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Bacterial Motility Is a Colonization Factor in Experimental Urinary Tract Infection

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In an experimental urinary tract infection of the mouse, colonization of the urinary bladder by isogenic strains of *Salmonella enterica* serovar Typhimurium was found to depend on the motility of the bacteria. Strains were obtained by genetic recombination between a highly motile O-6,7 and a poorly motile O-4,5,12 strain. The O antigen did not interfere with the colonization, whereas motility did; flagellated and motile O-6,7 and O-4,5,12 bacteria colonized the bladder equally well.

Urinary tract infection (UTI) has been studied experimentally in animal models in which bacteria are introduced into the mouse or rat bladder via a catheter (5, 15). *Escherichia coli* isolates derived from patients with acute pyelonephritis or cystitis are recovered from mouse bladders and kidneys in larger numbers than are strains of fecal origin (4–6). Adherence to and colonization of the urinary tract is an important step in establishing infection; this has been shown to depend on the presence of fimbriae such as P or common type 1 fimbriae on the uropathogenic bacteria (2, 15, 24).

In animal models, motility based on flagellation has been shown to contribute to intestinal colonization by *Campylobacter jejuni* and *Vibrio cholerae* (1, 17, 18) and by the nonfimbriated *Salmonella enterica* serovar Typhimurium (13, 16).

Lipopolysaccharide (LPS) has been shown to play a role in experimental *E. coli* or *S. enterica* serovar Typhimurium UTI of both LPS-responsive and -nonresponsive mouse strains (3, 21). The loss of the O antigen of LPS resulted in a disadvantage for the infecting *E. coli* strain (21). LPS-nonresponsive mice were more sensitive to the *E. coli* infection and were less able to eliminate the injected bacteria from their bladders and kidneys, probably because of a lack of LPS-induced chemotaxis of phagocytic cells.

We were interested in the possible role of the O antigen of LPS in UTI, because previous studies in the laboratory had shown it to be an important determinant in intraperitoneal infection with *S. enterica* serovar Typhimurium (14, 26, 27). The mechanism in that case had been shown to occur via activation of complement and subsequent opsonization of the bacteria (20). Complement activation could also be expected to generate chemotactic activity for phagocytic cells (7). We started the study with the strains from the previous experiments that had O antigens of either the virulent (O-4,12) or avirulent (O-6,7) type and tested the ability of the strains to colonize the mouse bladder in experimental UTI (4).

Female (CBA × C57BL/6)F_1 hybrid mice (6 to 8 weeks of age; bred at this Institute) were used in all experiments. These mice are wild type with respect to *lps* gene-dependent LPS responsiveness and Ity-dependent *Salmonella* susceptibility. The *S. enterica* strains studied, their origins, and their major properties are listed in Table 1. The O and H antigens of the recombinants were determined by slide agglutination in appropriately diluted and absorbed rabbit antisera by using cultures from nutrient or semisolid agar plates, respectively (9). Motility was tested at 37°C by inoculating the strains at the top of plastic tubes containing semisolid agar (9). When the bacteria reached the bottom of a tube, an inoculum was taken through a hole made in the bottom of the tube and stabbed in a semisolid agar plate. The H antigens were determined as described above. Motile strains swarmed to the bottom of the tube within 1 day, whereas poorly motile strains did so only after several days. The strains that failed to migrate at all were considered nonmotile.

The bacterial inoculum for infection of the mice was prepared from an overnight bacterial culture in static Luria broth at 37°C by 10-fold dilution in the same medium followed by ca. 2 h of growth under similar conditions to the appropriate concentration as indicated by the optical density of the culture (Klett-Summerson colorimeter with a red filter). The exact number of bacteria injected was determined by measuring the viable counts, and their motility characteristics were confirmed on semisolid agar plates.

The mice were anesthetized by intraperitoneal injection of 0.25 ml of sodium pentobarbital (6 mg/ml). The urinary bladder was emptied by gentle compression of the abdomen, and then 5×10^7 *Salmonella* bacteria in 0.05 ml of Luria broth were injected through a soft polyethylene catheter (4). Two mice were infected with each bacterial strain. After waking from anesthesia, the mice were allowed food and drink ad libitum.

After 24 h the mice were sacrificed by cervical dislocation. The bladder was removed aseptically, placed in 5 ml of saline in a disposable plastic bag, and homogenized (Colworth Stomacher 80 homogenizer; Seward Ltd., London, United Kingdom). Serial dilutions of the homogenate were plated on nutrient agar plates to determine the numbers of viable bacteria in them. The results are given as the geometric mean ($\log_{10}$) of bacteria found in the bladders of the two mice.

The first experiment with the previously characterized strains SH 3879 to SH 5770 showed differences between the strains, suggesting that either motility or the O antigen is decisive for colonization with *S. enterica* serovar Typhimurium in experimental UTI (Fig. 1). To separate the effects of the two properties, we isolated a new set of recombinant strains (SH 8552 to SH 8566) by conjugation (21, 25) between a highly motile O-6,7 donor strain (SH 5152) and a poorly motile O-4,5,12 recipient strain (SH 5671), selecting *his*$^+$

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TABLE 1. *S. enterica* strains used in this study

Strain	O:H antigens	Motility[a]	Description	Reference
SH 5152	6,7:b:−	+++	Hfr, derivative of *S. enterica* serovar Abony	19
SH 3879	4,5,12:i:1,2	+	*his ilv*, *S. enterica* serovar Typhimurium	25
SH 5671	4,5,12:i:1,2	+	*ilv*$^+$ recombinant derivative of SH 3879	25
SH 5673	4,5,12:−:−	−	*ilv*$^+$ recombinant derivative of SH 3879	25
SH 5770	6,7:g,m,s:1,2	+++	*his*$^+$ recombinant from SH 5152 −× SH 5671	25
SH 8552	6,7:b:1,2	+++	*his*$^+$ recombinant from SH 5152 −× SH 5671	This study
SH 8555	6,7:i:1,2	+++	*his*$^+$ recombinant from SH 5152 −× SH 5671	This study
SH 8558	4,5,12:b:1,2	+++	*his*$^+$ recombinant from SH 5152 −× SH 5671	This study
SH 8563	4,5,12:−:−	−	*his*$^+$ recombinant from SH 5152 −× SH 5671	This study
SH 8566	6,7:−:−	−	*his*$^+$ recombinant from SH 5152 −× SH 5671	This study
SH 8569	4,5,12:i:1,2	+++	Spontaneous motile variant of SH 5671	This study
SH 8570	4,5,12:b:1,2	+++	Spontaneous motile variant of SH 8563	This study

[a] Abbreviations: −, nonmotile; +, poorly motile; +++, highly motile.

recombinants. One hundred recombinants thus selected were streaked out on nutrient agar plates, and single colonies were reisolated. Of these 100 strains, 12 were autoagglutinable (rough) and were not characterized further. Of the remaining 88 smooth strains, 81 were O-6,7 like the donor. Sixty recombinants were motile like the donor, and 28 were nonmotile. Of the 60 motile recombinants, 39 (65%) had the donor type and 22 (37%) had the recipient type flagellin H, b, or i (one strain was positive for both i and b) (Table 2). Like the donor and the recipient, none of the recombinants expressed type 1 fimbriae as determined by mannose-sensitive agglutination of *Saccharomyces cerevisiae* cells (Oy Alko Ab, Helsinki, Finland) on glass slides with bacterial cultures in stationary Luria broth (10).

Motile SH 8552, SH 8555, and SH 8558 strains, as well as nonmotile SH 8563 and SH 8566 strains, were selected for testing in a urinary bladder infection (Fig. 1). Both sets included both O-4,5,12 and O-6,7 strains. The absence of flagellin in strain SH 8563 was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of whole bacteria of this strain and the motile variant strain SH 8570 (11). The SDS-PAGE gel was stained with Coomassie blue or immunostained (23) with flagellin b-specific antiserum (data not shown).

The motile strains colonized 100 to 1,000 times more effectively than the nonmotile ones. The nature of the O antigen did not influence the number of bacteria recovered; neither did the quality of the flagellin (b or i). In two experiments with the poorly motile and nonmotile O-4,5,12 strains (SH 5671 and SH 8563), all of the colonies recovered from the bladders were tested for motility. Of these, 12 of 14 and 13 of 14, respectively, were highly motile. Two representatives of these spontaneous motile variants, SH 8570 and SH 8569 (one from each experiment) colonized the mouse bladder effectively (Fig. 1).

It has been previously demonstrated that *E. coli* strains

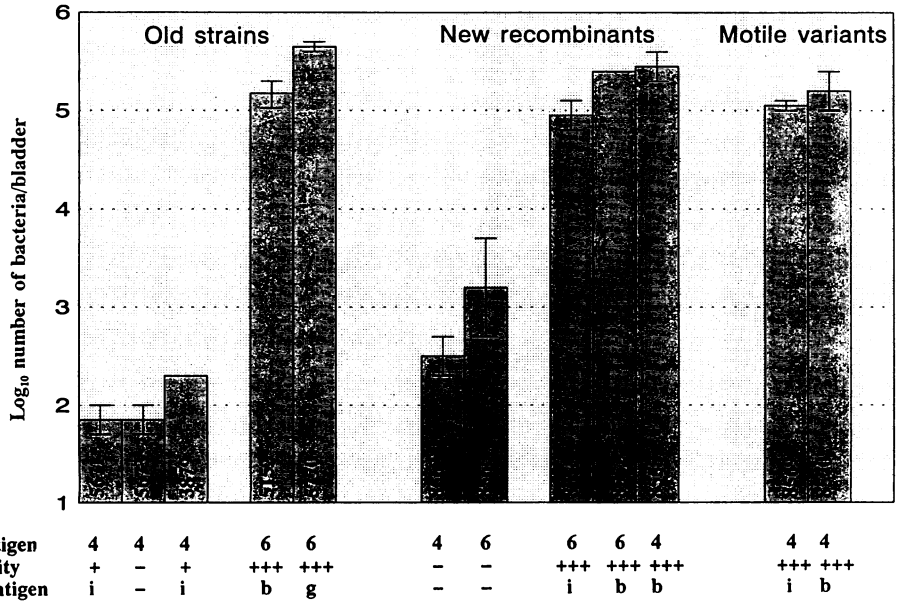

FIG. 1. The number of viable highly motile (+++), nonmotile (−), and poorly motile (+) *S. enterica* bacteria (O antigen 4,5,12 or 6,7; H1 antigen i, b, g, or non) in the urinary bladder of normal mice after transurethral challenge with 5×10^7 bacteria in three experiments with three sets of strains: old strains, new recombinants, and motile variants. Each column represents the geometric mean of the number of bacteria in entire bladders of two mice after 24 hours, and the vertical line shows the range (with two strains, SH 3879 and SH 8552, the same numbers of bacteria were recovered from two bladders). The asterisks (* or **) indicate the parent strain and its variant, respectively.

TABLE 2. Distribution of 88 *his*$^+$ smooth recombinants of *S. enterica* SH 5152 (6,7:b:−) and SH 5671 (4,5,12:i:1,2) according to their O and H antigens

O antigen	No. of recombinants	No. with H antigen:			
		b:1,2	i:1,2	i,b:1,2	Nonmotile[a]
6,7	81	37	21	0	23
4,5,12	7	1	0	1	5
Total	88	38	21	1	28

[a] Also nonagglutinable by the flagellar antisera.

that produce P or common type 1 fimbriae preferentially colonize the mucosa of the mouse or rat urinary bladder (5, 6, 15). Salmonellae do not contain P fimbriae, and the strains used in this study did not express type 1 fimbriae under the test conditions; therefore, they cannot be used as a basis for conclusions about colonization of the bladder by *S. enterica* strains.

In studies with HeLa cells (8, 12, 22), motility has been shown to increase invasion by *Salmonella* species. In studies involving oral challenge of mice, motility has had an effect on the virulence of *Salmonella* species not expressing type 1 fimbriae (13). The data presented here suggest that in experimental UTI, effective colonization by *Salmonella* species seems to be dependent on the ability of the bacteria to swim and thus make contact with the cells on the endothelial surface of the urinary bladder. An extrapolation of these findings is that the motility of the bacteria may be a virulence factor for lower UTIs, a hypothesis that should be tested in freshly isolated clinical material.

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