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The O4 Specific Antigen Moiety of Lipopolysaccharide but Not the K54 Group 2 Capsule Is Important for Urovirulence of an Extraintestinal Isolate of *Escherichia coli*

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Group 2 capsules and lipopolysaccharides are regarded as important virulence factors in extraintestinal isolates of *Escherichia coli*, but their specific contributions to bladder and renal infections, if any, are unknown. Proven isogenic derivatives deficient in the K54 antigen alone (CP9.137), the O4 antigen alone (CP921), or both the K54 and O4 antigens (CP923) were compared with their wild-type parent (CP9 [O4/K54/H5]) for growth in human urine in vitro and for virulence in vivo in a mouse model of ascending urinary tract infection (UTI). Growth of CP9.137 and CP921 was equivalent to that of CP9 in human urine. CP923 demonstrated a small but reproducible decrease in log-phase growth but achieved the same plateau density. In the mouse model of UTI, the isogenic mutant deficient in the O4 antigen alone (CP921) and, to a greater degree, the derivative deficient in both the K54 and O4 antigens (CP923) were significantly less virulent in nearly all parameters measured. In contrast, the K54 knockout derivative was as virulent as its parent, CP9, in causing bladder infection and nearly as virulent in causing renal infection. These results demonstrate an important role for the O4 antigen moiety of lipopolysaccharide in the pathogenesis of UTI. The possibility that the K54 antigen also plays a minor role cannot be excluded.

Urinary tract infection (UTI) is the most common extraintestinal infection caused by *Escherichia coli*. Identification and evaluation of virulence factors are necessary for a more complete understanding of the pathogenesis of *E. coli* UTI and may, in turn, lead to the logical development of new strategies for the prevention or treatment of UTI or other infections caused by this pathogen.

E. coli has the ability to produce two types of capsular polysaccharide, designated groups 1 and 2, each comprising a large number of distinct serotypes. This division has been made on the basis of chemical, physical, epidemiological, and microbiological characteristics. Most extraintestinal pathogenic isolates constitutively produce group 2 capsules (14). Certain group 2 capsular serotypes have been documented to contribute to pathogenesis in systemic models of infection (8, 24, 38). However, whether group 2 capsules contribute to the pathogenesis of UTI, and if so, by which mechanism, is unclear. It has been amply documented that group 2 capsules are more frequently present in strains that cause UTI than in fecal isolates (21, 22, 42). This epidemiologic association has led to speculation that group 2 capsules contribute to urovirulence by means of their ability to impede phagocytosis (6, 26, 47, 50) and to confer resistance to the bactericidal activity of serum (3, 7, 25, 39). However, in urine the function of neutrophils may be depressed, complement levels are low, and anticomplement activity may be present (2, 4, 5, 29, 46). Further, evidence supporting a role for group 2 capsules in the pathogenesis of UTI in a variety of in vivo models is contradictory. Studies that correlated the presence or amount of capsule with the ability to cause ascending renal infection or bacteremia either supported (28) or refuted (9, 23, 53) a role for capsule. Studies that compared capsule-positive and capsule-negative strains found that the capsule-negative derivatives caused less bladder and renal colonization and lethality than their capsule-positive counterparts; however, isogenic strains were not utilized (47, 54).

Likewise, O-specific antigens also have been implicated as possible virulence determinants for systemic infection (35, 43, 53) and in the pathogenesis of UTI. Strains isolated from patients with pyelonephritis and symptomatic cystitis are more likely to possess O-specific antigen than are asymptomatic bacteriuric or fecal isolates. Further, a subgroup of 8 to 12 serotypes are disproportionately represented among UTI isolates (15, 52, 55). It is not known, however, whether these serotypes are themselves urovirulence traits or whether they are linked to other such factors. Certain O-specific antigens may confer serum resistance (20, 33, 35, 36, 48), whereas others do not (40). Lipopolysaccharide (LPS) may also protect against phagocytosis (1, 26, 45). In vivo studies, also with nonisogenic strains, also suggest that LPS directly contributes to the development of UTI (47, 49).

CP9 (O4/K54/H5) is an extraintestinal isolate of *E. coli* that is being studied as a model pathogen (37). Proven isogenic derivatives of this strain have been constructed that are deficient in the group 2 K54 capsule alone (CP9.137), the O4-specific antigen moiety of LPS alone, or both the K54 capsule and the O4-specific antigen (CP923) (37, 40). No studies have been performed with true isogenic mutants to assess the role of the group 2 capsule and the O-specific antigen in models of ascending UTI (10). Therefore, we used these strains to evaluate whether these bacterial properties contribute to uropathogenesis. In this study, we examined the growth of these strains in vitro in human urine and their virulence in vivo in a mouse model of ascending unobstructed UTI.

Bacterial strains. Wild-type clinical strain CP9 is an *E. coli* blood isolate that has already been described (37). In brief, it is characterized by growth in 80% human serum, hemolysin

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production, an O4/K54/H5 serotype, the P pilus (class I PapG adhesin), the Prs pilus (class III PapG adhesin), the type 1 pilus, no known antibiotic resistance, absence of an aerobactin system (14a), and possession of a 36.2-kb cryptic plasmid (pJEG). The construction of the three proven isogenic derivatives of CP9 evaluated in this study also has already been described (37, 40). CP9.137 ($K54^-$ Kan^r) and CP921 ($O4^-$ Kan^r) were created by transposon mutagenesis with Tn*phoA* and Tn*phoA'1*, respectively. CP923 was created by transducing (T4) the Tn*phoA'-1* insertion from CP921 into a chloramphenicol-resistant, kanamycin-sensitive derivative of CP9.137, TR1377 ($K54^-$).

In vitro growth in human urine. (i) Collection and processing of human urine. Urine from healthy humans who had never experienced a UTI was used for studies assessing growth of strains in vitro. Because of the concern that there may be significant variability in urine collected from different subjects and that processing of urine may also be a variable, strains were tested for growth in urine that was (i) fresh and unfiltered, (ii) fresh and filtered with a 0.22 μm pore-size filter, and (iii) filtered and stored at 4°C. Further, urine from a single subject and a mixture of urine samples from six subjects were utilized. When growth in urine was evaluated, the urine used for overnight growth was collected and processed in the same manner as that used for the growth curve the next day.

(ii) Quantitation of in vitro growth. Strains were assayed for in vitro growth in L-B broth (41) and in human urine. The strain to be tested was grown overnight in 2 ml of medium with or without the appropriate antibiotic(s) (kanamycin at 40 μg/ml and chloramphenicol at 25 μg/ml). On the next day, bacterial cells grown overnight were added to the same fresh medium and incubated at 37°C until logarithmic-phase growth was reached. These cells were subsequently diluted and inoculated into the same fresh medium to achieve a starting concentration of approximately 1.0×10^2 to 1.0×10^3 CFU/ml, since this titer is at the lower end of the spectrum of what is considered significant for UTI in symptomatic young women (44). During incubation at 37°C, aliquots were removed at intervals and bacterial titers were determined by plating 10-fold serial dilutions in 1× phosphate-buffered saline in duplicate on appropriate media.

Mouse model of UTI. The mouse model used was based on that of Hagberg et al. (12), and inoculation conditions that have been shown in our laboratory to avoid inoculation-induced vesicoureteral reflux were used (18). Briefly, anesthetized 6- to 8-week-old female Swiss-Webster mice were inoculated transurethrally, with a Harvard infusion pump at 1.25 μl/g of body weight, with a suspension of one of the four test bacterial strains at various concentrations. Mice were killed 2 days after inoculation. Urine was collected from the bladder and cultured quantitatively, and the kidneys and bladders were harvested and hemisected; one half of each organ was cultured (quantitatively for bladders, semiquantitatively for kidneys), and the other half was processed for histopathological examination (17).

For semiquantitative kidney culture, the flat surface of each hemisected kidney was smeared on an agar plate and the number of colonies was counted after overnight incubation (30). A renal infection intensity score (0 to 5) was assigned as follows: 5, confluent growth; 4, nonconfluent growth but colonies too numerous to count; 3, >20 CFU; 2, 11 to 20 CFU; 1, 1 to 10 CFU; 0, no growth. A combined renal infection intensity score for each mouse was calculated as the sum of the scores for each mouse's two kidneys (18). For analysis, urine or organ cultures were considered positive if they demonstrated $\geq 10^2$ CFU/ml of urine, $\geq 10^2$ CFU/g of bladder tissue, or a renal infection intensity score of >1 (which reflects >10 colonies per plate).

Semiquantitative histopathological analysis of kidney and bladder sections, based on polymorphonuclear leukocyte density, was done by a blinded observer as previously described (16, 17). In kidney sections, polymorphonuclear leukocyte densities in the collecting system, within the mucosa, and in the submucosa each were graded as 0 (absent) to 3+ (maximal severity). An individual renal inflammation score was calculated as the sum of the scores for each of these three sites. Scores from each mouse's two kidneys were summed to give the animal's total kidney pathology score. Bladder sections were similarly evaluated for polymorphonuclear leukocyte density within the mucosa and interstitium and the degree of interstitial edema. A mouse's bladder pathology score was the sum of the scores for each of these three bladder histopathological parameters. A mouse's combined pathology score was calculated as the sum of its kidney and bladder pathology scores.

Phenotypic analyses. Bacterial isolates recovered from the bladders and kidneys of selected mice underwent analyses of LPS and capsular polysaccharide to ensure that inoculated strains were phenotypically stable.

LPS. Whole-cell lysates were used for LPS analysis. Lysates were prepared as previously described (13), separated by 14% polyacrylamide gel electrophoresis, and silver stained (51).

Capsular polysaccharide. Bacterial capsules were extracted by the method of Pelkonen et al., except that overnight cultures and L-B broth were used (34). The capsular material obtained from 10 ml of cells was resuspended in 50 μl of sterile water. Analysis was performed by immunoelectrophoresis and Cetavlon (hexadecyltrimethylammonium bromide) precipitation as developed by Orskov (32).

Statistical analyses. For statistical analysis of infection and histopathologic outcomes between different bacterial strains, results from mice inoculated with the same strain were combined to give larger comparison groups. Pairwise comparisons with respect to quantitative culture results and histopathologic scores between wild-type strain CP9 and each of its three knockout derivatives were made with the Mann-Whitney test, and comparisons of proportions were done with Fisher's exact test.

Growth in human urine in vitro. CP9 (wild type), CP9.137 ($K54^-$), CP921 ($O4^-$), and CP923 ($K54^-$ $O4^-$) were tested for growth in vitro in L-B broth and human urine. In L-B broth, there was no difference in growth between wild-type strain CP9 and its isogenic derivatives CP9.137, CP921, and CP923 (Fig. 1A). In human urine, CP9, CP9.137, and CP921 grew identically. In contrast, CP923 demonstrated a small but reproducible decrease in log-phase growth but nonetheless achieved the same plateau density (Fig. 1B). Representative growth curves of these strains grown in fresh, filtered, pooled human urine are shown in Fig. 1B.

Growth and survival in vivo in the mouse UTI model. The contribution of the K54 capsule and the O4-specific antigen to urovirulence was assessed in a mouse model of ascending unobstructed UTI. Mice were inoculated with various inocula of isogenic strains CP9 (wild type), CP9.137 ($K54^-$), CP921 ($O4^-$), and CP923 ($K54^-$ $O4^-$) transurethrally under nonrefluxing conditions and sacrificed after 48 h.

Cultures of urine, bladders, and kidneys from mice killed 48 h after inoculation demonstrated that wild-type strain CP9 and capsular mutant CP9.137 were similar in the ability to infect the lower and upper parts of the mouse urinary tract. This was true whether infection outcomes were evaluated as the pro-

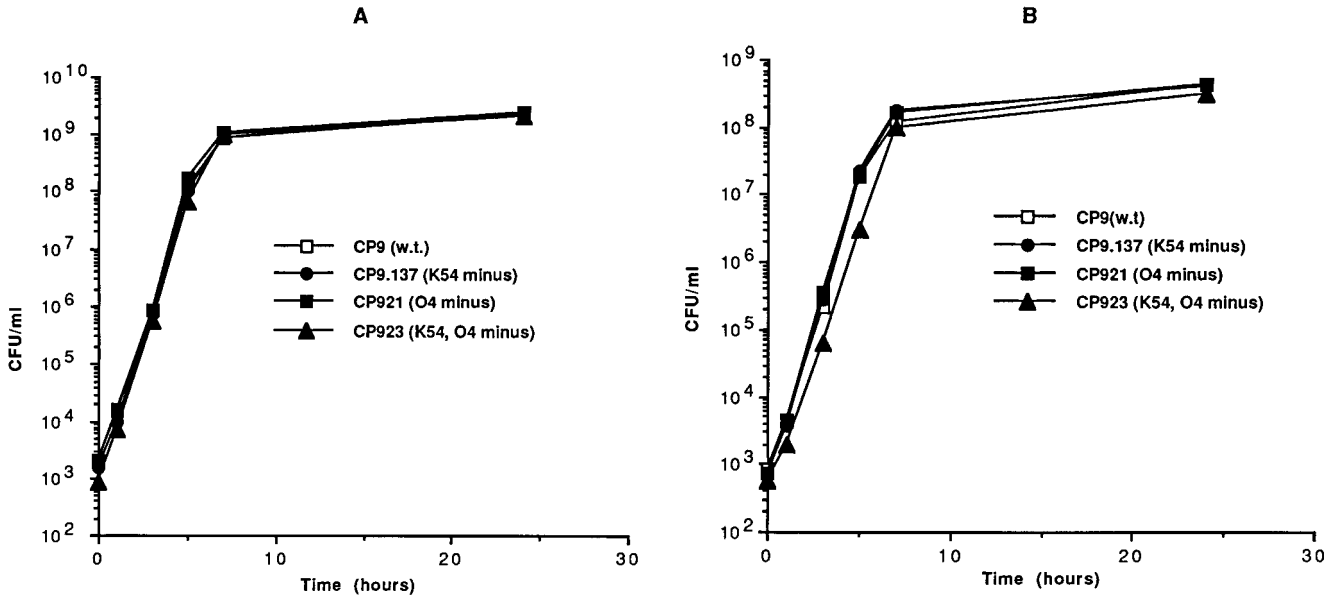

FIG. 1. Growth curves of CP9 (wild type [w.t.]), CP9.137 (K54$^-$), CP921 (O4$^-$), and CP923 (K54$^-$ O4$^-$) in L-B broth (A) and pooled human urine (B) incubated at 37°C and agitated at 125 rpm. Cultures were grown overnight at 37°C in the same medium with or without kanamycin (40 μg/ml) and chloramphenicol (25 μg/ml), diluted in fresh medium on the next day to achieve logarithmic growth, and then rediluted and inoculated into fresh medium to achieve a starting inoculum of 1.0×10^2 to 1.0×10^3 CFU/ml. Aliquots were removed over time, and viable titers were determined by plating serial 10-fold dilutions in duplicate.

portion of mice infected at a particular site, the mean number of kidneys infected per mouse, or the mean intensity of infection at each site (Tables 1 and 2). In contrast, the O4$^-$ derivative and the O4$^-$ K54$^-$ double mutant exhibited a markedly reduced ability to infect the mouse urinary tract as assessed by these same parameters, with the O4 single mutant appearing nearly as virulence impaired as the double mutant (Tables 1 and 2).

Histopathological findings with the four comparison strains paralleled the culture results (Tables 3 and 4). The wild-type strain (CP9) and the K54$^-$ mutant (CP9.137) stimulated similar degrees of inflammatory changes in the bladders and kidneys (Tables 3 and 4). In contrast, the mean inflammatory scores for the O4$^-$ mutant (CP921) and the O4$^-$ K54$^-$ double mutant (CP923) were only about half as great as the corresponding scores for CP9. These apparent differences approached statistical significance for the comparison of the combined (kidney plus bladder) pathology score between the wild-type strain and the O4$^-$ K54$^-$ double mutant (CP923).

Phenotypic analysis of isolates recovered after in vivo passage. Capsular polysaccharide and LPS analysis was performed on selected isolates from mouse urine, bladders, and kidneys to ensure that the mutations in CP9.137 (K54$^-$), CP921 (O4$^-$), and CP923 (K54$^-$ O4$^-$) were stable and that CP9 (wild type) did not undergo spontaneous capsule or LPS loss. Independent isolates from different sites and/or experiments were chosen for analysis. Twenty-five isolates from mice inoculated with CP9 (three bladder and four kidney isolates), CP9.137 (one bladder isolate and one kidney isolate), CP921 (three bladder and five kidney isolates), and CP923 (two bladder and five kidney isolates) were evaluated. No reversions to a capsule- or O-specific antigen-positive phenotype were observed in the

TABLE 1. Infection of the urine, bladders, and kidneys of Swiss Webster mice after challenge with CP9 and isogenic derivatives

Strain	Log_{10} inoculum size (CFU)	No. of mice in group	% of mice with positive urine culture	Mean log_{10} urine titer ± SEM	% of mice with bladder infection	Mean log_{10} bladder titer ± SEM	% of mice with kidney infection	Mean renal infection intensity score ± SEM	Mean no. of kidneys infected/mouse ± SEM
CP9 (wild type)	5.8	5	25	0.6 ± 0.6	0	0 ± 0	20	2.0 ± 1.0	0.4 ± 0.4
	6.9	5	60	1.9 ± 0.8	0	0.2 ± 0.2	60	3.8 ± 1.2	1.2 ± 0.5
	8.9	4	33[a]	2.5 ± 0.9	75	2.7 ± 0.9	100	5.8 ± 1.1	1.8 ± 0.3
	9.7	5	100	3.5 ± 0.7	100	4.9 ± 0.2	100	5.8 ± 1.2	2.0 ± 0.0
CP9.137 (K54$^-$)	6.8	4	0	0.0 ± 0.0	0	0.0 ± 0.0	50	2.5 ± 0.9	0.5 ± 0.3
	8.7	5	100	3.3 ± 0.4	80	2.7 ± 0.7	80	4.4 ± 0.7	1.6 ± 0.2
CP921 (O4$^-$)	6.6	7	14	0.4 ± 0.3	0	0.1 ± 0.1	14	1.7 ± 0.6	0.3 ± 0.2
	8.7	5	0	0.5 ± 0.3	40	1.6 ± 1.0	60	2.8 ± 1.0	0.8 ± 0.3
CP923 (K54$^-$ O4$^-$)	6.8	5	0	0.0 ± 0.0	0	0.0 ± 0.0	20	1.6 ± 0.6	0.2 ± 0.2
	7.7	5	20	0.5 ± 0.5	0	0.2 ± 0.2	40	2.0 ± 0.6	0.4 ± 0.4
	8.8	4	0	0.0 ± 0.0	25	0.9 ± 0.9	25	2.3 ± 0.6	0.8 ± 0.3

[a] Urine was unobtainable from one mouse.

TABLE 2. Compilation and statistical analysis of data in Table 1[a]

Strain	Mean $\log_{10}$ inoculum size (CFU)	No. of mice in group	% of mice with positive urine culture	Mean $\log_{10}$ urine titer ± SEM	% of mice with bladder infection	Mean $\log_{10}$ bladder titer ± SEM	% of mice with kidney infection	Mean renal infection intensity score ± SEM	Mean no. of kidneys infected/ mouse ± SEM
CP9	7.6	19	53	2.0 ± 0.4	42	1.9 ± 0.5	68	4.3 ± 0.6	1.1 ± 0.2
CP9.137[b]	7.9	9	56	1.8 ± 0.6	44	1.5 ± 0.6	67	3.6 ± 0.6	0.9 ± 0.3
CP921	7.5	12	8[c]	0.5 ± 0.2[c]	17[d]	0.8 ± 0.5[e]	33[f]	2.2 ± 0.5[g]	0.4 ± 0.2[g]
CP923	7.7	14	7[h]	0.2 ± 0.2[i]	7[j]	0.3 ± 0.3[g]	29[k]	1.9 ± 0.3[c]	0.3 ± 0.1[l]

[a] The Mann-Whitney test was used for continuous variables, and Fisher's exact test was used for proportions. All comparisons are versus CP9.
[b] For CP9 versus CP9.137, $P > 0.50$ for all comparisons.
[c] $P = 0.02$.
[d] $P = 0.24$.
[e] $P = 0.13$.
[f] $P = 0.08$.
[g] $P = 0.03$.
[h] $P = 0.01$.
[i] $P = 0.002$.
[j] $P = 0.047$.
[k] $P = 0.04$.
[l] $P = 0.007$.

isogenic mutants, and CP9 remained K54 and O4 antigen positive (data not shown).

Although group 2 capsules and O-specific antigens have been regarded as putative virulence factors in the pathogenesis of UTI, most of the studies that have evaluated their role have generated only epidemiological support for this hypothesis (21, 22, 42, 52, 55). Further, these studies have not always been in agreement (9, 23, 28, 53). No studies have been performed with true isogenic mutants and models of ascending UTI (10). As a result, the roles of the K- and O-specific antigens in promoting UTI and urosepsis have remained unclear.

Since uropathogens likely differ in multiple characteristics, a genetic approach that compares strains that differ only with respect to specific, defined traits is better suited for assessment of the contribution of individual bacterial factors to virulence. Therefore, in this study we evaluated a wild-type clinical isolate (CP9 [O4/K54/H5]) and isogenic, transposon-generated knockout derivatives deficient in the O4 antigen alone (CP921), the K54 antigen alone (CP9.137), or both antigens (CP923) to determine the specific contributions of the O4-specific antigen moiety of LPS and the group 2 K54 capsule to the pathogenesis of ascending bladder and renal infections. Our in vivo data strongly support the contention that the O4-specific antigen of itself plays an important role in the pathogenesis of UTI in vivo, since loss of this LPS component resulted in a decrease in all of the infection parameters measured in the mouse model of UTI. Loss of the K54 capsular polysaccharide by itself (CP9.137) did not significantly reduce infection of urine, the bladder, or the kidneys. However, loss of both the K54 and O4 antigens did result in seemingly larger decreases in all of the infection indices measured compared with loss of the O4 antigen alone. Therefore, these results do not exclude the possibility that the K54 antigen plays a minor role in the pathogenesis of ascending UTI.

Only loss of both the O4 and K54 antigens together, not loss of O4 alone, affected in vitro growth in human urine. Further, the observed difference was a minor but reproducible decrease in log-phase growth without a decrease in the plateau titer eventually reached. These findings suggest that the contribution of the O4 antigen to uropathogenesis is not mediated through growth in human urine.

We are aware of a single previous study that evaluated K and O antigen-deficient strains in an in vivo model of ascending, unobstructed UTI (47). This study demonstrated that a different O-specific antigen (O75) also contributed significantly to bacterial survival in both the bladder and kidneys. However, in contrast to our finding, a role also was demonstrated for a K antigen, albeit of a serotype different from the one we studied (K5). There are several possible explanations for this variance. First, isogenic mutants were not evaluated in the earlier study. Therefore, the possibility that a cryptic mutation(s) was responsible for the observed differences in these strains cannot be excluded. Secondly, not all K or O antigens may serve identical biologic functions. It is possible that certain O and K antigens, but not others, are important for growth and survival in the bladder and kidneys. Such a conjecture, however, awaits additional testing with other proven isogenic knockout derivatives. Finally, a number of differences exist between the mouse model systems utilized. We used Swiss-Webster, not C3H/HeN, mice; inoculated a single bacterial strain, not mixtures of bacteria; and inoculated bacteria into the bladder in a reduced volume and controlled rate to avoid reflux. Whether these differences are of importance is not known. Another study compared six different K antigen-positive parents (one each of K1, K2, or K23 and three of an unknown K type) and their spontaneous K antigen-deficient derivatives with respect to the ability to infect kidneys after intravenous injection.

TABLE 3. Histopathological evidence of urinary tract inflammation in mice challenged with CP9 or its isogenic derivatives

Strain	Log_{10} inoculum size (CFU)	No. of mice in group	Mean bladder pathology score ± SEM	Mean kidney pathology score ± SEM	Mean combined pathology score ± SEM
CP9 (wild type)	5.8	5	0.8 ± 0.8	3.0 ± 3.0	2.6 ± 1.8
	6.9	5	1.0 ± 0.8	4.2 ± 1.9	5.2 ± 2.5
	8.9	4	3.5 ± 1.3	3.0 ± 0.6	5.8 ± 1.1
	9.7	5	4.0 ± 1.1	6.0 ± 0.7	10.0 ± 1.1
CP9.137 ($K54^-$)	6.8	4	1.2 ± 1.2	1.0 ± 1.0	4.3 ± 1.6
	8.8	5	3.5 ± 0.9	5.8 ± 0.9	7.3 ± 1.8
CP921 ($O4^-$)	6.6	7	0.7 ± 0.4	1.8 ± 1.0	2.0 ± 1.1
	8.7	5	1.6 ± 1.2	4.3 ± 2.4	5.3 ± 3.3
CP923 ($O4^-$ $K54^-$)	6.8	5	0.6 ± 0.2	0.0 ± 0.0	0.5 ± 0.3
	7.7	5	0.8 ± 0.2	2.0 ± 1.4	2.4 ± 1.2
	8.8	4	1.5 ± 0.7	2.0 ± 1.2	4.0 ± 1.2

TABLE 4. Compilation and statistical analysis of data in Table 3[a]

Strain	Mean $\log_{10}$ inoculum size (CFU)	No. of mice in group	Mean bladder pathology score ± SEM	Mean kidney pathology score ± SEM	Mean combined pathology score ± SEM
CP9	7.6	19	2.3 ± 0.6	3.6 ± 0.8	5.9 ± 1.0
CP9.137[b]	7.9	9	2.2 ± 0.8	3.3 ± 1.1	5.8 ± 1.3
CP921[b]	7.5	12	1.1 ± 0.5	2.0 ± 0.9	3.3 ± 1.5
CP923	7.7	14	0.9 ± 0.2[c]	1.4 ± 0.6[d]	2.3 ± 0.7[e]

[a] The Mann-Whitney test was used for continuous variables, and Fisher's exact test was used for proportions.
[b] $P > 0.10$ for all comparisons of CP9 versus CP9.137 and CP921.
[c] $P = 0.38$ versus CP9.
[d] $P = 0.06$ versus CP9.
[e] $P = 0.037$ versus CP9.

These mutants also were not isogenic, and a model system fundamentally different from ours was used. Nonetheless, that study's findings were largely consistent with those of the present study in that loss of the K antigen had little effect on the ability of the test strains to infect kidneys.

In the present study, histopathological evaluation of mice infected with CP921 ($O4^-$) and CP923 ($O4^-$ $K54^-$) disclosed a diminished inflammatory response compared with CP9 (wild type). Since all of the comparison strains possess an intact, unaltered LPS lipid A moiety, which is a potent proinflammatory mediator (27), the presence of an inflammatory response, even with the mutants, is not surprising. If the diminished response documented with CP921 and CP923 is biologically significant, one possible explanation for this reduction might be that inflammation is proportional to bacterial titer and/or duration of persistence, independent of any direct effect of the O4 antigen on the inflammatory response. Alternatively, it might be that alterations in or absence of the O-specific antigen may affect the processing of and the resultant inflammatory response incited by lipid A (11).

Despite instillation into the bladder, our wild-type strain (CP9) and its isogenic derivatives demonstrated a higher prevalence of kidney infection than bladder infection. One explanation for this difference is that in this model system, the prevalence of bladder and renal infections is time dependent. Alternatively, it is possible that certain virulence traits enable *E. coli* strains to more successfully infect the bladder, whereas other traits are more important for renal infection. CP9 and its derivatives express the P pilus (class I PapG adhesin) and the Prs pilus (class III PapG adhesin), which is one virulence trait that appears to be more important for upper versus lower tract disease (15, 31). If there exists a separation in virulence determinants involved in infection at these two sites, this might explain why certain women with acute pyelonephritis present with flank pain and fever in the absence of symptoms of lower UTI (19). Data from this study and a previously published report (40) support the concept that the relative importance of at least some virulence determinants in extraintestinal strains of *E. coli* are site specific. The O4 antigen, but not the K54 antigen, is important for UTI, whereas the K54 antigen, but not the O4 antigen, is important for systemic infection after intraperitoneal injection into mice (40).

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