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Serum and Urogenital Antibody Responses to *Escherichia coli* Pili in Cystitis

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Antipili antibody (APA) was quantitated by enzyme-linked immunosorbent assay in the serum, urine, and vaginal secretions of 12 women with cystitis due to *Escherichia coli*. Pili were purified from the strain infecting each patient. The geometric mean titers of APA in serum taken at the initial visit were: immunoglobulin G (IgG), 23.7 ± 3.9; IgA, 4.1 ± 4.0; and IgM, 4.4 ± 5.7. No consistent change was observed between samples obtained at 3 and 6 weeks. APA in serum from 10 control patients was assayed against a pool of pili assembled from the 12 infecting isolates. The geometric mean titers of the control sera were: IgG, 8.4 ± 3.2; IgA, 9.6 ± 2.4; and IgM, 0. Antipili IgG and IgM were significantly greater in the infected than in the control sera ($P < 0.05$). APA was not detected in the urine or vaginal secretions from any infected or control patient. Antigenic relationships between the 12 infecting strains were investigated by indirect immune electron microscopy. All but 1 of 12 infecting strains shared antigenic determinants. Two patients had a second *E. coli* infection during the study. The pili of the reinfecting isolates showed partial antigenic cross-reactivity with the pili of the initial infecting bacteria. These observations indicate that although cystitis stimulates the development of serum APAs, it fails to stimulate local APA and thus may not engender any immunological barrier to subsequent recolonization.

Despite the availability of effective chemotherapeutic agents, recurrent urinary tract infection (UTI) remains a cause of considerable morbidity. Recent studies have focused on the importance of vaginal colonization in the pathogenesis of recurrent UTI. It has been found that women who are prone to recurrent UTI are more likely to have their vaginal vestibules colonized with enterobacteriaceae than women who are not at risk (18), and that this characteristic is due to altered adhesive characteristics of the vaginal epithelial cells (5, 7). An essential prerequisite for colonization is the ability of ingressing organisms to adhere to the vaginal mucosa. For most strains of *Escherichia coli*, adherence is mediated by pili (14, 15). Not all pili have the same properties, however, and there is considerable controversy about whether pili which cause mannose-resistant (MR) or mannose-sensitive (MS) hemagglutination are more important for virulence in the urinary tract (15, 21).

It is unclear whether immune mechanisms operate in the urinary or genital tracts to prevent colonization of the vagina with potential uropathogens. Svanborg-Eden and her colleagues reported that the urine of a patient with pyelonephritis inhibited the adherence of *E. coli* to uroepithelial cells and that this activity was removed by absorption with O antigen and to a lesser extent with pili (22). Other investigators have detected antibodies to *E. coli* surface antigens in urine and vaginal secretions (16, 20), but no studies have specifically quantitated antibodies to pilus antigen. The purpose of the present investigation therefore was to define the local and serum antibody response to *E. coli* pili in women with cystitis. The results indicate that strain-specific antipili antibodies are present in the sera of women with cystitis but absent from their vaginal secretions and urine. The failure of cystitis to stimulate local antipili antibody may account, in part, for the relatively high recurrence rate of lower UTI.

MATERIALS AND METHODS

Twenty-two female patients, 18 to 29 years old, who presented at the University of California–Los Angeles Student Health Center with symptoms of UTI were enrolled in the study. Twelve women had *E. coli* cystitis as documented by a midstream urine culture containing 10^5 or more colonies of *E. coli* per ml and symptoms consistent with cystitis, i.e., dysuria, frequent urination, and absence of fever and flank pain. Ten women whose urine failed to grow a significant bacterial pathogen served as controls.

Samples of serum, urine, and vaginal fluid were collected from all patients at their initial visit. Patients subsequently found to have a urine culture positive for

TABLE 1. Summary of clinical and immunological findings for 12 patients with *E. coli* cystitis

Patient no.	Previous UTI	Duration of symptoms	Antipili antibody titer (reciprocal dilution) in:							
			Initial serum			6-wk serum			Urine	Vaginal secretions
			IgG	IgA	IgM	IgG	IgA	IgM		
1	No	2 days	80	2	1	40	20	2	0	0
2	Yes	4 days	ND[a]	ND	ND	1	2	2	0	0
3	No	3 days	80	80	10	80	40	1	0	0
4	Yes	2 days	40	2	1	20	2	1	0	0
5	Yes	1 wk	10	2	1	2	2	1	0	0
6	Yes	1 wk	40	20	2	40	2	10	0	0
7	Yes	1 wk	10	10	80	80	20	20	0	0
8	No	1 day	10	1	10	20	1	1	0	0
9	Yes	3 days	1	2	80	2	80	80	0	0
10	No	2 days	20	1	10	20	1	1	0	0
11	No	2 mo	80	2	1	80	2	10	0	0
12	No	1 wk	80	10	1	80	2	1	0	0
Geometric mean titer			23.7	4.1	4.4	18.9	4.6	3.1	0	0

[a] ND, Not determined.

E. coli had additional samples obtained 3 and 6 weeks later. In two instances, patients experienced a second episode of *E. coli* cystitis, and additional samples were obtained at that time. Vaginal fluid was collected by the method of Stamey (17). The vagina was lavaged with 50 ml of distilled water, and particulate material was removed from the sample by centrifugation. The samples were lyophilized and stored at −79°C. They were reconstituted with 1 ml of distilled water for assay. The adequacy of the vaginal fluid collection procedure was assessed by determining the immunoglobulin content by radial immune assay on agar plates impregnated with anti-immunoglobulin antibody (Kallastad Laboratories, Chaska, Minn.).

E. coli was identified with API strips (Analytab Products, Plainview, N.Y.). The hemagglutination pattern of each strain and its purified pili was evaluated in microtiter plates by the method of Duguid et al. (3), with guinea pig and human type A erythrocytes used to detect MS and MR agglutination, respectively. Pili were immunotyped by indirect immune electron microscopy as described by Kelen et al. (8) with antisera raised in rabbits against seven clinical isolates of *E. coli*. These strains were found to bear antigenically distinct pili as determined by the presence of little or no cross-reacting antipili antibody by immune electron microscopy and double immune diffusion in agar (P. Rene and F. Silverblatt, unpublished data). A positive reaction was defined as unequivocal labeling of pili at an antiserum dilution of 1:128.

The pili in the isolates from each infected patient and the strains used for serotyping were purified from broth cultures by the method of Brinton (1). The pili of one strain that displayed MR hemagglutination was also purified from growth on agar by the method of Korhonen et al. (9). Pili were considered pure if found to be free of contamination by electron microscopy and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Antipili antibody was measured by a previously described enzyme-linked immunosorbent assay (ELISA) technique (2) that was adapted for 0.1-ml sample volumes. Polystyrene tubes (10 by 75 mm) were coated with 0.1 ml of a 10 μg/ml solution of purified pili in 0.05 M Tris buffer, pH 8. The tubes were incubated at 37°C for 18 h and washed three times with 2 ml of phosphate-buffered saline (pH 7) with 0.05% Tween 20 (PBST). Samples (0.1 ml) of twofold dilutions of serum, urine, or vaginal secretions were incubated at 37°C for 30 min and washed as before with PBST. Peroxidase-conjugated antihuman immunoglobulin G (IgG), IgM, or IgA (Miles Laboratories Inc., Elkhart, Ind.) was diluted 1:250 with PBST, and 0.1 ml of the diluted conjugate was added to each tube. The tubes were incubated at 37°C for 30 min and then washed as above with PBST. A 1-ml amount of a solution containing 0.003% of hydrogen peroxide and 0.05% *O*-phenyldiamine (Sigma Chemical Co., St. Louis, Mo.) in 5% methanol was added to each sample tube. The tubes were incubated for 3 h in the dark, and the reaction was stopped with 8 N hydrochloric acid. Distilled water (1 ml) was then added to each tube, and the optical density was measured at 490 nm with a Spectronic 20 spectrophotometer (Bausch & Lomb, Rochester, N.Y.). A tube without any patient sample was included with each run as a negative control. The antibody titer was expressed as the highest twofold dilution of sample at which the absorbance was 0.05 optical density units greater than it was in a control tube without antibody. For statistical comparisons, titers were converted to their logarithms, and Student's *t* test for two means was employed. With this technique, antipili antibody was detected in a sample of serum obtained from a rabbit immunized with pili which was diluted to contain about 100 ng of specific antipili antibody (Silverblatt, unpublished data).

RESULTS

The clinical and immunological characteristics of the patients with *E. coli* cystitis are given in Table 1. IgG antipili antibodies were present in the serum of almost every patient at the time of the initial visit. The geometric mean titers (± standard deviation) were: IgG, 23.7 ± 3.9;

TABLE 2. Pilus immunogroup and hemagglutination pattern of *E. coli* cystitis strains

Strain from patient no.[a]:	Immunogroup[b]							Hemagglutination	
	1	2	3	4	5	6	7	MS[c]	MR[d]
1	+	−	−	−	+	−	+	+	−
2	−	−	−	−	−	−	−	−	−
3	+	+	−	+	+	−	−	+	−
4	+	+	+	−	+	−	−	+	+
5	+	+	+	−	−	−	−	+	−
6	+	−	−	+	−	−	−	+	−
7(A)	+	+	−	−	−	−	−	+	−
7(B)	+	−	+	+	+	+	−	+	+
8(A)	+	+	+	−	+	−	+	+	−
8(B)	+	−	+	+	−	+	−	+	−
9	+	+	+	−	+	−	−	+	+
10	+	+	+	−	+	−	−	+	+
11	+	−	+	−	+	−	−	+	+
12	+	+	+	−	+	−	−	+	−

[a] Patients no. 7 and 8 were each reinfected; the original strain is designated A, and the reinforcing strain is designated B.
[b] Reactivity of pili by immune electron microscopy to antiserum against seven prototypical strains.
[c] Agglutination of guinea pig erythrocytes by pili or bacteria at 22°C inhibited by mannose.
[d] Agglutination of human erythrocytes by pili or bacteria at 4°C not inhibited by mannose.

IgA, 4.1 ± 4.0; and IgM, 4.4 ± 5.7. There did not appear to be any relation between the initial level of antibody and the duration of symptoms or history of UTI. The immunoreactivity and hemagglutination patterns of the pili of the infecting strains are given in Table 2. All but one strain reacted with at least one of the seven antisera, and most reacted with three or more. Patients no. 7 and 8 had a second UTI during the course of the study, and in both instances the reinfecting strain shared antigenic determinants with the original strain. All but one strain caused MS agglutination. Five strains gave MR hemagglutination as well. The hemagglutination patterns of purified pili were similar to those of whole bacteria.

Antibodies appeared to be directed to the specific pili of the infecting strain, as little or no activity was found in the serum of four cystitis patients against a pool of pili that were immunologically unrelated to their infecting strains (Table 3). Patient no. 7, although not representative, manifested an eightfold rise in IgG titer and a fourfold drop in IgM antibodies during the course of her illness (Fig. 1). This patient developed a recurrence with an antigenically related strain (Table 2) when her serum IgG titer was 1:40. Other patients, however, showed no consistent pattern of response; titers at 3 (data not shown) and 6 weeks rose, fell, or remained constant.

The clinical and immunological characteristics of 10 control patients are given in Table 4. Antipili antibody activity in the sera of control patients was assayed by using pili pooled from isolates from all of the patients. The geometric mean titers (± standard deviation) were: IgG, 8.4 ± 3.2; IgA, 9.6 ± 2.4; and IgM, 0. The IgG and IgM titers for the control patients were significantly lower than those for the cystitis patients ($P < 0.05$). In contrast to the cystitis patients, there did appear to be a relationship between a history of prior UTI and the level of IgG antipili antibody in the serum of the controls.

The immunoglobulin concentration in twelve vaginal secretions selected randomly from among the control and UTI patient samples was 7.87 ± 8.52 mg/dl for IgG and 1.55 ± 2.80 mg/dl for IgA. These values are similar to previously published results (20) and indicate that the procedure for collecting the secretions was adequate. There were no detectable antipili antibodies of any class found in the urine or vaginal secretions of any cystitis or control patient. Negative results were obtained when either MS or MR pili were used as the test antigen.

DISCUSSION

A number of explanations have been proposed to account for the frequency with which infec-

TABLE 3. Specificity of antipili IgG antibody in sera[a] of cystitis patients

Serum from patient no.:	Homologous pili		Heterologous pool	
	Titer	Immunoactivity	Titer	Immunogroups in pool
5	1:10	1, 2, 3	1:2	4, 5, 7, 8
6	1:40	1, 4	1:2	2, 3, 5, 6, 7
7	1:80	1, 2	1:2	3, 5, 6, 7
11	1:80	1, 3, 5	1:2	2, 4

[a] Sample containing the maximum titer.

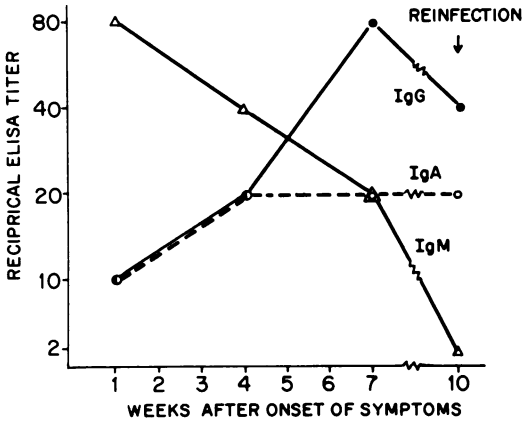

FIG. 1. Antipili antibody response in the serum of patient no. 7 with *E. coli* cystitis. Antibody was measured by the ELISA technique with class-specific anti-immunoglobulins and pili purified from the strain infecting the patient.

tions of the lower urinary tract recur. It is commonly accepted that successive reinfecting strains differ antigenically, so that antibodies produced to one strain offer no protection against subsequent bacterial challenge. More recently, evidence has also been presented both for and against the hypothesis that women prone to recurrent infection constitute a separate population that can be distinguished from normals by the enhanced ability of bacteria to adhere to their vaginal epithelial cells (5, 10).

The results of the present study provide an additional explanation for the prevalence of recurrent cystitis—lower UTIs appear to stimulate little or no antipili antibody in the urogenital fluids. Pili are the principle attachment organelle of *E. coli* (4), and recent evidence from this and a previous study suggests that the pili of many urinary isolates are antigenically similar (13). Failure to mount an immune response to the pili of an infecting strain may provide no immunological barrier to subsequent colonization. We had an opportunity to substantiate this hypothesis in two of our subjects who became reinfected during the course of the study. In both instances, the pili of the reinfecting strain were antigenically related to the pili of the initial strain.

We considered the possibility that our failure to detect antipili antibody was due to the insensitivity of our assay or to the use of the wrong type of pili. The ELISA test has been used in previous studies to quantitate antibodies to pili in the sera of patients with upper UTI (13) and to other bacterial antigens in lower UTI (12, 16). In the present study, however, we had to adapt our standard ELISA procedure to smaller sample volumes because of the limited quantity of vaginal secretions available. With this modified technique we were able to detect activity in a sample of rabbit antiserum diluted to contain about 100 ng of antipili antibody. It is nonetheless possible that lesser amounts of antibody were present but not detected in the vaginal secretions of our patients. To circumvent the controversy over whether MR or MS hemagglutinating pili are more important for virulence in the urinary tract, we used pili purified from the strain infecting each patient to measure her antibody response. In addition, because cultivation in broth may favor the expression of MS pili (1), we specifically purified both MS and MR pili from

TABLE 4. Summary of clinical and immunological findings for 10 control patients

Patient no.	Diagnosis	Previous UTI	Antipili antibody titer (reciprocal dilution) in:				
			Serum			Urine	Vaginal secretions
			IgG	IgA	IgM		
1C	NSU[a]	No	2	10	0	0	0
2C	NSU	No	2	20	0	0	0
3C	Herpes cervicitis	Yes	10	10	0	0	0
4C	Herpes cervicitis	Yes	20	20	0	0	0
5C	Candida vaginitis	Yes	20	20	0	0	0
6C	Chlamydia cervicitis	Yes	20	20	0	0	0
7C	NSU	No	20	10	0	0	0
8C	NSU	Yes	20	10	0	0	0
9C	NSU	No	1	2	0	0	0
10C	NSU	Yes	10	2	0	0	0
Geometric mean titer			8.1	9.6	0	0	0

[a] NSU, Nonspecific urethritis.

the strain infecting one patient. In no instance was any antibody detected.

The presence of little or no antipili antibody in the urine or vaginal secretions of our patients is consistent with earlier observations that cystitis rarely engenders the production of local antibodies (24). This is believed to be due to the superficial nature of the infection. Pyelonephritis, on the other hand, provokes an intense inflammatory response and is associated with the appearance of antibody-producing cells in the kidney (11). This distinction is the basis of the antibody-coated bacteriuria test which is used to localize the site of infection in the urinary tract (23). Some studies have found that urine and vaginal secretions from women with lower UTI contain small amounts of antibody to the O antigen of *E. coli* (16, 20). O antigens may stimulate more of a local immune response than pili can because of the powerful immunogenicity of lipopolysaccharide antigens.

Although not found in the urogenital fluids, antipili antibodies were detected in the serum of our patients with cystitis. These antibodies appeared to be specific for the pili of the infecting strain, as titers were fivefold or more higher against homologous pili than against a pool of immunologically unrelated pili. It should be noted, however, that we have previously found much higher titers of antipili antibody in the sera of some patients with bacteremic pyelonephritis (13). For most patients, antipili antibodies were already present in the serum at the time of onset of symptoms. Longitudinal studies have shown that colonization of the vaginal mucosa often precedes acute infection (19), and it is possible that the preexisting antibodies represent a response to this carriage. Antipili antibodies were also present in the sera of the uninfected controls but at a significantly lower titer. This low level of activity may represent a response to intestinal carriage or residual antibody to prior *E. coli* infection because, except for one control patient, there was a correlation between a history of prior UTI and antibody titers of 1:10 or greater. This concept is also supported by the lack of any IgM antipili antibody in the control sera. IgM antibodies are usually produced during primary exposure and are relatively short lived. Alternatively, the use of a pool of antigens to detect antibody in the controls may have proportionately reduced the apparent antibody level.

Finally, this study provides information that may be useful for the development of a pilus-based UTI vaccine. Most of our strains reacted with two or more pili serogroups. Although this observation may merely reflect antigenic cross-reactivity, it also suggests that each strain had more than one type of pilus. The latter possibility is supported by the demonstration that both MR and MS hemagglutinins were present on some strains. A successful vaccine may need, therefore, to include multiple serogroups of pili. Our results also support the concept that in conferring protection, it may be more important that antipili antibody be present in the secretions that bathe the vaginal mucosa than in the serum, as two of our patients became reinfected despite the presence of antibodies in their sera.

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