INFECTION AND IMMUNITY, Dec. 1978, p. 790-797
0019-9567/78/0022-0790$02.00/0

Vol. 22, No. 3
Printed in U.S.A.

Secretory Immunoglobulin A and G Antibodies Prevent Adhesion of *Escherichia coli* to Human Urinary Tract Epithelial Cells

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Received for publication 19 July 1978

The adhesion of *Escherichia coli* to human urinary tract epithelial cells was inhibited by commercial gamma globulin, the total immunoglobulin fraction of human breast milk and urine, as well as the isolated immunoglobulin G and secretory immunoglobulin A fractions of urine from patients with acute pyelonephritis. Urinary anti-O6 antibodies reduced the adhesion of several O6 strains. Absorption of antibodies to the lipopolysaccharide of the adhering strain markedly decreased the antiadhesive capacity of all the immunoglobulin preparations, whereas elimination of antibodies to the capsular polysaccharide antigen consistently had a small but not significant effect. When urine was absorbed with whole, live bacteria of the patients' infecting strains, the antiadhesive effect completely disappeared. Absorption with bacteria lacking pili only partially reduced this effect.

Attachment to mucosal surfaces may be a prerequisite for various bacteria colonizing the mucosa and/or infecting underlying tissue (for a review, see reference 17). *Escherichia coli* isolated from patients with symptomatic urinary tract infections attach more frequently to human urinary tract epithelial cells in vitro than do *E. coli* from patients with asymptomatic urinary tract infections or normal fecal *E. coli* (19a, 20). The adhesive ability of *E. coli* strains strongly correlates to the presence of pili on the bacterial surface (20). Bacteria belonging to the O groups most often found causing pyelonephritis in children attach better than do strains of other O groups, whereas no such relation between the K antigen type and attachment has been found (19a).

Bacterial attachment may be influenced by various factors present on or adjacent to the mucosa, such as indigenous bacteria (17), mucus (26), and antibodies (5, 6, 24-26, 29; L. Å. Hanson and P. Brandtzaeg, *in* E. R. Stiehm and V. A. Fulginiti (ed.), *Immunological Disorders in Infants and Children*, in press). Antibodies of secretory immunoglobulin A (SIgA) type dominate quantitatively in secretions (24; Hanson and Brandtzaeg, in press), and much interest has been focused on the possible role of such antibodies in mucosal infections (5, 25, 26; Hanson and Brandtzaeg, in press). In studies with *Vibrio cholerae*, protection by SIgA was associated with decreased bacterial attachment to intestinal mucosa (6), and saliva containing SIgA decreased the adhesion of streptococci to the lining of the oral cavity (29). Urine from patients with *E. coli* urinary tract infections often contains antibodies against the infecting strain, particularly of SIgA but also of the immunoglobulin G (IgG) class, whereas IgM antibodies are rare (9; A. Sohl-Akerlund, S. Ahlstedt, L. Å. Hanson, and U. Jodal, Acta Pathol. Microbiol. Scand. Sect. C, in press). By using an in vitro test system, we have analyzed to what extent specific antibodies of IgG and SIgA classes against various bacterial surface structures are capable of inhibiting the attachment of urinary *E. coli* isolates to human urinary tract epithelial cells.

MATERIALS AND METHODS

Bacteria. Three attaching *E. coli* strains of the serotypes O4K3, O25K nontypable, and O21K2, recently isolated from urine of three girls aged 7 to 10 years with acute pyelonephritis and five attaching *E. coli* O6K2 strains, previously isolated from the urine of five patients with acute pyelonephritis (19a), were used to test the anti-adhesive effect of antibodies. Serotyping was performed as described earlier (10, 14). For diagnostic criteria, see reference 15.

Antibody sources. Commercially available gammaglobulin (165 g/liter, Kabi, Sweden) as well as a pool of human breast milk with an SIgA content of 1.0 g/liter (18) taken from healthy Swedish mothers were used as sources of IgG and SIgA antibodies, respectively.

Twenty-four-hour portions of urine were collected

from the three girls with nonobstructive acute pyelonephritis attending the Children's Hospital, Göteborg, for their first known urinary tract infection. Urine samples were also obtained from a 4-year-old boy with myelomeningocele, kidney damage, and recurrent urinary tract infections, the present infection caused by a nonattaching *E. coli* O6K2 strain. The samples were collected between the days 7 and 10 after onset of symptoms, when urine antibody levels usually are high (Sohl-Åkerlund et al., in press), and stored frozen at −20°C until used.

The immunoglobulin fractions of milk and urine were obtained by precipitation twice with 50% saturated ammonium sulphate. The precipitates, obtained by centrifugation and dialysis against phosphate-buffered saline (PBS, pH 7.2) for 48 h were restored to 100% (milk) or 1% (urine) of the original volume by addition of PBS.

Quantitation of antibodies. The enzyme-linked immunosorbent assay (ELISA) (4) was used as previously described (7).

The solid-phase antigens were as follows: (i) the supernatant of boiled *E. coli* bacteria of the infecting strain; (ii) lipopolysaccharide (LPS) of *E. coli* O6 and O4, prepared by hot phenol-water extraction (28); and (iii) acidic capsular polysaccharide, K antigen, purified by Cetavlon precipitation (10). Anti-human IgG, IgA, and IgM alkaline phosphatase conjugates were purchased (Orion, Helsinki, Finland), and conjugates with rabbit anti-human secretory component (SC) (Dakopatts, Copenhagen, Denmark) were prepared as described elsewhere (1).

Titers are given as the dilution of antibody preparation giving absorbance at 400 nm of 0.2 above background, after reacting the tube-bound enzyme with substrate for 100 min.

Absorption of antibody. Anti-LPS and anti-K antibodies were eliminated by absorbing once or twice with freeze-dried LPS or K antigen added to a final concentration of 10 mg/ml to the respective immunoglobulin preparations. After incubation during rotation at 37°C for 30 min, absorption was continued without rotation at 4°C overnight. Urine was also absorbed with whole, live bacteria. A 1-ml amount of the concentrated immunoglobulin preparation was mixed with the sediment from 3 ml of an *E. coli* brain heart infusion broth culture grown without agitation for 16 h and incubated as described above. Bacteria were subsequently eliminated by centrifugation. One urine sample was also absorbed with whole, live *E. coli* grown shaking to prevent pili formation. Presence or absence of pili was ascertained by electron microscopy with negatively stained bacteria as described earlier (20).

Affinity chromatography. The IgG and SIgA fractions were purified from urine by subjecting the urinary immunoglobulin preparations to affinity chromatography columns with anti-human IgG (Orion, Helsinki, Finland) or anti-human SC covalently linked to Sepharose as previously described (23). The material passing and the immunoglobulin fraction bound to and specifically eluted from the columns with acid buffer (pH 3) (22) were dialyzed and concentrated to the original sample volume before use.

Adhesion inhibition. Adhesion testing was done as previously described (19). Sixteen-hour bacterial cultures in brain heart infusion broth were harvested by centrifugation and resuspended in PBS. A total of 10^8 bacteria was preincubated with antibodies at 37°C for 30 min during rotation. Control bacteria in PBS were incubated accordingly. To the bacterial suspensions with or without antibodies were added 10^5 human urinary tract epithelial cells, obtained from the sediment of a healthy female's morning urine, and PBS to a volume of 1 ml. The mixtures were incubated during rotation at 37°C for 60 min. Unattached bacteria were then eliminated by repeated washing. The number of bacteria adhering to each of 40 epithelial cells was counted by direct-light microscopy by using a Bürker chamber. Adhesion is given as the mean number of bacteria per epithelial cell. Dead cells were excluded by addition of trypan blue.

Statistical evaluations. Statistical evaluations were performed as described earlier (19) with an ordinary chi-square test. When comparisons were based on experiments performed on different days, the chi-square test was applied separately to the data from each day and pooled together (1).

Procedure controls. A total of 90 to 100% of the specific anti-*E. coli* LPS antibodies in milk and urine were recovered in the ammonium sulphate precipitates of the immunoglobulin fractions.

The O6 LPS was prepared from an *E. coli* O6K13 strain, the O4 LPS was from an O4K12 strain, K2 was from an O2K2 strain, and K3 was from an O nontypable K3 strain. Cross-reactions because of possible impurities in the antigen preparation was controlled by absorption experiments with the ELISA. No effect on anti-O antibodies was seen after absorption with K antigen and a very slight decrease in anti-K antibodies after absorption with O antigen, indicating good specificity of the antigens.

Because all urine samples contained antibiotics due to treatment, the ammonium sulphate-precipitated fractions were tested for presence of these antibiotics. Paper disks moistened with standardized volumes of the urinary immunoglobulin fractions were applied to agar previously inoculated with sensitive test strains (8). None of the fractions inhibited bacterial growth.

About 10^7 bacteria per ml, i.e., 10% of the number used for adhesion testing, remained in the supernatants of urines absorbed with whole, live bacteria. To compensate for this, 10^7 bacteria were added per ml to the PBS controls in experiments with urine absorbed with whole, live bacteria.

To find the optimal conditions for adhesion inhibition by antibody, preincubations with antibodies of bacteria and epithelial cells were separately made. No marked difference between the procedures was found. Preincubation of bacteria and antibody was used as the test procedure.

A nonspecific effect of protein on bacterial attachment was not likely because bovine serum albumin at concentrations of 0.01 to 40 mg/ml had no significant effect on attachment.

RESULTS

Inhibition of adhesion by antibodies. Antibodies to *E. coli* O6K2 from commercial gam-

maglobulin rich in IgG and human breast milk rich in IgA were used to study whether antibodies of these classes would inhibit the adhesion of *E. coli* to human urinary tract epithelial cells. The effect of different amounts of IgG or IgA antibody on *E. coli* adhesion is shown in Fig. 1. High concentrations of the IgG and IgA antibody preparations inhibited adhesion. Gammaglobulin diluted 1/50 to 1/10,000 increased binding of *E. coli* to the epithelial cells above control levels, whereas still lower amounts of antibody had no effect. No such increased adhesion was observed with any concentration of milk antibodies. The amount of milk and gammaglobulin in various experiments needed to decrease adhesion to 50% of the control was highly reproducible (Table 1).

Antibody specificity. The gammaglobulin and human milk used contained antibodies against the O6 and K2 antigens found on the *E. coli* O6K2 strain used as a model strain for adhesion testing. Absorption with O6 LPS, eliminating the anti-O6 LPS antibodies, significantly ($P < 0.01$) reduced the antiadhesive effect of milk and gammaglobulin (Table 2). Further absorption with K2 polysaccharide did not significantly decrease the antiadhesive effect of milk or gammaglobulin ($P > 0.10$). No effect on adhesion was observed after addition of 0.1 to 25 mg of LPS or K antigen per ml to the PBS controls.

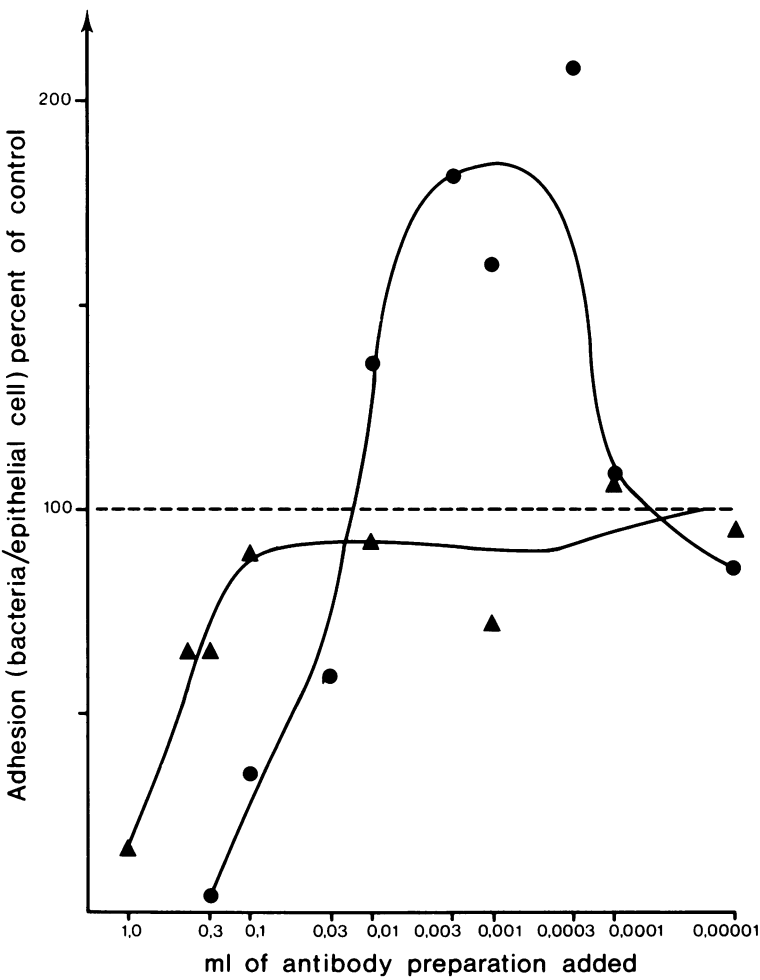

FIG. 1. *Dose response relationship between the amount of antibody preparation added per milliliter of test mixture (containing 10^8 bacteria and 10^5 epithelial cells) and the effect on the adhesion of E. coli O6 to human urinary tract epithelial cells (= mean number of bacteria attached to 40 epithelial cells). Symbols: ---, adhesion without addition of antibody; ●, after addition of commercial gammaglobulin (mean of 9 experiments); and ▲, after addition of the immunoglobulin fraction of a human milk pool (mean of 7 experiments).*

Urinary antibodies. The immunoglobulin fraction of urine from the patient with kidney damage and pyelonephritis due to an *E. coli* O6K2 strain partly inhibited the adhesion of five O6 *E. coli* strains not identical with the infecting strain. Absorption with O6 LPS eliminated the antiadhesive effect of urine, tested against one of the five strains (Table 3). Elimination of the anti-K2 antibodies had no significant effect (Table 3).

The immunoglobulin fraction of urine from the patient with acute pyelonephritis due to an *E. coli* O4K3 strain inhibited the adhesion of the infecting strain. Elimination of the anti-K3 antibodies by absorption did not significantly decrease the antiadhesive effect of urine ($P > 0.05$) (Fig. 2). A marked decrease in antiadhesive effect was observed after absorption with O4 LPS ($P < 0.01$). Absorption with whole, live nonpiliated bacteria eliminated all anti-O4 and anti-K3 antibodies, but provided no further decrease in the antiadhesive effect as compared with the LPS absorption. Absorption with live, piliated bacteria, completely abolished the antiadhesive effect of the urine ($P < 0.05$) (Fig. 2).

The immunoglobulin fraction of urine from each of three patients with acute pyelonephritis was found to inhibit adhesion of the patient's own *E. coli* strain. Absorption with the homologous live bacteria in all instances abolished this antiadhesive effect ($P < 0.05$).

Separated immunoglobulin fractions. To analyze whether urinary antibodies of IgG and SIgA classes also had antiadhesive activity the isolated IgG and SIgA fractions of urine were tested.

Urine from the patient with kidney damage, containing considerable levels of specific antibodies to O6 of both IgG and IgA but not IgM

TABLE 1. *Reproducibility of the antiadhesive effect of anti-O6 antibodies in human milk and commercial gammaglobulin on the adhesion of E. coli O6 to human urinary tract epithelial cells*

Source of antibodies	No. of experiments	Vol of antibody preparation required for 50% adhesion inhibition[a] (ml)	Anti-O6 titer (ELISA)	
			IgG	IgA
Gamma globulin[b]	14	0.12 ± 0.01	100,000	<10
Human milk pool[c]	7	0.43 ± 0.02	<10	1,000

[a] Total volume of 1.0 ml with 10^8 *E. coli* bacteria and 10^5 epithelial cells. Means ± standard error of the mean.
[b] Concentration, 165 g/liter, Kabi, Sweden.
[c] Concentration, 1.0 g of SIgA per liter.

TABLE 2. *Antiadhesive effect of anti-LPS antibodies in human milk and commercial gammaglobulin on the attachment of E. coli O6 to human urinary tract epithelial cells*

Source of antibodies	Anti-O6 titer (ELISA)		Adhesion[a] (bacteria/epithelial cell)	Significance[b] of the decrease in antiadhesive effect
	IgG	IgA		
Milk	<10	1,000	10	
Milk absorbed with O6 LPS	<10	10	26	$P < 0.01$
Saline control			54	
Gammaglobulin	100,000	<10	0	
Gammaglobulin absorbed with O6 LPS	<10	<10	12	$P < 0.01$
Saline control			39	

[a] Mean of two experiments.
[b] Chi-square test for the median.

TABLE 3. *Effects of K2 and O6 LPS absorption on the antiadhesive effect of urine containing anti-O6K2 antibodies on an E. coli O6K2 strain different from that of the infected patient*

Source of antibodies	ELISA titer								Adhesion[a] (bacteria/epithelial cell)	Significance[b] of decrease in antiadhesive effect compared with unabsorbed urine
	Anti-O6				Anti-K2					
	IgG	%[c]	IgA	%[c]	IgG	%[c]	IgA	%[c]		
Urine	3,000	100	3,000	100	500	100	160	100	14	
Urine absorbed with K2 polysaccharide[d]	3,000	100	3,000	100	<10	<2	<10	<6	17	$P > 0.01$
Urine absorbed with O6 LPS[d]	<10	<1	<10	<1	400	80	126	79	35	$P < 0.001$
Saline control	—		—						37	

[a] Mean of two experiments.
[b] Chi-square test for the median.
[c] Percentage of antibodies in whole urine.
[d] A 10-mg amount of purified LPS or K antigen per ml of urine.

classes, was applied to a column with anti-SC antibodies coupled to Sepharose. Both the SIgA fraction bound to and specifically eluted from the gel and the material passing the column, i.e., IgG and some IgA devoid of SC, retained much of the adhesion inhibiting effect of unfractionated urine (Table 4).

In another experiment urine containing specific antibodies, particularly of IgA class, was applied to an anti-SC column and on a column with antibodies to human IgG covalently coupled to Sepharose. As shown in Table 5 the material passing the anti-IgG column, i.e., the total IgA fraction contaminated with some IgG, retained the entire antiadhesive effect of the total immunoglobulin fraction. The SIgA fraction, isolated from the anti-SC column, was almost equally effective. The purified IgG fraction containing low levels of specific antibodies had no effect (Table 5).

DISCUSSION

Antibodies inhibiting attachment of *E. coli* to human urinary tract epithelial cells were found in urine from patients with acute pyelonephritis, in a pool of human breast milk containing mostly IgA antibodies, and in commercial gammaglobulin. Absorption of antibodies directed against O antigen markedly reduced the antiadhesive effect of all antibody preparations used. Elimination of antibodies to the K antigen had a slight but not significant effect. An adhesion-inhibiting effect of urinary components other than antibodies is less likely because isolated immunoglobulin fractions retained most of the antiadhesive effect of urine. Absorption with live bacteria of the infecting strain eliminated the antiadhesive effect of urine, whereas urine absorbed with live bacteria, grown shaking to prevent pili formation, retained some antiadhesive effect, al-

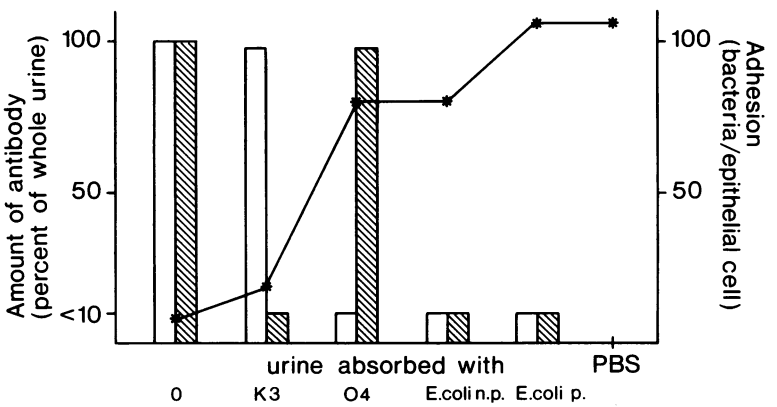

FIG. 2. *Adhesion inhibition by urinary antibodies against different bacterial surface structures. The effect of urine from a patient with acute pyelonephritis due to an E. coli O4K3 strain on the attachment of the infecting strain. Symbols: □, O4 antibodies and ▨, K3 antibodies, in percentage of the total urinary immunoglobulin fraction, as registered with the ELISA; *, adhesion, mean number of bacteria attached to 40 epithelial cells. p, piliated; n.p., nonpiliated E. coli O4K3.*

TABLE 4. *Antiadhesive effect of total immunoglobulin in urine compared with that of IgG and SIgA fractions*

Source of antibodies	Anti-O6 titer (ELISA)				Adhesion[a] (bacteria/epithelial cell)	Significance of the difference in antiadhesive effect compared with the saline control
	IgG	%[b]	IgA	%[b]		
Total immunoglobulin	3,000	100	3,000	100	11	$P < 0.05$
SIgA[c]	<1	<1	1,000	33	27	$P < 0.05$
IgG + non-SC-IgA[d]	1,800	60	160	3	22	$P < 0.05$
Saline control					38	

[a] Mean of two experiments.
[b] Percentage of antibodies in whole urine.
[c] Material bound to and specifically eluted from a column with anti-human-SC covalently coupled to Sepharose.
[d] Material passing the anti-SC column.

TABLE 5. *Antiadhesive effect of total immunoglobulin and the isolated IgG, IgA, and SIgA fractions in urine on the attachment of the infecting E. coli O4K3 strain*

Antibody preparation	Anti-O6 titer (ELISA)				Adhesion (bacteria/epithelial cell)	Significance[b] of differences in antiadhesive effect compared with the saline control
	IgG	%[a]	IgA	%[a]		
Total immunoglobulin	40	100	6,000	100	28	$P < 0.001$
Total IgA[c]	6	15	5,400	90	30	$P < 0.001$
SIgA[d]	1	3	4,800	80	36	$P < 0.001$
Total IgG[e]	38	95	100	2	79	$P > 0.10$
Saline control	0	0	10	0	79	

[a] Percentage of antibodies in whole urine.
[b] Chi-square test for the median.
[c] Material passing a column with human IgG covalently coupled to Sepharose.
[d] Material bound and specifically eluted from a column with anti-human SC covalently linked to Sepharose.
[e] Material bound and specifically eluted from the column described in footnote *c*.

though all anti-O and anti-K antibodies were eliminated. Thus, antibodies directed against bacterial surface structures other than O and K antigens, possibly pili, may be capable of inhibiting *E. coli* attachment. The mechanism underlying the antiadhesive effect of antibodies is at present not known. Agglutination of bacteria, steric hindrance due to antibodies binding to any bacterial surface structure or interaction with specific receptors on bacteria or epithelial cells, may well be of importance and explain the findings in the present study.

By comparing the amounts of antibody needed to reduce bacterial attachment to 50% of the control, experiments from different days could be compared with high reproducibility in spite of the day variation of 15 to 20% in the control adhesion (19). IgG levels of 0.01 to 2% of those inhibiting attachment instead increased adhesion. This might illustrate a function proposed for IgG, that of binding antigens to mucosal surfaces (26). Thus, trapping of antigens by IgG antibodies present on the intestinal mucosa of rats immunized intraperitoneally has been shown (27), and an Fc receptor for IgG on the intestinal epithelia of neonatal rats has recently been described (3).

Increased levels of urinary and serum antibodies against O antigen of the infecting *E. coli* strain are a consistent finding in patients with acute pyelonephritis. In a recent study all patients over 3 months of age with acute pyelonephritis and 80% of girls with asymptomatic bacteriuria had urinary anti-O antibodies (9). Such anti-O antibodies have been shown to give some protection against hematogenous and ascending pyelonephritis in rats (11, 13) and to be capable of efficiently inhibiting adhesion as demonstrated in the present study. The role of the *E. coli* LPS for adhesion is, however, poorly understood. Although the *E. coli* O groups most often found in patients with acute pyelonephritis have higher adhesive ability than strains of less common O groups, poorly adhering strains have been found within all O groups studied (19). Because the LPS covers 10 to 40% of the bacterial surface area, anti-O antibodies may possibly bind in high enough concentration to cause agglutination and steric hindrance and thus inhibit adhesion.

In the enriched urinary immunoglobulin fractions used in the present study, anti-K antibodies were found. However, urinary antibodies directed against the K3 and K2 antigens did not significantly diminish adhesion of *E. coli* O4K3 and O6K2. Both this low antiadhesive effect and the low frequency of K antibodies usually found in patients with urinary tract infections (9, 12) might be explained by the presence of only small amounts of K antigen on the infecting strain, when present in the urinary tract of the patient. In spite of the limited effect on adhesion of anti-K antibodies in the present study, such antibodies may, however, well be efficient in preventing attachment of heavily encapsulated strains. For example anti-K antibodies protected against ascending pyelonephritis in rats caused by a heavily encapsulated *E. coli* strain (13).

In a recent study, a significant correlation was found between presence of pili and adhesive ability in *E. coli* strains isolated from patients with urinary tract infection (20). Because pili are antigenic (16), antibodies to pili may be present in urine of patients with urinary tract infections caused by piliated and adhering *E. coli* strains. Recently, antiserum against purified type 1 pili from *E. coli* K-12 was shown to prevent adhesion of that strain to monkey kidney cells (16). Our results with only partial elimination of antiadhesive antibodies after absorption of the urine with a nonpiliated variant of the original strain also

suggest a protective function of antibodies to bacterial pili.

In urine from patients with urinary tract infections, specific antibodies of the IgA class dominate, IgG are commonly found, but IgM antibodies are rare (9; Sohl-Åkerlund et al., in press). The higher proportion of IgA antibodies in urine than in serum and the parallelism between the levels of IgA and SC-containing antibodies in urine (9; Sohl-Åkerlund et al., in press) suggest a predominance of locally formed IgA antibodies in urine. In the present study antibodies of IgG and SIgA types were efficient in blocking *E. coli* attachment in vitro. Because the ELISA does not allow direct quantitative comparison of antibodies of different classes, the antiadhesive efficiency of IgG and SIgA could not be compared. A greater effectiveness of SIgA than of IgG might, however, be expected, because gammaglobulin with 100-fold higher ELISA antibody titers was only slightly more efficient in blocking adhesion than milk.

Because 30% of patients with their first attack of acute pyelonephritis have a recurrence within a year and 70% of those have a relapse within 3 months (30), a protective effect of urinary antibodies has been doubted. However, *E. coli* strains causing reinfection usually differ from the original strain with regard to O and K antigens (2). Our results indicate that urinary antibodies to one O antigen prevent the attachment of any strain of that O group. Antiadhesive antibodies in the urinary tract of the patient might thus be one factor selecting the reinfecting strain.

ACKNOWLEDGMENTS

This study was supported by grants from the Medical Faculty, University of Göteborg; the Swedish Medical Research Council (grants 215 and 338); and the Ellen, Walter and Lennart Hesselman Foundation for Scientific Research.

The fruitful discussions with L. Å. Hanson and Jan Holmgren and the technical assistance of Ann-Marie Jonsson, Britt-Marie Lavstrand, and Helena Lomberg are much appreciated. We also thank Barbro Carlsson for collecting the milk and Ulf Jodal for collecting the urine from two of the patients.

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