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Protection Against *Escherichia coli*-Induced Urinary Tract Infections with Hybridoma Antibodies Directed Against Type 1 Fimbriae or Complementary D-Mannose Receptors

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Hybridoma antibodies directed against quaternary structural epitopes of the type 1 fimbrial adhesin of *Escherichia coli* or against D-mannose, the sugar determinant in the complementary host cell receptor, prevented the attachment of mannose-sensitive *E. coli* to various eucaryotic cells. Passive intraperitoneal administration of the fimbria-specific or D-mannose-specific antibodies protected mice against retrograde colonization with mannose-sensitive *E. coli* instilled into their urinary bladders. Monoclonal antibodies directed against fimbrial subunits rather than quaternary structural epitopes or against *N*-acetylgalactosamine rather than D-mannose residues lacked protective activity. These studies provide evidence that bacterial colonization can be blocked or interrupted by antibodies directed against either the adhesin or the complementary host cell receptor of pathogenic microorganisms.

The initiation of infections by bacteria that attack the host at mucosal surfaces is mediated by molecular recognition between ligand molecules (or adhesins) on the surface of the bacteria and receptor molecules on the surface of the susceptible host (2). A number of examples of such specific interactions between bacteria and host surfaces have been described previously (2). A classical example is the type 1 fimbria-mediated attachment of various species of *Enterobacteriaceae* to mannose-containing receptors of mucosal epithelial cells (3, 9, 12, 13). Purified type 1 fimbriae of *Escherichia coli* agglutinate mannose-containing yeast cells and guinea pig erythrocytes and bind to several other mammalian cell types (3, 9, 12, 13). Binding of *E. coli* cells or their isolated fimbriae to each of these cell types is blocked specifically by solutions of D-mannose and its derivatives or by the D-mannose-specific lectin concanavalin A (9). Immune sera directed against the fimbriae have been shown to prevent the attachment of the related *E. coli* to epithelial cells in vitro and to prevent ascending (or retrograde) colonization of the kidneys in a rat model of *E. coli*-induced pyelonephritis (14, 15).

Recently, we prepared hybridoma antibodies against specific structural conformations of type 1 fimbriae of *E. coli*. A quaternary structure-specific antibody, but not two subunit-specific antibodies, prevented the attachment of type 1 fimbriae and related *E. coli* to mannose-containing eucaryotic cells (1). In this paper, we describe the protective properties of these antiadhesive antibodies in a mouse model of *E. coli* induced pyelonephritis. In addition, we studied the protective properties of hybridoma antibodies directed against D-mannose contained in the complementary receptor on the mucosal surface of the urinary tract. We present evidence to show that bacterial colonization can be prevented by monoclonal antibodies directed against either the bacterial adhesin or the complementary host cell receptor.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. In addition to the Cold Spring Harbor K-12-derived strain (CSH50) of *E. coli* used to prepare type 1 fimbriae, we used a urinary tract isolate, *E. coli* CI5 (nontypable). The latter strain and its isolated fimbriae reacted as well as the CSH50 strain and its fimbriae with each of the fimbria-specific monoclonal antibodies used in these studies. The clinical isolate rather than the CSH50 strain was used in the challenge studies of mice because it has been reported that K-12-derived strains are incapable of causing experimental infections (5). The bacteria were cultured in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) under static conditions at 37°C for 24 to 48 h (1).

Preparation of specific monoclonal antibodies. The preparation of hybridoma clones from which the antifimbrial antibodies were derived for the present study was described in detail in a previous report (1). Two sugar-specific monoclonal antibodies, A07 (D-mannose specific) and F12 (*N*-acetylgalactosamine specific), were obtained in a similar fashion by immunizing mice with a purified salivary glycoprotein of human origin (Babu et al., manuscript submitted for publication). Briefly, nonsecreting SP2/0-Ag14 myeloma cells were fused with spleen cells obtained from mice immunized with the glycoprotein. The cells were fused with polyethylene glycol, suspended in complete growth medium containing 0.83% methylcellulose, hypoxanthine, aminopterin, and thymidine, and seeded onto 30-mm tissue culture dishes. The cultures were incubated in a humidified atmosphere of 5% CO_2–95% air at 37°C until clones developed. Clones were picked and transplanted into 96-well plates containing growth medium which included hypoxanthine and thymidine. Normal spleen cells were added to the cultures as feeder cells. Culture medium was assayed for antibody after 1 week. Antibody-producing cells were expanded and recloned. Ascites fluid was obtained by injecting cloned antibody-producing cells into the peritoneal cavities of Pristane-primed BALB/c mice (1). Control ascites fluid lacking specific

antibodies was prepared in the same manner by injecting unfused myeloma cells (1).

Screening for sugar-specific monoclonal antibodies. The specificity of the monoclonal antibodies was determined by the inhibition of enzyme-linked immunosorbent assays (ELISA). Twofold dilutions of the appropriate sugar were made in 0.5 ml of 0.2 M phosphate–0.15 M NaCl (PBS; pH 7.4); 0.5 ml of a diluted ascites fluid was added to each dilution of sugar. The mixtures were then incubated at 37°C for 30 min and assayed by standard ELISA procedures (1), using glycoprotein-coated trays. The sugars tested included D-mannose, α-methyl-D-glucoside, *N*-acetylglucosamine,α-methyl-D-mannoside, *N*-acetylgalactosamine, arabinose, fucose, D-galactose, and sialic acid.

Assays of adherence of bacteria to mouse bladder mucosa. The association of *E. coli* with mouse bladder mucosal surfaces in the presence of sugar-specific monoclonal antibodies was assayed by a modified method of Fader and Davis (4). Briefly, groups of three mice were killed by cervical dislocation, and their urinary bladders were exposed. Urine was removed from the bladder with a needle and syringe, and immediately thereafter 100 μl of ascites fluid containing either the D-mannose-specific or *N*-acetylgalactosamine-specific antibodies was injected through the same needle. The bladders from the control animals were injected with control ascites fluids. After 15 min of incubation, 200 μl of PBS containing 10^9 *E. coli* was instilled into each bladder. The bladders were then incubated for an additional 20 min, after which the mucosa of the bladders were exposed and washed repeatedly with sterile PBS. Control experiments were performed to assure that the *E. coli* test strain used for the in vivo studies adhered to the bladder mucosal surfaces in a mannose-specific fashion. For this purpose, mouse bladders were injected with *E. coli* cells suspended in 2% α-methyl-D-mannoside, α-methyl-D-glucoside, or *N*-acetylgalactosamine, then incubated, and washed as above. All bladders were then homogenized, and the number of viable bacteria which remained associated with the bladder mucosa was determined by colony counts of serial dilutions of the homogenates onto plates of MacConkey agar (see below). The results are recorded as the mean number of CFU per bladder ($n = 3$). It should be noted that no attempt was made to distinguish between adherence to individual uroepithelial cells as opposed to mucins coating the mucosal surfaces.

Mouse protection assays. The protection studies were carried out in Swiss White mice undergoing water diuresis induced by the method of Keane and Freedman (7). Briefly, male mice weighing 25 to 30 g were given 5% glucose in water as the sole source of drinking water for 4 days before bacterial challenge. At 24 h before challenge, the animals were injected intraperitoneally with 250 μl of control ascites fluid or with test ascites fluids containing hybridoma antibodies against either type 1 fimbriae or sugar moieties. After anesthetization with methoxyfluorane (Abbott Laboratories, North Chicago, Ill.), the urinary bladders of the mice were exposed, and the urine was aspirated through a 30-gauge needle. Each bladder was then inoculated through the same needle with 50 μl of bacterial suspension (10^8 CFU/ml in PBS. After 5 days, the animals were sacrificed by cervical dislocation, and the bladders and kidneys were removed with sterile precautions. The organs were homogenized in PBS, and 0.1-ml samples of serial 10-fold dilutions of the homogenate were inoculated onto MacConkey agar to determine the number of CFU per organ. In addition, blood and urine were collected from mice at various intervals before they were sacrificed to determine the presence of antifimbrial and receptor-specific antibodies.

Antibody assays. The antibodies in ascites fluids and in the serum or urine of passively immunized mice were assayed by ELISA as previously described (1). Before the urines were assayed for antibodies they were dialyzed against PBS. Fimbria-specific antibodies were assayed in fimbria-coated ELISA trays, whereas sugar-specific antibodies were assayed in salivary glycoprotein-coated trays.

RESULTS

Antiadhesive properties of adhesin-specific and receptor-specific monoclonal antibodies. The in vitro antiadhesive properties of the fimbrial quaternary structure-specific hybridoma antibody (CD3) and the lack of antiadhesive properties of the subunit specific antibody (AA8) against *E. coli* type 1 fimbriae have been reported previously (1). In this study, the antiadhesive properties of two additional monoclonal antibodies raised against a salivary glycoprotein were examined. The specificity of these monoclonal antibodies (A07 and F12) for D-mannose and the *N*-acetylgalactosamine residues, respectively, is illustrated in Fig. 1 and 2. D-Mannose or α-methyl-D-mannoside, but not α-methyl-D-glucoside, inhibited binding of A07 antibody to the salivary glycoprotein in a dose-dependent manner as demonstrated by the inhibition of ELISA. Similarly, *N*-acetylgalactosamine specifically inhibited the binding of F12 antibody to the glycoprotein (Fig. 2).

The antiadhesive properties of the sugar-specific antibodies were assessed by in vitro and in situ experiments utilizing cheek epithelial cells and urinary bladders, respectively, from mice of the same strain employed in the following in vivo experiments. To assure the D-mannose specificity of the attachment of our *E. coli* CI5 test strains, we performed preliminary experiments in which the adherence of the bacteria was tested in the absence and presence of sugars.

The mean number of bacteria binding to the cheek epithelial cells was 54 ± 23 per epithelial cell in the absence of sugar, 12 ± 4 in the presence of α-methyl-D-mannoside, and 50 ± 24 in the presence of *N*-acetylgalactosamine. α-methyl-

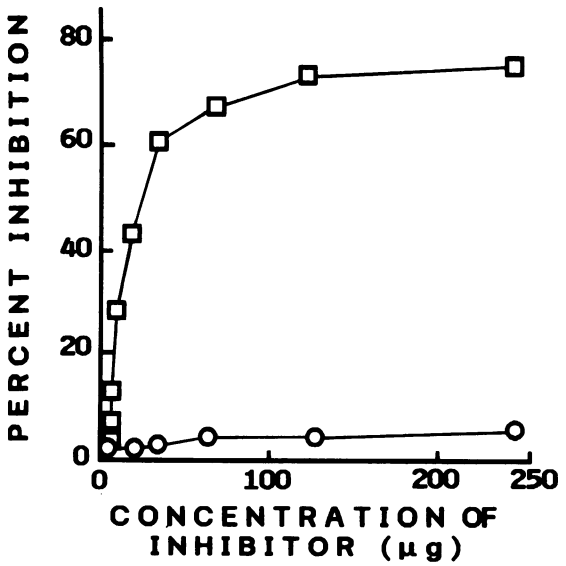

FIG. 1. ELISA inhibition assays of antibody A07 with salivary glycoprotein, using α-methyl-D-mannoside (□) and α-methyl-D-glucoside (○) as inhibitors.

D-mannoside similarly reduced the association of *E. coli* with mouse bladder mucosal surfaces by 89% as compared with control without sugar. The control sugars *N*-acetylgalactosamine and α-methyl-D-glucoside had no significant effects. These results confirmed the D-mannose specificity of the binding of cells of *E. coli* to the mouse epithelial cells and bladder mucosal surfaces.

Next we studied the effects of the sugar-specific monoclonal antibodies in these assays. The mean number of bacteria binding to the epithelial cells was 54 ± 23 per epithelial cell in the absence of antibody. The D-mannose-specific antibody reduced binding to 18 ± 6 per epithelial cell, whereas the *N*-acetylgalactosamine-specific antibody had only a small inhibitory effect (45 ± 29). Similar results were obtained in situ; the D-mannose-specific antibody markedly reduced the association of *E. coli* cells with the urinary bladders (Table 1). Although the *N*-acetylgalactosamine-specific antibody also was inhibitory, the degree of inhibition was two orders of magnitude less than that observed with the D-mannose specific antibody (Table 1). Neither of the sugar-specific monoclonal antibodies reacted with cells of *E. coli* by ELISA. These results suggest, therefore, that the antibody molecules exert their antiadhesive properties by binding to sugar moieties on the mucosal surface of mouse bladders. The failure of *N*-acetylgalactosamine to block adherence suggests that the inhibition observed with the antibody against this sugar is mediated by steric hindrance.

Protection against experimental urinary tract infection with fimbria-specific and D-mannose-specific monoclonal antibodies. To determine the protective properties of these antiadhesive hybridoma antibodies, we used the experimental mouse model of ascending pyelonephritis described by Keane and Freedman (see above). The mice were passively immunized with monoclonal antibodies as described above. Before the mice were challenged intravesically with type 1-fimbriated *E. coli*, passive transfer of antibody was confirmed by ELISA of serum and urine samples, using purified type 1 fimbriae and salivary glycoprotein as solid-phase antigens. Serum antibody levels were measured every 8 h for 72 h, and the highest antibody titer was observed 24 h after passive immunization. At this time the serum titers of the various monoclonal antibodies ranged from 1:800 to 1:1,200. In the urine, fimbria-specific antibody titers of 1:10 were obtained. Although no attempts were made to detect Fab fragments as such, if present they would have been detected in our assay system, which employed antibodies against both heavy and light chains. No sugar-specific antibodies were detected in the urine.

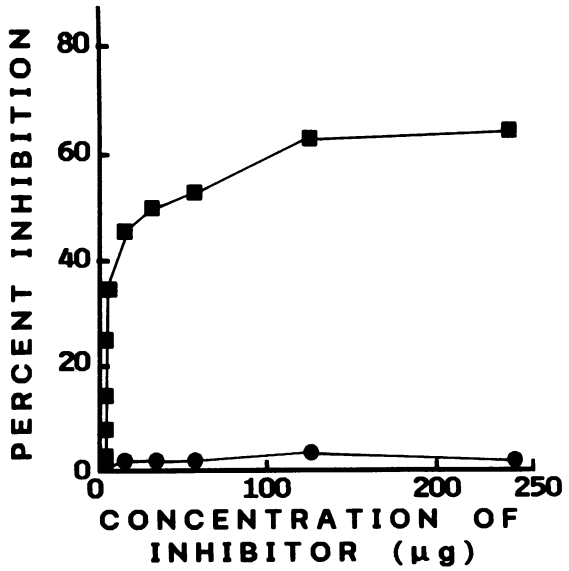

FIG. 2. ELISA inhibition assays of antibody F12 with salivary glycoprotein, using *N*-acetylgalactosamine (■) and *N*-acetylglucosamine (●) as inhibitors.

TABLE 1. Inhibition of the association of type 1-fimbriated *E. coli* with mouse bladder mucosa by sugar-specific monoclonal antibodies

Monoclonal antibody used to pretreat mouse bladders[a]	Sugar specificity	CFU of bacteria per bladder[b]
AO7	D-Mannose	1.3×10^3
F12	*N*-Acetylgalactosamine	2.3×10^5
Control ascites	None	3.4×10^8

[a] Urine from each bladder was removed with a syringe, and 100 μl of ascites fluid was introduced. After 15 min of incubation, 200 μl containing 10^9 CFU of *E. coli* was instilled into the bladder.

[b] Mean of three mice.

Only the fimbrial, quaternary structure-specific hybridoma antibody, and the D-mannose-specific monoclonal antibody were able to protect the kidneys against infection by *E. coli* CI5 ($P < 0.001$) (Table 2). Bacterial colonization of the urinary bladder also was significantly reduced by the quaternary structure-specific antibody ($P < 0.01$) but not with the D-mannose-specific monoclonal antibody. Both fimbrial subunit-specific antibody (AA8) and the *N*-acetylgalactosamine-specific antibody (F12) lacked any protective effect (Table 2). These results indicate that monoclonal antibodies directed against intact type 1 fimbriae or the complementary D-mannose receptor protect experimental animals against ascending urinary tract infections by the related type 1-fimbriated *E. coli*.

DISCUSSION

Our data provide definitive evidence that antibodies directed against either mannose residues on host mucosal surfaces or a bacterial adhesin can protect against the colonization of certain mucosal surfaces by pathogenic bacteria. Although not proven, we speculate that our monoclonal antibodies prevent colonization by blocking initial bacterial attachment to urinary tract surfaces. The speculation is based on the following findings: (i) the antibodies directed against both the bacterial adhesin and against mannose residues on host cells are antiadhesive in situ; (ii) both antibodies prevent renal colonization; (iii) the injected fimbria-specific monoclonal antibodies appear in the urine; and (iv) no protective activity was observed with the fimbrial subunit-specific monoclonal antibody which was shown to lack antiadhesive properties in vitro (1). Interestingly, the *N*-acetylgalactosamine-specific monoclonal antibody was found to inhibit the association of *E. coli* with mouse bladders to a limited degree in situ. Although the reason for the observed inhibitory effect remains unclear, it is possible that the mucosal surface of the bladder contains *N*-acetylgalactosamine residues, and antibodies reacting with these residues block the attachment of the *E. coli* cells by steric hindrance.

We do not know why the D-mannose-specific antibody had no protective activity in vivo against bladder colonization even though the antibody was found to be antiadhesive in situ. It is possible that these antibodies were adsorbed by

TABLE 2. Protection of mice against urinary tract infection by passive immunization

Monoclonal antibody[a]	IgG subclass	Antigenic specificity	Kidney		Bladder	
			Infectivity rate[b]	Mean CFU of *E. coli*[c]	Infectivity rate[b]	Mean CFU of *E. coli*[c]
Control ascites	2a	None	16/16	3.3×10^6	16/16	1.8×10^8
CD3	1	Fimbrial, quaternary structure	3/16	6.3×10^3	16/16	6.5×10^5
AA8	1	Fimbrial, subunit	16/16	1.9×10^6	16/16	9.4×10^7
A07	3	D-Mannose	2/16	1.1×10^3	16/16	8.5×10^7
F12	3	*N*-Acetylgalactosamine	14/14	6.9×10^6	14/14	4.0×10^7

[a] Each mouse was injected 24 h before challenge with 0.2 ml of ascites fluid containing monoclonal antibody CD3, AA8, A07, or F12. Control ascites fluid was raised in mice inoculated with unfused myeloma cells.
[b] Number infected/number challenged.
[c] Isolated from each homogenized kidney or bladder 5 days after intravesicular challenge.

D-mannose-containing receptors higher in the urinary tract or by soluble urinary mucin, which is rich in mannose residues (10).

Although several studies have demonstrated prevention of ascending experimental urinary tract infections by active and passive immunizations against the bacterial adhesin (11, 14, 15), it is not clear how these antibodies exerted their protective effect. In the present study, and in the recent study of Silverblatt et al. (15), low levels of antibody were detectable in the urine of the immunized animals, suggesting that either antibodies or antigen-binding fragments passed into the urinary space. However, it is quite conceivable that greater levels of antibodies may be released into the urinary tract after kidney damage. Indeed, it is a common clinical observation that antibody-coated bacteria can be detected in the urine of patients with acute kidney infections (16). Thus, we speculate that the adhesin- and receptor-specific antibodies exert their protective effect on the surface of the urinary tract after passage into the urinary space. Whether bacterial damage to the glomerular walls enhances excretion of antibody in our mouse model remains to be investigated.

We are aware that *E. coli* strains possess adhesins other than mannose-sensitive type 1 fimbriae. In fact, the P fimbria-mediated attachment (mannose-nonsensitive) of many uropathogenic *E. coli* strains has gained considerable importance since the recent discovery that these fimbriae bind specifically to α-D-Galp-(1-4)-β-Galp residues of globohexosylceramides composing the p^k blood group substance present on most normal uroepithelial cells (6, 8). Moreover, antibodies against P fimbriae appear to protect experimental animals against renal infections with P-fimbriated *E. coli* (11). We further recognize that a controversy exists as to the relative role of type 1 fimbriae versus P fimbriae or other mannose-nonsensitive fimbriae in the colonization and infection of the urinary tract (6, 8, 14, 15). Our purpose has not been to resolve this controversy but rather to gain insight into the in vivo significance of interaction of a well-defined surface appendage, such as type 1 fimbriae, with its complementary receptor on host mucosal surfaces. Our data provide the most definitive evidence that serious bacterial infections may be prevented by the administration of antibodies directed toward either the bacterial adhesin or the host cell receptor.

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