INFECTION AND IMMUNITY, May 1987, p. 1224–1232
0019-9567/87/051224-09$02.00/0
Copyright © 1987, American Society for Microbiology
Vol. 55, No. 5

Bacterial Virulence versus Host Resistance in the Urinary Tracts of Mice

C. SVANBORG-EDÉN,[1] L. HAGBERG,[1] R. HULL,[2] S. HULL,[2]* K.-E. MAGNUSSON,[3] AND L. ÖHMAN[3]

Department of Clinical Immunology, University of Göteborg,[1] Department of Medical Microbiology, University of Linköping,[3] Sweden, and Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030[2]

Received 20 October 1986/Accepted 30 January 1987

The relative contributions of host resistance and bacterial virulence were analyzed in a mouse model for ascending urinary tract infection. The congenic mouse strains C3H/HeJ and C3H/HeN were used in parallel. They differ in their reactivity to lipopolysaccharide (LPS) and susceptibility to experimental urinary tract infection. C3H/HeJ cells are susceptible to infection and are nonresponders to LPS (*Lps^d Lps^d*), whereas C3H/HeN cells respond to LPS and are resistant to infection (*Lps^n Lps^n*). The *Escherichia coli* pyelonephritis isolate GR-12, serotype O75K5, expressing adhesins specific for globoseries glycolipids (P fimbriae) and for mannosides (type-1 fimbriae), and its derivatives deficient in these factors were used, either singly or in combination, to establish experimental infections. In C3H/HeN mice, the relative persistence of *E. coli* was inversely proportional to its phagocytosis in vitro. Loss of the O75 and K5 antigens increased the tendency toward hydrophobic interaction, promoted phagocytosis, and reduced persistence in the kidneys. This was not the case in C3H/HeJ mice, in which O75$^-$ and K5$^-$ serotypes persisted in the same extent as did the parent strain. The total number of bacteria recovered from the kidneys of C3H/HeJ mice was about 1,000-fold higher than the number recovered from kidneys of C3H/HeN mice 24 h after infection. Previous studies have demonstrated a delayed influx of polymorphonuclear leukocytes into the urinary tracts of C3H/HeJ mice. The results are consistent with the hypothesis that phagocyte activation through LPS is a major defense mechanism against *E. coli* in the kidney, a property in which C3H/HeJ mice are deficient.

Urinary tract infections (UTI) result from the interaction of *Escherichia coli* strains with a susceptible host (39). Each step in the pathogenic process is determined by the balance between host resistance and bacterial virulence. The virulent strain possesses the sum of determinants required to colonize the intestines, ascend into and persist in the urinary tract, and induce infection. In the resistant host, pathogenesis is interrupted by host defense mechanisms. In the present study, determinants of bacterial virulence and host resistance in an experimental UTI mouse model are analyzed.

The definition of virulence in uropathogenic *E. coli* is based largely on epidemiologic evidence (7, 23, 26, 42). Characteristics which are prevalent in isolates causing the more severe infections, but rare in other strains, are termed virulence factors. Pyelonephritogenic *E. coli* belong to a limited number of O:K:H serotypes or electrophoretic types, adhere to uroepithelial cells, are resistant to the bactericidal action of serum, and produce hemolysin (7, 29, 34, 35, 42, 45). The clonal structure of *E. coli* populations results in a nonrandom coexpression of virulence factors. Since wild-type isolates differ by multiple characteristics, genetic manipulations are required to assess the contribution of individual virulence traits (14, 15). In this way, adherence specific for Galα1→4Galβ-containing receptors was recently shown to increase the persistence of *E. coli* in the kidneys of mice; the combination of adhesins with Galα1→4Gal and mannose specificity was optimal in the bladder (15).

Specific and innate mechanisms of host resistance combine to protect the urinary tract against infection (8, 31). Although immunity, induced by hyperimmunization, has been shown to protect against infection, specific immunity does not explain the natural resistance to infection (5, 20, 38). In contrast, resistance to *E. coli* UTI in the mouse was related to lipopolysaccharide (LPS) responsiveness (13, 16). T-cell-deficient (*nu/nu*), B-cell-deficient (*xid*), or macrophage-deficient (A/J) mice were shown to clear *E. coli* from the kidneys at a rate similar to that of their normal counterparts (41). The LPS nonresponder mouse strain C3H/HeJ (*Lps^d Lps^d*) has about 1,000-fold-higher *E. coli* counts than does strain C3H/HeN (*Lps^n Lps^n*) from kidneys within 24 h after infection (41, 46). The aim of the present study was to analyze the contribution of individual bacterial virulence traits in mouse strains of high or low natural susceptibility to infection.

MATERIALS AND METHODS

Bacteria. The *E. coli* strains used were derived from the wild-type pyelonephritis isolate GR-12 (43). This strain is of serotype O75:K5:H$^-$, has adhesins specific for the globoseries glycolipid receptors (GS) and for mannosides (MS), is resistant to serum killing, and does not produce hemolysin.

The pedigrees of the derivatives of GR-12 are shown in Fig. 1 and Table 1.

Isolation of nonadherent mutants. An exponentially growing culture of *E. coli* GR-12 was treated with nitrosoguanidine as described elsewhere (47). The survival frequency was 60% after treatment. After overnight incubation at 37°C, the mutagenized culture was diluted, and the dilutions were spread on L agar (22) to yield isolated colonies. Individual colonies were tested for the ability to hemagglutinate human erythrocytes in the presence of D-mannose. For isolation of

* Corresponding author.

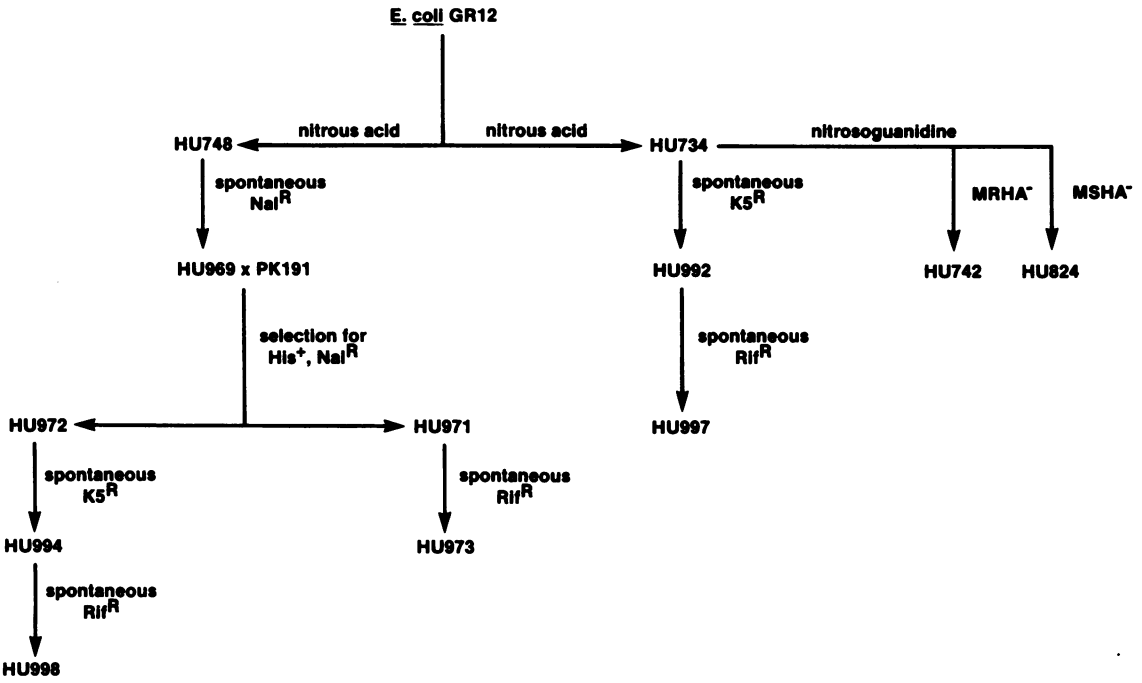

FIG. 1. Pedigrees of the *E. coli* GR-12 derivatives used in the present study. Abbreviations: $K5^R$, resistance to bacteriophage K5; Nal^R, resistance to nalidixic acid; Rif^R, resistance to rifampin.

MS-negative mutants, survival frequency after nitrosoguanidine treatment was 47%, and guinea pig erythrocytes were used for hemagglutinations. Of 300 colonies tested for D-mannose-resistant hemagglutination, 3 were negative. On initial screening for reversion to hemagglutination positive, a single mutant, HU742, was found to be stable. By using a more rigorous enrichment procedure for revertants on the basis of separation of erythrocyte-adherent bacteria with a glycerol discontinuous gradient, the frequency of reversion of HU742 was found to be less than 10^{-5}. HU824 retained the GS adhesins, and HU742 retained the MS adhesins from the parental strains. Other traits, including serotype, electrophoretic type, serum resistance, and plasmid pattern, remained unaltered. Nalidixic acid-resistant (50 μg/ml) variants of HU824 and HU742 were selected by using gradient plates. The growth rates of these strains and of all other mutant derivatives were the same as for the parent strains.

Isolation of mutants with defects in the LPS side chain. HU973 and HU972 were constructed in the following way. GR-12 was mutagenized with nitrous acid (28), and a nonreverting histidine auxotroph, HU748 (*his*), was identified after two cycles of ampicillin-D-cycloserine enrichment (9). A spontaneous nalidixic acid-resistant derivative of HU748, HU969, was selected on agar containing 100 μg of nalidixic acid per ml. HU969 was mated with an *E. coli* K-12 Hfr donor, PK191 (*E. coli* Genetic Stock Center, Yale University, New Haven, Conn.), for 1 h on filters as previously described (19, 25). Histidine-independent exconjugants were tested for coinheritance of the *rfb* locus of *E. coli* K-12 (3), by using a commercially prepared anti-O75 serum (Difco Laboratories, Detroit, Mich.) in a slide agglutination assay. HU972 was O^-, and HU971 was O75. HU971, the his^+ O75 exconjugant, was plated on agar containing 200 μg of rifampin per ml to select a spontaneous rifampin-resistant mutant (HU973).

TABLE 1. Bacterial strains

Strain	Relevant characteristics[a]	Reference or source
PK191	*E. coli* K-12 Hfr	25
GR-12	O75K5H⁻; GSMS, ColV⁺, Ser^r Hly^-	15
HU734	GR-12 *lac* Ser^r	—[b]
HU972	HU969 his^+ O^- Ser^s	—[b]
HU973	HU971 Rif^r Ser^r	—[b]
HU997	HU992 Rif^r Ser^r	—[b]
HU998	HU994 Rif^r Ser^s	—[b]
HU742	HU734 MS^- Ser^r	15
HU824	HU734 GS^- Ser^r	15

[a] Abbreviations: Hly, hemolysin expression, Ser, resistant (r) or sensitive (s) to killing in serum.
[b] —, Strain prepared for this report.

Isolation of mutants in capsular polysaccharide. The bacteriophage specific for the K5 polysaccharide antigen was kindly provided by G. Schmidt, Borstel, Federal Republic of Germany. The phage was propagated in *E. coli* Bi8337-41. The phage was precipitated from chloroform-treated cultures in L broth with ammonium sulfate and purified by centrifugation on a discontinuous cesium chloride gradient (12).

For selection of colonies resistant to the K5 phage, HU734 and HU972 were grown on L agar. One drop of suspension containing K5-specific phage was added. Spontaneously resistant colonies were picked and checked either for their ability to be agglutinated by O75 antiserum without previous boiling (HU992) or for rough-colony morphology (HU994). Spontaneously rifampin-resistant mutants of HU992 (HU997) and HU994 (HU998) were selected as described above.

Culture conditions. *E. coli* HU824 Nal^r and HU742 Nal^r were maintained lyophilized or for limited periods of time on tryptic soy agar (TSA; Difco) with 50 μg of nalidixic acid per ml. *E. coli* HU973, HU997, and HU998 were maintained on TSA plates with rifampicin (200 μg/ml). For infection and in vitro testing, the resistant strains were passaged on TSA free

of antibiotics. Other growth media used were lactose-bromthymol blue agar and tryptic soy broth.

Adhesins. GS adhesins were defined by mannose-resistant agglutination of human erythrocytes of blood group P_1 but not $\bar{p}$ (39). Latex beads with covalently linked Galα1→4Gal were used as controls. MS adhesins were defined by mannose-sensitive agglutination of guinea pig erythrocytes (15, 40).

O and K serotypes. The presence of O75 antigen was determined by agglutination with anti-O75 antiserum. Presence of the K5 antigen was determined by K5-specific phage (12).

Serum resistance. The bactericidal effect of human serum was assessed according to the method of Olling et al. (34); resistance signifies killing of less than 50% of the organisms, intermediate signifies killing of 50 to 99%, and sensitive signifies >99% killing.

Physiochemical surface properties. (i) Aqueous two-phase partitioning. To assess surface charge and hydrophobicity, the bacteria were partitioned at 4°C in an aqueous two-phase system formed by dextran 500 T (6.2% wt/wt) and polyethylene glycol (PEG) 6000 (4.4% wt/wt) in a 0.03 M Tris buffer (pH 7.3). The change of partition accompanying the addition of positively charged bis-trimethylamino PEG or palmitoyl PEG at 10 and 5% of total PEG concentration, respectively, was used as a measure of surface charge and hydrophobicity (21). Briefly, bacteria were labeled with 3,4,5-[^{3}H]leucine (NET-460; New England Nuclear Corp., Boston, Mass.) by adding 25 μCi to 10 ml of tryptic soy broth. After being washed three times, 0.1 ml of a bacterial suspension (about 5×10^8 cells per ml) was added to a phase system with 2 ml of bottom phase (rich in dextran), 2 ml of top phase (rich in PEG), and 0.2 ml of ligand PEG (bis-trimethylamino PEG or palmitoyl PEG). After being mixed and separated (30 min), 0.5-ml samples were withdrawn for determination of bacteria in the bottom and top phases and at the interface by using an automatic scintillation counter (Rack-Beta; LKB Wallac, Turku, Finland).

(ii) Hydrophobic interaction chromatography. The bacteria were analyzed for surface hydrophobicity and charge by using a modification of procedures described previously (21, 40). Hydrophobicity was tested by the binding of [^{3}H]leucine-labeled bacteria (0.2 ml; 5×10^8 cells per ml) to octyl-Sepharose or Sepharose-CL-4B (Pharmacia, Uppsala, Sweden) equilibrated with phosphate-buffered saline (PBS) alone or PBS supplemented with 40% ethylene glycol (EG). D-Mannose and D-galactose were added at 0.1 M, since it was recently found that D-mannose partly prevents the hydrophobic interaction of type 1-fimbriated *E. coli* (33). The bacteria were suspended in the equilibration liquids.

Negative charge was assayed by the binding of the bacteria (0.2 ml; 5×10^8 cells per ml) to DEAE-Sepharose-CL-6B (Pharmacia) equilibrated with PBS. Bacteria were subsequently eluted with 2-ml portions of PBS, followed by PBS with 0.5 or 1.0 M NaCl. In both assays, the gels were poured into ordinary Pasteur pipettes. The tips of the pipettes were filled with siliconized glass wool, onto which was placed a nylon net (70-μm pores) and about 1 ml of each of the different gels.

Interaction with PMNs. (i) Preparation of PMNs. Polymorphonuclear leukocytes (PMNs) were isolated from EDTA-treated peripheral human blood by dextran sedimentation and Hypaque-Ficoll gradient (Winthrop Laboratories, Div. Sterling Drug Inc., New York, N.Y.) centrifugation (4). After separation, the remaining erythrocytes were removed by hypotonic lysis, and the PMNs were washed and suspended in Krebs-Ringer phosphate buffer supplemented with 10 mM glucose (KRG) and 1% human serum albumin (KABI, Stockholm, Sweden).

Phagocytosis was assayed by the fluorescence quenching method to discriminate between extracellular and intracellular bacteria (32).

(ii) Fluorescein-isothiocyanate labeling. Fluorescein isothiocyanate labeling was done in 1 ml of 0.2 M carbonate buffer (pH 9.5) containing 10^9 bacteria and 1 mg of fluorescein isothiocyanate. The mixture was incubated at room temperature (22°C) for 30 min and washed three times in PBS (pH 7.2).

(iii) Adherence to PMNs. The PMN suspension (100 μl; 5×10^5 cells per ml) was added to one spot of multispot glass slides (14 mm diameter; Dynatech Laboratories, Zurich, Switzerland) and allowed to adhere for 15 min at 37°C in a moist chamber. Nonadhering cells were removed by washing in warm (37°C) KRG, and then 100 μl of the fluorescein-isothiocyanate-labeled bacteria (2×10^8) was added. After 30 min of incubation at 37°C, the glass slides were rinsed in cold (4°C) KRG and immediately examined with an incident-light fluorescence microscope with phase-contrast optics (magnification, ×1,250; Carl Zeiss, Inc., Oberkochen, Federal Republic of Germany). The total number of bacteria that adhered to 100 PMNs was determined.

(iv) Ingestion by PMNs. After the adherent bacteria had been counted, 3 drops of crystal violet (0.6 mg/ml in 0.15 M NaCl) were added to the spot for a few seconds. The attached bacteria lost their fluorescence, whereas the ingested bacteria were not reached by the dye and remained fluorescent.

Chemiluminescence. Chemiluminescence response was assayed in a Lumacounter 2080 (Lumac AG, Zurich, Switzerland) in the presence of luminol (Sigma Chemical Co., St. Louis, Mo.). The PMN suspension (0.1 ml; 5×10^5 cells), the bacterial suspension (0.1 ml; 2×10^8), and luminol (0.1 ml; 1 mg/ml) were mixed at 37°C, and the chemiluminescence response was recorded continuously.

Mouse strains. C3H/HeJ mice (original breeding stock; Jackson Laboratory, Bar Harbor, Maine) and C3H/HeN (original breeding stock; Charles River, United Kingdom, Ltd., Margate, Kent, England) were bred and maintained at the animal facilities, Department of Clinical Immunology, Göteborg, Sweden. Female mice older than 6 weeks were used and matched for age in each experiment.

Experimental infection. The mixed infection protocol was presented in detail previously (14, 15). Briefly, derivatives of *E. coli* GR-12 to be compared were mixed before injection. The components of the mixtures were separated according to differences in antibiotic resistance or lactose fermentation. After infection and sacrifice of the mice, the tissues were plated on agar with the appropriate selection, and the relative recovery of each inoculum component was determined in each mouse.

(i) Inoculum. The bacterial cell density was adjusted by A_{597} to a concentration of 10^9 bacteria per ml by dilution in PBS (300 mosmol/liter, pH 7.2). In the mixed-infection experiments, equal numbers of bacteria from each strain (10^9 bacteria per ml) were mixed in the same inoculum. The concentration of each strain in the *E. coli* HU997-HU973 and HU997-HU998 mixtures was easily determined on lactose-bromthymol blue agar plates, since *E. coli* HU973 and HU998 fermented lactose and formed yellow colonies, whereas *E. coli* HU997 remained blue. The concentration of each strain in the *E. coli* HU973-HU972 and HU972-HU998 mixtures was determined by serial dilutions on TSA and

TABLE 2. Hydrophobic interaction chromotography of the *E. coli* derivatives

Elution buffer and gel[a]	% Bacteria retained in gel[b]			
	O75K5	O⁻K5	O75K⁻	O⁻K⁻
PBS				
Sepharose	3	6	4	6
Octyl-Sepharose	8	12	23	38
PBS + 0.1 M D-mannose				
Sepharose	2	3	4	14
Octyl-Sepharose	2	8	21	39
PBS + 0.1 M D-galactose				
Sepharose	3	4	4	2
Octyl-Sepharose	4	12	23	31
PBS + 40% EG[c]				
Sepharose	2	4	5	5
Octyl-Sepharose	4	5	8	21
PBS + EG + 0.1 M D-mannose				
Sepharose	1	4	6	19
Octyl-Sepharose	3	4	13	22
PBS + EG + 0.1 M D-galactose				
Sepharose	8	8	6	5
Octyl-Sepharose	5	5	9	19

[a] Sepharose, Sepharose CL-4B.
[b] O75K5, HU973; O⁻K5, HU972; O75K⁻, HU997; O⁻K⁻, HU998. The values are given without standard error for clarity; the coefficient of variation (± standard error of the mean) between identical experiments was 2.2 ± 0.2% (n = 9).
[c] Vol/vol.

TSA-rifampin plates. HU973 Rif^r and HU998 Rif^r were quantitated as the number of colonies on the TSA-rifampin plates, and HU972 was quantitated by subtraction of the colonies on TSA-rifampin from those on TSA. Control experiments with passage of HU973 Rif^r or HU998 Rif^r on antibiotic-free medium or in mice did not show segregation of the antibiotic marker.

(ii) Infection procedure. The animals were anesthetized by ether inhalation. The bladder was emptied by gentle compression of the abdomen. Immediately thereafter, a soft polyethylene catheter (outer diameter, 0.61 mm; Kebo Grave, Göteborg, Sweden) adapted to a needle (0.4 by 20 mm gauge) on a 1-ml tuberculin syringe was transurethrally inserted into the bladder. A 0.05-ml portion of the inoculum was injected. The catheter was withdrawn immediately, and no further manipulations were done. The ether anesthesia lasted for about 30 s, after which time the mice were allowed free access to food and water.

(iii) Bacterial recovery from tissues. After 24 h, animals were sacrificed by cervical dislocation. Kidneys and bladders were removed aseptically. The tissues were homogenized in 5 ml of PBS in disposable plastic bags with a Stomacher 80 homogenizer (Seward Medical UAC House, London, England). Serial dilutions of the homogenate were spread on appropriate agar plates. The bacterial recovery was calculated as the logarithm of CFU in a 0.1-ml homogenate.

Statistics. The relative recovery from each animal of strains I and II in the inoculum mixture was calculated as the ratio of strain I to strain II. The recovery from a group of mice was expressed as the geometric mean (i.e., the mean of the logarithms of the CFU per 0.1 ml of tissue homogenate) and mean ratio, with standard error factor.

TABLE 3. Ion-exchange chromatography of the *E. coli* derivatives

Elution buffer	% Bacteria[a] retained in gel[b]			
	O75K5	O⁻K5	O75K⁻	O⁻K⁻
PBS	92 ± 2[c]	98 ± 0	81 ± 1	97 ± 0
PBS with 0.5 M NaCl	22 ± 1	42 ± 3	13 ± 1	47 ± 2
PBS with 1.0 M NaCl	6 ± 1	19 ± 1	8 ± 1	39 ± 1

[a] See Table 2, footnote *b*.
[b] DEAE-Sepharose-CL-6B.
[c] Data presented as average ± range of duplicate experiments.

RESULTS

Characteristics of the GR-12 derivatives. The adhesins, serotype determinants, and serum resistance of the GR-12 derivatives are shown in Table 1. Removal of adhesins did not affect the O or K serotype. The O⁻, K⁻, or O⁻K⁻ variants retained the ability to produce the adhesins of the wild-type parent. Manipulation of the adhesins did not affect the resistance to serum killing. In contrast, loss of the O75 antigen was associated with conversion to serum sensitivity. Thus, the O⁻K5 and the O⁻K⁻ derivatives were sensitive to killing in serum (Table 1). Loss of the K5 antigen alone did not affect serum sensitivity.

Both LPS and capsular polysaccharide influenced the surface charge and hydrophobicity of the GR-12 derivatives (Tables 2 to 4). The affinity for the hydrophobic octyl-Sepharose gel was low for the O75K5 parent and O⁻K5 recombinant, but higher for the O75K⁻ mutant (Table 2). The derivative deficient in both O75 and K5 antigens (O⁻K⁻) was most hydrophobic, with 38% retained in the gel. When the surface tension of the suspending medium and the hydrophobic attraction between the bacteria and the column were reduced by the addition of 40% EG, the numbers of O75K5, O⁻K5, and O75K⁻ in the column were reduced to background values, whereas the O⁻K⁻ derivate remained associated (21%) with the gel. The K5 antigen appeared to contribute more than did the O75 antigen to the overall hydrophilic character, since the O75K⁻ was more hydrophobic than the O⁻K5 or O75K5 parent (Table 2). Little effect was obtained with either 0.1 M D-galactose or D-mannose, and the binding to unsubstituted Sepharose was similar for all bacteria.

The O⁻K⁻ and O⁻K5 mutants were the most negatively charged, as shown by the lower elution with the high-salt

TABLE 4. Partitioning of *E. coli* derivatives in aqueous two-phase systems

Bacteria[a]	Partition (%) ± range[b]			Change of partition (%)[c]	
	Top phase	Bottom phase	Interface	PEG-TMA	PEG-P
O75K5	7 ± 1	87 ± 6	6 ± 5	+28	+1
O⁻K5	5 ± 0	76 ± 2	19 ± 2	+22	−15
O75K⁻	25 ± 2	25 ± 4	50 ± 2	+11	+3
O⁻K⁻	3 ± 0	50 ± 8	47 ± 7	+47	+17

[a] See Table 2, footnote *b*.
[b] PEG and dextran. Duplicate experiments; coefficient of variation ± standard error of the mean, 4.2 ± 0.7% (n = 33).
[c] PEG-TMA, bis-trimethylamino PEG; PEG-P, palmitoyl PEG. The percent change equals the sum of the two differences i and ii. (i) The percent bacteria in the top phase after addition of the ligand PEG minus the percent bacteria in the top phase before addition of the ligand PEG. (ii) The percent bacteria in the bottom phase before addition of the ligand PEG minus the percent bacteria in the bottom phase after addition of the ligand PEG.

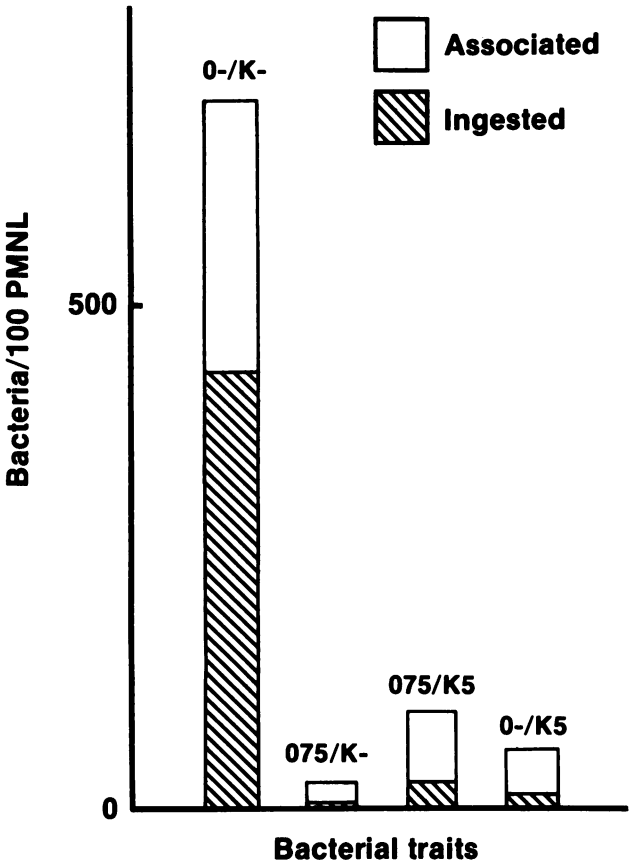

FIG. 2. Association with and ingestion by human PMNs of *E. coli* derivatives differing in O and K antigen.

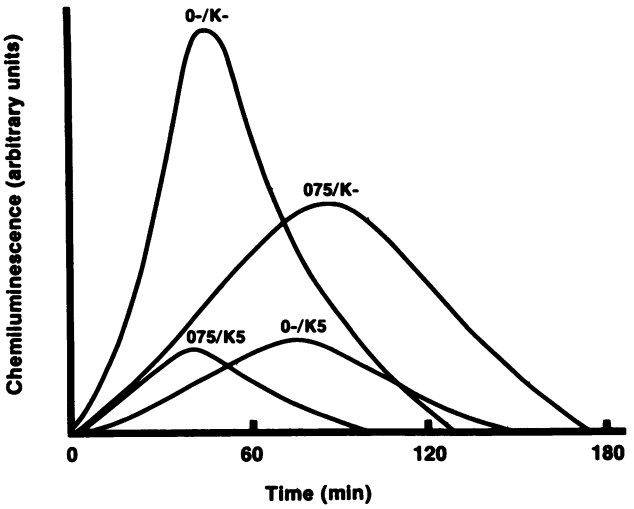

FIG. 3. Chemiluminescence response to *E. coli* derivatives differing in O and K antigens.

buffer from DEAE-Sepharose compared with that for the O75K5 and O75K$^-$ strains (Table 3). This probably indicates that the K5 antigen added negatively charged groups to the cell envelope, but that deeper structures, such as were exposed in the O$^-$K$^-$ mutant, contributed as well. Parallel results were obtained with positively charged bis-trimethylamino PEG in two-phase partitioning (Table 4). The change of partition was +47% for the O$^-$K$^-$ mutant and +28 and 22%, respectively, for the O75K5 and O$^-$K5 strains. The effect of palmitoyl PEG on the partition of bacteria in the dextran-PEG system confirmed that the O$^-$K$^-$ strain was most hydrophobic (change of partition, 17%) (Table 4).

Interaction with PMNs. The interaction of the *E. coli* strains with PMNs was investigated in two ways: by the association and ingestion of each strain (Fig. 2) and by the activation (evocation) of a chemiluminescence response (Fig. 3). The O$^-$K$^-$ strain both bound to and was engulfed to the greatest extent by the PMNs, whereas there was little difference between the other strains. On a relative basis, the associations were 100, 2, 7, and 21% for the O$^-$K$^-$, O75K$^-$, O$^-$K5, and O75K5 strains, respectively. For the same bacteria, the percentages of engulfed bacteria were 61, 13, 21, and 25%, respectively. The average values for repeated experiments are shown in Table 5. Evidently, the K5 capsule strongly prevented association to and, to a lesser extent, ingestion by the PMNs.

The chemiluminescence response was more complex since it varied in intensity (maximum value), kinetics (time before maximum value), and total activity (time-response integral) among the strains. The O$^-$K$^-$ strain caused the most intense, rapid, and large response (Fig. 3). On a relative basis, the peak values and integrals were 100 and 100, 57 and 98, 23 and 33, and 21 and 19% for the O$^-$K$^-$, O75K$^-$, O$^-$K5, and O75K5 strains, respectively (Fig. 3). It thus appears that the capsular antigen (K5) reduced the chemiluminescence to a greater extent than did the LPS (O75).

In previous experiments, variants with MS adhesins bound to the PMN and activated a chemiluminescence response. The mutant with Galα1→4Galβ-binding adhesins only did not activate such a response. This was attributed to the lack of Galα1→4Galβ receptors in human PMNs (40).

Persistence in the mouse urinary tract. The effect of changes in O antigen or capsule on bacterial persistence in the mouse urinary tract was studied in two ways: (i) the mutant lacking the trait of interest was compared with the parental strain retaining this trait, and (ii) the mutant lacking both O and K antigens was compared with the derivatives with either O or K. The effect of changes in adhesins was examined by comparison of the GSMS parental strain with the GS— or —MS mutants or of the GS— and —MS mutants with each other. Each comparison was made by mixed infections and in parallel in C3H/HeJ and C3H/HeN mice.

The results of the infection with the O and K antigen mutants in the mice are shown in Table 6. The total recoveries from C3H/HeN mice ranged from 2 to 331 colonies, regardless of the virulence of the infecting strain. Within this range, differences were found. Both the O75 and K5 antigens contributed significantly to bacterial survival. The O75 de-

TABLE 5. Interaction with PMNs of *E. coli* derivatives

Bacteria[a]	Association[b] (% ± SEM)	Ingestion[b] (% ± SEM)	Chemiluminescence[b] peak value (% ± SEM)
O75K5	5 ± 2	1 ± 0	13 ± 6
O$^-$K5	8 ± 2	22 ± 8	3 ± 0
O75K$^-$	5 ± 0.3	8 ± 4	21 ± 8
O$^-$K$^-$	100 ± 18	25 ± 8	100 ± 12

[a] See Table 2, footnote *b*.
[b] Relative values. Values shown are means of three experiments with different preparations of PMNs and bacteria.

TABLE 6. Infectivity of *E. coli* polysaccharide derivatives in the urinary tracts of C3H/HeN and C3H/HeJ mice

Inoculum mixture I/II[a]	Mouse strain	Bacterial recovery[b] from:			
		Kidneys		Bladders	
		Geometric mean	Ratio of I to II (SEF)	Geometric mean	Ratio of I to II (SEF)
O75K5/O⁻K5	C3H/HeN	37/3.4	11.0 (1.85)	4.1/1.7	2.41 (2.18)
	C3H/HeJ	3,160/3,470	0.99 (1.41)	191/8.3	23.0 (1.62)
O75K5/O75K⁻	C3H/HeN	245/53.7	4.56 (2.16)	25/3.6	6.9 (2.21)
	C3H/HeJ	9,550/20,900	0.46 (1.37)	67.6/72.4	0.93 (1.53)
O75K⁻/O⁻K⁻	C3H/HeN	331/2.0	165 (1.71)	132/8.9	14.8 (2.75)
	C3H/HeJ	20,400/2,690	7.58 (1.47)	339/2.57	132 (1.87)
O⁻K5/O⁻K⁻	C3H/HeN	158/5.4	29.3 (2.00)	3020/14	216 (3.55)
	C3H/HeJ	28,200/1,620	17.4 (1.40)	52.5/8.71	6.03 (1.48)

[a] See Table 2, footnote *b*.
[b] Mean of two experiments, 10 mice per group. Geometric mean is the mean of the logarithms of the CFU/0.1 ml of tissue homogenate. Values shown are antilogs. SEF, Standard error factor.

rivative had an 11-fold advantage in the kidneys over the O⁻K5, and the O75K⁻ had about a 160-fold advantage over the O⁻K⁻ derivative. Deletion of the K5 antigen had a lesser effect (about 5-fold increase of O75K5 over O75K⁻ and a 29-fold advantage of O⁻K5 over O⁻K⁻). The results were parallel for kidneys and bladders, except for the O⁻K5 strain, which had a 200-fold advantage over the O⁻K⁻ derivatives in the bladders.

The results from the C3H/HeJ mice differed from those from the C3H/HeN mice (Table 6). Regardless of which derivative was used for infection, the level of bacterial recovery was 100- to 1,000-fold greater in C3H/HeJ mice than in C3H/HeN mice. Deletion of the O75 or K5 antigens did not significantly affect the persistence in the kidneys of C3H/HeJ mice (ratio, about 1.0 for O75K5 over O⁻K5 and 0.5 for O75K5 over O75K⁻). Compared with the O⁻K⁻ mutant, O75K⁻ or O⁻K5 had a small but significant advantage (ratio, about 8 for O75K⁻ over O⁻K⁻ and about 17 for O⁻K5 over O⁻K⁻) (Table 6). In the bladders of the C3H/HeJ mice, the O75 antigen gave a significant advantage.

The adhesins contributed to the same extent to bacterial persistence in both kidneys and bladders in C3H/HeJ and C3H/HeN mice (Table 7). The GSMS derivative had an advantage over the GS— and —MS derivatives in kidneys and bladders. The GS— derivative persisted in higher numbers than did the —MS derivative in the kidneys; the —MS derivative persisted significantly better than did the GS— derivative in the bladders. In the bladders of C3H/HeN mice, the GSMS parent had a greater advantage over the GS— derivative than in C3H/HeJ mice.

DISCUSSION

The relative contributions of host resistance and bacterial virulence were analyzed in a mouse model of ascending UTI. This approach was made possible by two lines of work: (i) the development of a series of mutants of a wild-type pyelonephritis strain, differing in virulence factors, and (ii) the identification of a mouse strain susceptible to infection. The results demonstrated a major influence of host resistance on the persistence of *E. coli* in the mouse urinary tract regardless of the virulence of the infecting strain. Adherence contributed significantly to bacterial persistence in both resistant and susceptible mice; O and K antigens were more important in the resistant hosts than in the susceptible hosts.

Some of the bacterial mutants were isolated after treatment with a potent mutagen. Nitrosoguanidine is well known for its propensity to elicit secondary auxotrophic and temperature-sensitive mutations within 2 min of the selected site; estimations of the frequency of secondary mutant phenotypes range from 1 to 8% (1, 36). Nitrosoguanidine was selected for use only after several unsuccessful attempts with milder mutagenic agents. Direct enrichment by hemad-

TABLE 7. Infectivity of *E. coli* adhesin mutants in the urinary tracts of C3H/HeN and C3H/HeJ mice

Inoculum mixture I/II[a]	Mouse strain	Bacterial recovery[b] from:			
		Kidneys		Bladders	
		Geometric mean	Ratio of I to II (SEF)	Geometric mean	Ratio of I to II (SEF)
GSMS/—MS	C3H/HeN	240/13.8	17.4 (2.23)	282/93.3	3.02 (2.50)
	C3H/HeJ	33,900/2,750	12.3 (1.69)	490/178	2.75 (2.24)
GS—/—MS	C3H/HeN	166/16.2	10.2 (1.93)	9.55/51.3	0.19 (1.97)
	C3H/HeJ	17,400/3,470	5.01 (1.58)	13.0/158	0.08 (2.69)
GSMS/GS—	C3H/HeN	417/6.46	64.6 (2.45)	53,700/8.71	6,170 (2.58)
	C3H/HeJ	19,500/457	42.7 (1.97)	158/15.5	10.2 (1.69)

[a] GSMS, HU734; GS—, HU824; —MS, HU742.
[b] Mean of two experiments. 10 mice per group. Geometric mean is the mean of the logarithms of the CFU/0.1 ml of tissue homogenate. Values shown are antilogs. SEF, Standard error factor.

sorption of nonadherent mutants was not possible because of the presence of nonadherent-phase variants in the population. Furthermore, the HU734 line was refractory to in vitro insertion mutagenesis. The quality of the mutants was controlled. HU742 and HU824 have no additional auxotrophic or temperature-sensitive mutations, and several definable virulence determinants were unaltered. The growth rates of HU734, HU742, and HU824 in urine did not differ. To address the concern that there may be two different secondary mutant alleles, one near the MS adherence gene in HU824 and one near the GS adherence gene(s) in HU742, affecting hitherto unknown genes associated with in vivo survival, a genetically distinct set of strains was constructed. A smooth, nonadherent fecal *E. coli* strain was transformed with recombinant plasmids carrying genes encoding the GS, MS, or GSMS adhesins. When these strains were tested for colonizing potential, results paralleled those obtained with the mutants (15, 18).

Other surface antigens were altered without chemical mutagenesis. The loss of the K5 antigen from HU734 and HU972 occurred through spontaneous events selected by resistance to bacteriophage K5 (12). Spontaneous mutations are predominantly point mutations with a minority (3%) of small deletions (36). The deletions of O75 antigen were transferred with an O^- *E. coli* K-12 donor. Such interclonal matings are inefficient. Even though the selected gene, *his*, is very near the Hfr origin (2 min), no his^+ recombinants were detected until 45 min after mixing of the donor and recipient. Thus, the matings resulted in the transfer of only a small region of the chromosome. Both $O75^-$ and $O75^+$ strains used in this study were his^+ recombinants obtained from the same mating and they presumably differed only at the *rfb* locus, at genes tightly linked with *rfb*, and at those between *rfb* and the Hfr origin. Genes in this region in *E. coli* K-12 potentially associated with virulence include *flu* (expression of piliation) and *non* (expression of M antigen or colonic acid) (11, 35). However, the Hfr donor used is presumed to carry wild-type alleles at these loci, and the recombinants used in this study were capable of both fimbriae and capsule expression. Although it cannot be rigorously excluded that one recombinant and not the other inherited an unknown gene affecting bacterial survival in vivo, we believe that such an event is unlikely.

The construction of isogenic sets of bacteria allowed us to measure the effects of the O and K antigens and adhesins on the physiochemical surface properties of *E. coli*. Previously, the contribution of individual bacterial components to charge and hydrophobicity was implied from comparisons of wild-type strains differing in multiple characteristics (33, 37). The O^-K^- mutant was most hydrophobic as measured either by octyl-Sepharose chromatography or two-phase partitioning with palmitoyl PEG. The $O75K^-$ variant was less hydrophobic, and the O^-K5 and O75K5 strains had the lowest hydrophobic interaction. The high negative charge of the O^-K^- derivative may at first seem contradictory, since it lacked acidic capsular material. It has previously been shown that deep rough (*rfe*) mutants of *Salmonella typhimurium* and *Salmonella minnesota* are hydrophobic and negatively charged (21). This was explained by the exposure of negatively charged ketodeoxyoctanate and phosphate groups.

The charge and hydrophobicity correlated directly with the interaction of phagocytes, as measured by attachment, membrane irritation, and ingestion. The most hydrophobic and most negatively charged mutant O^-K^- gave maximal responses, and the $O75K^-$ mutant produced the second highest signal in both assays. The stimulation of oxidative metabolism by the $O75K^-$ mutant, as evidenced by chemiluminescence production (2, 10), is remarkable because the association with and uptake in the PMNs was small. This clearly indicates that further studies must be done to elucidate the role of other bacterial surface structures as passive amplifiers or attenuators of chemiluminescence. Certain stimuli, e.g., formyl-methionyl-leucyl-phenylalanine (6), generated a two-peak response with one extracellular and one intracellular event. The $O75K^-$ strain generated only a delayed second peak (6, 10). The discrepancy between the induction of chemiluminescence and the induction of uptake and killing has been emphasized previously (32). In this study, ingestion by phagocytes was reduced by the presence of both the O75 and the K5 antigens.

The effect of specific adhesins on chemiluminescence dominated over charge and hydrophobicity (40). The MS adhesins overcame the hydrophilicity of the wild-type strain by binding to mannose-containing receptors on the phagocytes. The mutant expressing adhesins specific for Galα1→4Galβ-containing receptors bound to and activated the PMNs poorly, because these receptors were not available on the phagocytes.

The mouse infection model was designed to analyze determinants of the initial stages of host-parasite interaction in the urinary tract. The endpoint measured was bacterial survival in kidneys and bladders at 24 h after infection. Although the localization of bacteria at that time has been difficult to assess by histologic techniques, we assume that bacteria are primarily colonizing the mucosal surfaces, with invasion as a rare event (17). The pronounced difference in bacterial numbers between C3H/HeJ and C3H/HeN as early as 24 h after infection suggested that C3H/HeJ mice are deficient in host factors controlling the initial clearance of bacteria. The effectors of host resistance were sought among cells known to exhibit deficient reactivities with LPS, e.g., lymphocytes, macrophages, and fibroblasts. C3H/HeJ mice were shown to have a defective recruitment of PMNs into the urinary tract during the first days after experimental infection (R. Shahin, I. Engberg, L. Hagberg, and C. Svanborg-Edén, J. Immunol., in press).

Comparisons of the relative bacterial persistence in the C3H/HeJ and C3H/HeN mice revealed differences in two types. (i) Regardless of the *E. coli* derivative used, the number of bacteria recovered 24 h after infection was about 1,000-fold higher in the kidneys of C3H/HeJ than in those of C3H/HeN mice. (ii) The relative recoveries of mutants deficient in O or K antigen differed between the mouse strains, but manipulation of adhesive characteristics gave largely parallel results. The difference was inversely related to the interaction with phagocytes in vitro. In the C3H/HeN mice, the loss of either the O75 or the K5 antigen or both in combination resulted in a disadvantage for the bacteria both in kidneys and bladders compared with the parent strain, and in vitro phagocytosis was increased for these mutants. Thus, the bacterial persistence in vivo in C3H/HeN correlated with the susceptibility to phagocytosis in vitro. In the C3H/HeJ mice, loss of neither the O75 nor the K5 antigen had a significant effect on bacterial persistence in the kidneys. The O^-K^- mutant, however, was at a disadvantage. Taken together, these results support a defect in phagocyte-mediated clearance in the kidneys of C3H/HeJ mice and a role for LPS as the inductory signal for the normal inflammatory response in C3H/HeN mice. The consequence of deficient recruitment of inflammatory cells in C3H/HeJ mice appeared to be enhanced persistence not only of the O75K5

mutant, but also of the O75K$^-$ and O$^-$K5 mutants which normally would be cleared by the inflammatory response.

Changes of the O and K antigens have been shown to alter bacterial virulence in several animal models (27, 30). The mechanisms previously suggested to be involved are lysis by antibody-dependent and independent mechanisms and increased susceptibility to phagocytosis caused by changes in charge and hydrophobicity. Numerous studies have attempted to analyze the influence of O or K antigens on the sensitivity to the bactericidal effect of serum (extensively reviewed in reference 44). *E. coli* with incomplete O antigens, rough, was susceptible to killing in serum. It was suggested that the O antigen shields the cell membrane against complement factors, although the LPS core was suggested as the actual target for the bactericidal components of serum. The present study clearly demonstrates that removal of the O75 antigen is sufficient to render the *E. coli* strain susceptible to killing in serum, independent of the K antigen. Adhesins had no significant influence.

The epidemiologic basis for the definition of virulence in *E. coli* causing UTI has been differences in the severity of infection in resistant hosts, i.e., children without known defects of the urine flow (39, 42). In contrast, patients inherently susceptible to UTI, i.e., those with reflux and renal scarring, were infected with *E. coli* strains not expressing these virulence traits (24). This suggested a pronounced effect of host resistance on the selection of bacteria able to cause acute pyelonephritis and is consistent with the present study in which C3H/HeJ mice were more susceptible to infection than were C3H/HeN mice and permitted the persistence of bacteria of lower virulence. The role of LPS-induced effector mechanisms in patients with UTI remains to be explored.

ACKNOWLEDGMENTS

These studies were supported by U.S. Public Health Service grant no. AI21009 from the National Institutes of Health; Swedish Medical Research Council grant no. 215, 6251; The Swedish Board for Technical Development; The Medical Faculty, University of Göteborg; Kabi-SSA; King Gustav V 80-Year Fund; and The Medical Research Council of the Swedish Life Insurance Companies and Östergötlands Läns Landstings Forskningsfond.

We thank Claes Dahlgren for valuable comments on the manuscript and I. Engberg and B. Larsson for skillful technical assistance.

LITERATURE CITED

1. **Adelberg, E. A., M. Mandel, and G. C. C. Chen.** 1965. Optimal conditions for mutagenesis by *N*-methyl-*N*-nitro-*N*-nitrosoguanidine in *E. coli* K-12. Biochem. Biophys. Res. Commun. **18**:788–795.
2. **Allen, R. C., R. L. Stjernholm, and R. H. Steele.** 1972. Evidence for the generation of an electronic excitation state(s) in human polymorphonuclear leukocytes and its participation in bactericidal activity. Biochem. Biophys. Res. Commun. **47**:679–684.
3. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
4. **Böyum, A.** Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. Suppl. **97**:77–89.
5. **Braude, A. I.** 1975. Opposing effects of immunity to endotoxin: hypersensitivity versus protection, p. 69–74. *In* B. Urbaschek, P. Urbaschek, and E. Netor (ed.)., Gram-negative bacterial infections and mode of endotoxin actions: pathophysiological, immunological, and clinical aspects. Springer-Verlag, New York.
6. **Briheim, G., O. Stendahl, and C. Dahlgren.** 1984. Intra- and extracellular events in luminol-dependent chemiluminescence of polymorphonuclear leukocytes. Infect. Immun. **45**:1–5.
7. **Caugant, D. A., B. R. Levin, I. Ørskov, F. Ørskov, C. Svanborg-Edén, and R. K. Selander.** 1985. Genetic diversity in relation to serotypes in *Escherichia coli*. Infect. Immun. **49**:407–413.
8. **Cox, C., and F. Hinman.** 1961. Experiments with induced bacteriuria, vesical emptying and bacterial growth on the mechanism of bladder defense to infection. J. Urol. **86**:739–748.
9. **Curtiss, R., III, L. J. Charamella, C. M. Berg, and P. E. Harris.** 1965. Kinetic and genetic analysis of D-cycloserine inhibition and resistance in *Escherichia coli*. J. Bacteriol. **90**:1238–1250.
10. **Dahlgren, C., and O. Stendahl.** 1983. Role of myeloperoxidase in luminol-dependent chemiluminescence of polymorphonuclear leukocytes. Infect. Immun. **39**:736–741.
11. **Diderichsen, B.** 1980. *flu*, a metastable gene controlling surface properties of *Escherichia coli*. J. Bacteriol. **141**:858–867.
12. **Gupta, D. S., B. Jann, G. Schmidt, J. R. Golecki, I. Ørskov, F. Ørskov, and K. Jann.** 1982. Coliphage K5, specific for *E. coli* exhibiting the capsular K5 antigen. FEMS Microbiol. Lett. **14**:75–78.
13. **Hagberg, L., D. E. Briles, and C. Svanborg-Edén.** 1985. Evidence for separate genetic defects in C3H/HeJ and C3HeB/FeJ mice, that affect susceptibility to gram-negative infections. J. Immunol. **134**:4118–4122.
14. **Hagberg, L., I. Engberg, R. Freter, J. Lam, S. Olling, and C. Svanborg-Edén.** 1983. Ascending, unobstructed urinary tract infection in mice caused by pyelonephritogenic *Escherichia coli* of human origin. Infect. Immun. **40**:273–283.
15. **Hagberg, L., R. Hull, S. Hull, S. Falkow, R. Freter, and C. Svanborg-Edén.** 1983. Contribution of adhesion to bacterial persistence in the mouse urinary tract. Infect. Immun. **40**: 265–272.
16. **Hagberg, L., R. Hull, S. Hull, J. R. McGhee, S. Michalek, and C. Svanborg-Edén.** 1984. Difference in susceptibility to gram-negative urinary tract infection between C3H/HeJ and C3H/HeN mice. Infect. Immun. **46**:839–844.
17. **Hagberg, L., J. Lam, C. Svanborg-Edén, and J. W. Costerton.** 1986. Interaction of pyelonephritogenic *E. coli* with tissue components in the mouse urinary tract. J. Urol. **136**:165–172.
18. **Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. Infect. Immun. **33**:933–938.
19. **Hull, S. I., R. A. Hull, B. H. Minshew, and S. Falkow.** 1982. Genetics of hemolysin of *Escherichia coli*. J. Bacteriol. **151**:1006–1012.
20. **Kaijser, B., P. Larsson, and S. Olling.** 1978. Protection against ascending *Escherichia coli* pyelonephritis in rats and significance of local immunity. Infect. Immun. **20**:78–81.
21. **Kihlström, E., and K. E. Magnusson.** 1980. Adhesion to HeLa cells of LPS mutants of *Salmonella typhimurium* and *Salmonella minnesota* in relation to their physico-chemical surface properties. Cell Biophys. **2**:177–189.
22. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–196.
23. **Lidin-Janson, G., L. A. Hanson, B. Kaijser, K. Lincoln, U. Lindberg, S. Olling, and H. Wedel.** 1977. Comparisons of *Escherichia coli* from bacteriuric patients with those from feces of healthy school children. J. Infect. Dis. **136**:346–353.
24. **Lomberg, H., M. Hellström, U. Jodal, H. Leffler, K. Lincoln, and C. Svanborg-Edén.** 1984. Virulence-associated traits in *Escherichia coli* causing first and recurrent episodes of urinary tract infection in children with reflux. J. Infect. Dis. **150**:561–569.
25. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587–607.
26. **Mabeck, C. E., F. Ørskov, and I. Ørskov.** 1971. *Escherichia coli* serotypes and renal involvement in urinary tract infection. Lancet **i**:1312–1317.
27. **Mäkela, P. H., V. V. Valtonen, and M. Valtonen.** 1973. Role of O-antigen (lipopolysaccharide) factors in the virulence of *Salmonella*. J. Infect. Dis. **128**(Suppl.):81–85.
28. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 135–139. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

29. **Minshew, B. H., J. Jorgensen, G. W. Counts, and S. Falkow.** 1978. Association of hemolysin production, hemagglutination of human erythrocytes, and virulence for chicken embryos of extraintestinal *Escherichia coli* isolates. Infect. Immun. **20:** 50–54.
30. **Nakano, M., and K. Saito.** 1969. Chemical components in the cell wall of *Salmonella typhimurium* affecting its virulence and immunogenicity in mice. Nature (London) **222:**1085–1086.
31. **Norden, C. W., G. M. Green, and E. H. Kass.** 1968. Antibacterial mechanisms of the urinary bladder. J. Clin. Invest. **47:**2689–2700.
32. **Ohman, L., J. Hed, and O. Stendahl.** 1982. Interaction between human polymorphonuclear leucocytes and two different strains of type 1 fimbriae-bearing *Escherichia coli*. J. Infect. Dis. **146:**751–757.
33. **Ohman, L., K. E. Magnusson, and O. Stendahl.** 1985. Mannose-specific and hydrophobic interaction between *Escherichia coli* bacteria and human polymorphonuclear leucocytes: influence of bacterial culture period. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **93:**125–131.
34. **Olling, S., L. A. Hanson, J. Holmgren, U. Jodal, K. Lincoln, and U. Lindberg.** 1973. The bactericidal effect of normal human serum on *E. coli* strains from normals and from patients with urinary tract infections. Infection **1:**23–28.
35. **Ørskov, I., F. Ørskov, B. Jann, and K. Jann.** 1977. Serology, chemistry, and genetics of O and K antigens of *Escherichia coli*. Bacteriol. Rev. **41:**667–710.
36. **Schwartz, D. O., and J. R. Beckwith.** 1969. Mutagens which cause deletions in *Escherichia coli*. Genetics **61:**371–376.
37. **Stendahl, O., B. Normann, and L. Edebo.** 1979. Influence of O and K antigens on the surface properties of *Escherichia coli* in relation to phagocytosis. Acta Pathol. Microbiol. Scand. Sect. B **87:**85–91.
38. **Svanborg-Edén, C., B. Andersson, L. Hagberg, L. A. Hanson, H. Leffler, G. Magnusson, and T. Soderström.** 1983. Receptor analogues and anti-pili antibodies as inhibitors of attachment *in vivo* and *in vitro*. Proc. N.Y. Acad. Sci. **409:**580–592.
39. **Svanborg-Edén, C., L. M. Bjursten, L. Hagberg, R. Hull, S. Hull, U. Jodal, H. Leffler, and H. Lomberg.** 1984. New aspects of pathogenesis and treatment of urinary tract infections in relation to bacterial adherence, p. 117–125. *In* H. Losse, A. W. Asscher, A. E. Lison, and V. T. Andriole (ed.), Pyelonephritis V. Georg Thieme Verlag, New York.
40. **Svanborg-Edén, C., L.-M. Bjursten, R. Hull, R. Hull, K.-E. Magnusson, Z. Moldovano, and H. Leffler.** 1984. Influence of adhesins on the interaction of *Escherichia coli* with human phagocytes. Infect. Immun. **44:**672–680.
41. **Svanborg-Edén, C., L. Hagberg, D. Briles, J. McGhee, and S. Michalek.** 1985. Susceptibility to *Escherichia coli* urinary tract infection and LPS responsiveness, p. 385–391. *In* E. Skamene (ed.), Genetic control of host resistance to infection and malignancy. Alan R. Liss, Inc., New York.
42. **Svanborg-Edén, C., L. Hagberg, L. A. Hanson, T. Korhonen, H. Leffler, and S. Olling.** 1981. Adhesion of *Escherichia coli* in urinary tract infection. CIBA Found. Symp. **80:**161–187.
43. **Svanborg-Edén, C., R. Hull, S. Falkow, and H. Leffler.** 1983. Target cell specificity of wild type *E. coli* and mutants and clones with genetically defined adhesins. Prog. Food & Nutr. Sci. **7:**75–89.
44. **Taylor, P. W.** 1983. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. Microbiol. Rev. **47:** 46–83.
45. **Väisänen-Rhen, V., J. Elo, E. Väisänen, A. Siitonen, I. Ørskov, F. Ørskov, S. B. Svenson, P. H. Mäkelä, and T. K. Korhonen.** 1984. P-fimbriated clones among uropathogenic *Escherichia coli* strains. Infect. Immun. **43:**149–155.
46. **Watson, J., K. Kelly, M. Largen, and B. A. Taylor.** 1978. The genetic mapping of a defective LPS response gene. J. Immunol. **120:**422–424.
47. **Willetts, N. S., and D. W. Mount.** 1969. Genetic analysis of recombination-deficient mutants of *Escherichia coli* cotransducible with *thyA*. J. Bacteriol. **100:**923–934.