype.

The present study showed that THP had no inhibitory effect when used in physiological concentrations. Other authors have reported an inhibitory effect when high concentrations (>250 μg/ml) are used (8, 9). Such concentrations (>250 μg/ml) are not physiological, however, because normal THP excretion is between 20 and 200 mg/24 h. We did not find any differences between the THP isolated from diabetic women and that from control women. Therefore, it is not probable that a difference between diabetic and nondiabetic THP plays a role in the pathogenesis of the increased prevalence of bacteriuria in women with diabetes.

In conclusion, E. coli with type 1 fimbriae adhere to the uroepithelial cells from diabetic women twice as well as to those from nondiabetic women. This adherence is related to the regulation of diabetes. This mechanism may play a role in the pathogenesis and increased prevalence of bacteriuria in diabetic women.

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References