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Mannose-Specific Adherence of *Escherichia coli* Freshly Excreted in the Urine of Patients with Urinary Tract Infections, and of Isolates Subcultured from the Infected Urine

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The mannose-specific adherence to yeast cells of *Escherichia coli* excreted in the urine of patients with urinary tract infections was compared with that of isolates from the same urine after growth of the bacteria in broth. The results revealed that although *E. coli* excreted in only 2 of 24 urine specimens exhibited mannose-specific adherence, about half of the broth cultures from these specimens did so. Examination of representative specimens of *E. coli* excreted in urine showed that coating antibodies, mannose-containing glycoproteins, and encapsulation were not responsible for the lack of the mannose-specific adherence. Our results suggest that *E. coli* strains that are genetically capable of exhibiting mannose-specific adherence may, when growing in the bladder, be in a phase of growth which suppresses the phenotypic expression of this trait. Mannose-specific adherence is indicative of the presence on the bacterial surface of adhesins (lectins) that bind the organisms to mannose residues on both epithelial and phagocytic cells. We propose that whereas at the initial stages of infection the bacteria may benefit from their ability to bind to mannose residues on epithelial cells, loss of this ability at the later stages of the infection is also beneficial, since the bacteria can no longer adhere to mannose residues on phagocytes, and are thus resistant to nonimmune phagocytosis.

The adherence of numerous strains of gram-negative bacteria to animal cells is specifically inhibited by D-mannose or its derivatives, i.e., it is mannose specific (MS) (7, 15, 21). The MS adherence appears to be mediated by MS adhesins (lectins) present on the surface of the bacteria. The adhesins may be in the form of type 1 fimbriae (7, 10, 19). The phenotypic expression of the MS adhesin or type 1 fimbriae is controlled by various environmental factors, most importantly the number of laboratory passages and the phase and condition of bacterial growth (4, 7, 15). Type 1 fimbriae are best produced by organisms growing for 48 h in static conditions in broth but not on agar (7), and the presence of 1% glucose in the medium suppresses their formation (16). It is not known when, if at all, the MS adhesin is formed by bacterial pathogens during the course of natural infection.

The present study was undertaken to compare the MS adhesin activity of *Escherichia coli* excreted in the urine of patients with urinary tract infection with that of *E. coli* isolated and subcultured in vitro from the same urine. We found that, whereas in most cases the organisms excreted in the urine did not display MS activity, about half of the strains developed such activity upon being isolated and subcultured in vitro.

MATERIALS AND METHODS

The study group (24 patients) was comprised of elderly individuals, 21 females and 3 males, with a mean age of 75 years (range, 60 to 85). The patients each had a continuous indwelling catheter with a close sterile drainage unit, commercially available, preconnected with a sterile closed urinary drainage bag. None of the patients was under antibiotic treatment for at least 1 week before urine collection. The drainage bags, containing the output of the patients' urine over 3 to 4 h after the bags were changed early in the morning, were chilled and brought to the laboratory soon after collection. Viable bacterial counts on Trypticase soy agar plates were made from each urine specimen by serial 10-fold dilutions of the urine with sterile saline. Three to four colonies were picked and transferred to agar slants for identification by standard biochemical techniques and for further cultivation. Only urine samples containing a pure culture of more than 10^5 colony-forming units of *E. coli* per ml of urine were included in the study.

The bulk of the urine in the drainage bags was centrifuged at 3,000 × g for 30 min in the cold, the

pellet was resuspended in 5 ml of phosphate-buffered saline (PBS), pH 7.4, and the suspension was centrifuged at 200 × *g* for 10 min to remove large cells and cell debris. The supernatant was carefully collected and centrifuged at 2,000 × *g* for 15 min. The bacterial pellet was resuspended in 0.5 ml of PBS, and the number of organisms was counted by serial twofold dilution in a Petroff-Hausser chamber; the bacterial suspension was then adjusted to contain 10^9 cells per ml. Viable counts of these bacterial suspensions revealed that more than 90% of the organisms were alive.

Cultivation of bacteria in vitro. *E. coli* isolates from each urine were cultivated in 5 ml of brain heart infusion broth for 48 h at 37°C under static conditions for only one passage. After growth, the bacteria were harvested by centrifugation, resuspended in PBS, counted, and adjusted to contain 10^9 cells per ml as described above.

Determination of mannose binding and hemagglutinating activities. Two rows of serial twofold dilutions of each bacterial suspension in PBS (25 μl) were dispensed in microtiter plates. To each well in the first row 25 μl of a yeast suspension prepared as described elsewhere (14) was added; to each well in the second row 25 μl of washed 3% human group A erythrocytes was added. The plates were then gently shaken. Yeast agglutination, which is a measure of mannose binding activity (14), was scored after 30 min at room temperature, and hemagglutination was scored after the plates were kept overnight at 4°C. It was found that this method is sensitive down to 10^7 fully fimbriated bacteria, which possess good mannose binding activity, per ml (14). To establish whether the agglutination was MS or mannose resistant (MR) the bacteria (10^9 cells per ml) were suspended in PBS containing 2.5% methyl α-D-mannoside (Pfanstiehl Laboratories, Waukegan, Ill.) before the assay. In some experiments, the suspension was incubated for 10 min at room temperature, then washed twice with PBS free of sugar and resuspended in PBS for determination of mannose binding activity as described above.

Other tests. Formation of capsule was determined by India ink stain of bacterial suspension as described (6). The presence of coating antibody in bacteria shed in the urine was determined by using fluorescent antihuman serum (24), and the presence of pili was determined by electron microscopy (6).

RESULTS

MS adhesins in *E. coli* excreted in urine were detected in only 2 of the 24 specimens (Fig. 1). These two specimens contained somewhat more colony-forming units of *E. coli* (Table 1) than did the other 22 specimens. In contrast to bacteria excreted in the urine, MS adhesin was detected in 11 of 24 strains isolated and subcultured from the individual urine specimen (Fig. 1). In contrast, MR activity was detected in bacteria excreted in urine from seven patients, but only four isolates from these patients developed detectable MR hemagglutination upon subculturing in broth. This is not surprising since MR activity has been reported to be best expressed in certain *E. coli* isolates grown in agar rather than in broth (7).

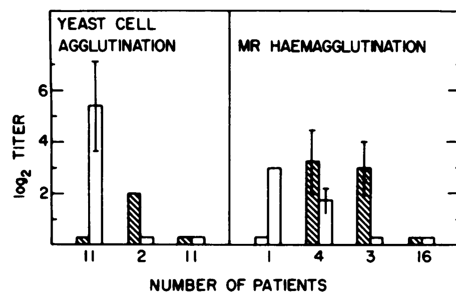

FIG. 1. *Yeast cell aggregation by E. coli obtained from patients with urinary tract infections. In all cases yeast agglutination was inhibited by mannose (MS). Hemagglutination of human group A erythrocytes was, as a rule, MR.*

TABLE 1. *MS adherence by E. coli excreted and growing in vitro in urine of patients with urinary tract infections*

Urine specimen from patient no.	Bacteria[a] growing in infected urine collected from patients after: 3 h (at 4°C) CFU/ml	3 h (at 4°C) MS adherence	24 h (at 37°C) CFU/ml	24 h (at 37°C) MS adherence
1	5×10^5	<1	2×10^8	2
2	2×10^6	<1	5×10^8	3
3	5×10^8	2	ND	ND
4	2×10^8	2	ND	ND

[a] MS adherence is expressed as the $\log_2$ of highest dilution of bacterial suspension (10^9/ml) which agglutinated a standard suspension of yeast cells. Viable counts are expressed as colony-forming units (CFU) on agar per milliliter of urine sample. ND, Not determined.

Two urine specimens which contained organisms lacking MS adhesin were incubated at 37°C overnight. The number of viable organisms in these specimens increased from 5×10^5 and 2×10^6 to 2×10^8 and 5×10^8, respectively, during the incubation period. Bacteria harvested from the incubated urine possessed low levels of MS adhesins (Table 1).

To ascertain that the lack of MS activity in the excreted bacteria was indeed due to lack of production of the adhesin, they were examined for the presence on their surface of capsule, coating antibody, mannose-containing glycoproteins, and fimbriae. Three of seven specimens contained capsulated organisms, while in all three other specimens examined, the bacteria lacked coating antibody or pili of any kind on their surface. Washing of the bacteria from these

latter three specimens with a solution of 2.5% methyl α-D-mannoside, followed by extensive washing with PBS, failed to reveal any MS activity, suggesting that mannose-containing glycoproteins which might block MS adhesin of the bacteria were not present on the surface of these organisms.

In a separate set of experiments, an *E. coli* isolate which had a $\log_2 6$ titer with yeast cells was allowed to drain from a bottle into the drainage system. The MS activity of bacteria collected in the bag was the same as that of the original culture, suggesting that no selective removal of bacteria with mannose-binding activity occurred during drainage.

DISCUSSION

The results of the present study show that *E. coli* excreted in the urine of elderly patients lacks MS activity. In about half of the cases, the organisms were genotypically capable of producing the MS adhesins when subcultured and grown in vitro, suggesting that growth conditions in the bladder do not permit the phenotypic expression of the adhesin on the *E. coli* surface.

It is unlikely that the development of MS adhesins by strains subcultured in vitro is due to selective outgrowth of spontaneously arising variants of MS bacteria (4), since for this to occur several successive cultivations are needed (7). Among the factors which were reported to repress fimbriae and MS adhesin synthesis was the phase of growth. MS adherence to erythrocytes (7), yeast cells (9), epithelial cells (15, 20), and phagocytic cells (2), as well as formation of type 1 fimbriae (7), are poorly expressed by organisms harvested at the logarithmic phase of growth, but are fully expressed by the organisms harvested at late stationary phase of growth. Growth in vitro resembles "chemostat conditions" in that it allows continuous proliferation under conditions of constant supply of limited nutrients and removal of excess bacterial population and its deleterious products (10). It follows that strains of *E. coli* which are genotypically competent to express MS adhesins and were growing in the bladder of the patients examined in this study were in a phase of growth which represses formation of the adhesins, similar to bacteria at the logarithmic phase of growth in vitro. This conclusion is supported by our findings that (i) *E. coli* excreted in urine, which lacked MS adhesin, acquired this activity after 24 h of growth in the urine in vitro, and (ii) the two urine specimens which contained *E. coli* with detectable MS adhesin had more bacteria than the urine specimens with bacteria lacking the adhesins, suggesting that in these two specimens the organisms reached stationary phase of growth, which permitted the phenotypic expression of the MS adhesin.

Our results can be best explained by postulating that infective *E. coli* organisms possessing MS adhesin undergo phase transition at certain stages of the infectious process which does not permit the expression of the adhesins. Since the adhesins enable the organism to adhere to both epithelial cells (7, 15, 17) and phagocytic cells (2, 3, 24), the bacteria may benefit from the inability to express the MS activity in vivo after infection has been initiated. Thus, the presence of MS adhesin on bacterial surfaces may be crucial in initiating infection, as it enables the organisms to adhere to the epithelial cells in order not to be swept away by the flow of urine. The absence, however, of the adhesins on bacterial surfaces at subsequent stages of the infection in deeper tissue enables the organisms to escape ingestion and killing by phagocytic cells which are capable of binding the bacteria via MS adhesin.

In support or agreement with the above hypothesis are reports showing that (i) adherence of certain uropathogenic *E. coli* to uroepithelial cells is MS (20, 25), indicating that mannose or mannose-like residues on the urinary epithelium are available for the binding of MS adhesin-containing organisms; (ii) experimental infection of urinary tract by *E. coli* or *Klebsiella pneumoniae* possessing MS adhesin can be markedly and specifically blocked either by methyl α-D-mannoside (1, 11) or by anti-type 1 fimbriae antibodies (N. Sharon, Y. Eshda, F. J. Silverblatt, and I. Ofek, *in* K. Elliott, ed., *Adhesion and Micro-Organisms Pathogenicity*, in press), showing that adherence to mannose residues in the urinary tract may be important in initiating infection; (iii) antibodies to type 1 fimbriae of *E. coli* are produced by patients with pyelonephritis (18), attesting to the presence in the body of fimbriated organisms at a certain stage of the infection; and (iv) well over half of the numerous urinary isolates of *E. coli* examined are genotypically capable of producing MS adhesins, although a high proportion of the MS isolates also produce MR adhesins (5, 8).

The production of low levels of MR adhesin was noted in the present study in bacteria excreted in urine in about one-third of the patients, suggesting that there may be a role for this adhesin in the pathogenesis of urinary tract infections by *E. coli* which are genotypically capable of producing both MS and MR adhesins (10, 13). In support of this are the preliminary findings that urine isolates of *E. coli* possessing MR adhesin do not bind to human polymorpho-

nuclear leukocytes (S. H. Susman, personal communication; I. Ofek and A. Peri, unpublished data). It would seem likely, therefore, that in a nonimmune host the MS adhesins play a part in the initiation of infection rather than in its subsequent persistence. A similar conclusion was formed in studies on experimental infection with *Proteus mirabilis* (22), which shifts from a phase in which the organisms are fimbriated and possess the capacity to bind to both epithelial and phagocytic cells, as well as to initiate retrograde infection, to a phase in which the organisms lack all of these characteristics.

Urinary tract infections are commonly initiated by the fecal flora. It is of interest, therefore, to establish whether *E. coli* strains in this flora possess MS adhesins which can potentially bind the organisms to epithelial cells to initiate colonization of the urinary tract surfaces.

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LITERATURE CITED

1. **Aronson, M. O., O. Medalia, L. Schori, N. Sharon, and I. Ofek.** 1979. Preventation of colonization of the urinary tract of mice with *Escherichia coli* by blocking of bacterial adherence with methyl α-D-mannopyranoside. J. Infect. Dis. **139**:329-332.
2. **Bar-Shavit, Z., R. Goldman, I. Ofek, N. Sharon, and D. Mirelman.** 1980. Mannose-binding activity of *Escherichia coli*: a determinant of attachment and ingestion of the bacteria by macrophages. Infect. Immun. **29**:417-424.
3. **Bar-Shavit, Z., I. Ofek, R. Goldman, D. Mirelman, and N. Sharon.** 1977. Mannose residues on phagocytes as receptors for the attachment of *Escherichia coli* and *Salmonella typhi*. Biochem. Biophys. Res. Commun. **78**:455-460.
4. **Brinton, C. C., Jr.** 1977. The pilation phase syndrome and the uses of purified pili in disease control, p. 33-70. *In* Proceedings of the 13th Joint U.S.-Japan Conference on Cholera, Atlanta, Ga., September, 1977. Department of Health, Education and Welfare Publication no. 78-1590. National Institutes of Health, Bethesda, Md.
5. **Crichton, P. B., and D. C. Old.** 1980. Differentiation of strains of *Escherichia coli*: multiple typing approach. J. Clin. Microbiol. **6**:635-640.
6. **Cruickshank, R., J. P. Duguid, B. P. Marmion, and R. H. A. Swain (ed.).** 1975. Medical microbiology, p. 42. Churchill Livingstone, London.
7. **Duguid, J. P., S. Clegg, and M. J. Wilson.** 1979. The fimbrial and non-fimbrial haemagglutinins of *Escherichia coli*. J. Med. Microbiol. **12**:213-227.
8. **Duguid, J. P., and D. C. Old.** 1980. Adhesive properties of Enterobacteriaceae, p. 186-217. *In* E. H. Beachey (ed.), Bacterial adherence receptors and recognition, series B, vol. 6. Chapman and Hall, London.
9. **Eisenstein, B. I., I. Ofek, and E. H. Beachey.** 1979. Interference with mannose binding and epithelial cell adherence of *Escherichia coli* by sublethal concentrations of streptomycin. J. Clin. Invest. **63**:1219-1228.
10. **Ellwood, D. C., and D. W. Tempest.** 1972. Effects of environment on bacterial wall content and composition. Adv. Microbiol. Physiol. **7**:83-115.
11. **Fader, R. C., and C. P. Davis.** 1980. Effect of piliation on *Klebsiella pneumoniae* infection in rat bladders. Infect. Immun. **30**:554-561.
12. **Korhonen, T. K.** 1979. Yeast cell agglutination by purified enterobacterial pili. FEMS Microbiol. Lett. **6**:421-425.
13. **Korhonen, T. K., and C. Svanborg-Eden.** 1980. Binding of purified *Escherichia coli* pili to human urinary tract epithelial cells. FEMS Microbiol. Lett. **7**:237-240.
14. **Ofek, I., and E. H. Beachey.** 1978. Mannose binding and epithelial cell adherence of *Escherichia coli*. Infect. Immun. **22**:247-254.
15. **Ofek, I., and E. H. Beachey.** 1980. Bacterial adherence. Adv. Intern. Med. **25**:503-532.
16. **Ofek, I., E. H. Beachey, B. I. Eisenstein, M. L. Alkan, and N. Sharon.** 1979. Suppression of bacterial adherence by subminimal inhibitory concentrations of β-lactam and aminoglycoside antibiotics. Rev. Infect. Dis. **1**: 832-837.
17. **Ofek, I., D. Mirelman, and N. Sharon.** 1977. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. Nature (London) **265**:623-625.
18. **Ottow, J. C. G.** 1975. Ecology, physiology and genetics of fimbriae and pili. Annu. Rev. Microbiol. **29**:79-108.
19. **Rene, P., and F. J. Silverblatt.** 1980. Serological response to *Escherichia coli* in pyelonephritis, p. 782-783. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease, vol. 1. The American Society for Microbiology, Washington, D.C.
20. **Salit, I. E., and E. C. Gotschlich.** 1977. Haemagglutination by purified type 1 *Escherichia coli* pili. J. Exp. Med. **146**:1169-1181.
21. **Schaeffer, A. J., S. K. Amondsen, and L. N. Schmidt.** 1979. Adherence of *Escherichia coli* to human urinary tract epithelial cells. Infect. Immun. **24**:753-759.
22. **Silverblatt, F. J., and L. S. Cohen.** 1979. Antipili antibody affords protection against experimental ascending pyelonephritis. J. Clin. Invest. **64**:333-336.
23. **Silverblatt, F. J., J. S. Dryer, and S. Schauer.** 1979. Effect of pili on susceptibility of *Escherichia coli* to phagocytosis. Infect. Immun. **24**:218-223.
24. **Silverblatt, F. J., and I. Ofek.** 1978. Effects of pili on susceptibility of *Proteus mirabilis* to phagocytosis and on adherence to bladder cells, p. 49-59. *In* E. H. Kass and W. Brumfitt (ed.), Infections of the urinary tract. University of Chicago Press, Ltd., Chicago.
25. **Thomas, V., A. Shelokov, and M. Forlaud.** 1974. Antibody-coated bacteria in the urine and the site of urinary tract infection. N. Engl. J. Med. **290**:588-590.
26. **Van den Bosch, J. F., U. Verbrom-Sohmer, P. Postma, J. de Graaff, and P. D. M. MacLaren.** 1980. Mannose-sensitive and mannose-resistant adherence to human uroepithelial cells and urinary virulence of *Escherichia coli*. Infect. Immun. **29**:226-233.