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Determinants That Increase the Serum Resistance of *Escherichia coli*

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The *rfb* locus, determining biosynthesis of O8-specific lipopolysaccharide side chains, was transferred to a rough mutant of *Escherichia coli*; recombinants producing a complete lipopolysaccharide were more resistant to the complement-mediated bactericidal action of human serum than the rough recipient. Inheritance of the *his*-linked genes for K27 antigen production did not alter the response to serum. The serum resistance of strains carrying O8 side chains, but not of strains with incomplete lipopolysaccharides, was further increased by inheritance of plasmids R1 and NR1.

Many gram-negative bacteria are susceptible to the bactericidal action of human and animal sera; the lethal action of serum is attributable to activated components of either the classical (6) or alternative (4) complement pathways. Serum-resistant strains, invariably of smooth colonial morphology, can be isolated from a variety of mammalian infections, and current evidence supports the contention that resistance to serum may be an important determinant of virulence in certain infections (18). Although the basis of serum resistance is not well understood, it has been suggested that a number of envelope components, such as O-side chain moiety of lipopolysaccharide (2, 15), acidic exopolysaccharide K antigens (6), and outer membrane proteins (19), are able to protect the bacterial cell against complement attack. Also, it has been demonstrated recently that certain conjugative plasmids are able to increase the resistance of suitable enterobacteria to the bactericidal action of serum (1, 3, 9, 17, 20).

Since it is likely that serum resistance has a multifactorial basis (15, 19), strains of *Escherichia coli* carrying combinations of surface antigens and conjugative plasmids have been constructed, and the sensitivity of the recombinants to human serum has been determined. For these experiments, *E. coli* F470 (*his* Str^r), a derivative of strain E56b [serotype O8:K27(A):H^- (13)], was used as recipient. F470 is an *rfb* (lipopolysaccharide O8 side chain-deficient) mutant that synthesizes the complete lipopolysaccharide R1 core (13) and produces no K27 antigen due to a point mutation in the *his*-linked genes that are, together with *trp*-linked genes, responsible for biosynthesis of this antigen (14). F470 carries no detectable extrachromosomal deoxyribonucleic acid and is extremely sensitive to human serum; no colonial survivors were detectable after 1 h of incubation (37°C) of 2.5×10^5 exponential-phase bacteria per ml in 75% serum. Hfr donor strains F639 (derived from *E. coli* F445 by G. Schmidt, Freiburg), F459 (Hfr 59) (13), and F445 (Hfr 45) facilitated the transfer of the *his*-linked K27 antigen alone, the *his*-linked O8 antigen alone, and both the O8 and K27 antigens simultaneously (Table 1).

Ninety-six percent of his^+ recombinants from cross F639 × F470 inherited the K27 antigen and were inagglutinable in R1 antiserum. Inheritance of the K27 antigen had no effect on survival in serum; all nine $K27^+$ recombinants examined were as serum sensitive as *E. coli* F470. Recombinants inheriting the *rfb* locus determining biosynthesis of the lipopolysaccharide O8 side chain, a mannan (8), were killed by serum but only after a delay of 1 h (Fig. 1), confirming that although O-side chains do not determine complete serum resistance they are likely to be responsible for a lag in the serum killing effect (6). Ten recombinants from the F459 × F470 cross were found to produce the K27 antigen in addition to the O8 antigen; both donor and recipient strains were derived from *E. coli* E56b, and loss of K antigen by both strains resulted from point mutations at different chromosomal sites (7). K27-carrying hybrids presumably arose by intragenic recombination, as previously suggested by Olson and co-workers (7). The responses to serum of these recombinants were found to be identical to those obtained with the O8:K^- recombinants. The majority of his^+ recombinants from the cross between strain F470 and O8:K27 donor strain F445 agglutinated in both O8 antiserum and K27 antiserum (Table 1), indicating that although they had inherited loci determining the biosynthesis of both antigens, these hy-

TABLE 1. *Analysis of his⁺ recombinants selected from crosses between rough recipient F470 (his) and various Hfr donor strains*[a]

Hfr donor	Donor serotype	No. of recombinants of serological type:				
		O8:K⁻	O8:K27	O8:K27i	O⁻:K27	O⁻:K⁻
F639	O⁻:K27	0	0	0	115	5
F459	O8:K⁻	106	10[b]	0	0	4
F445	O8:K27	0	3	112[c]	0	5

[a] The mating procedure described by Schmidt and co-workers (12) was used. Mating mixtures were diluted in TM buffer and streaked onto minimal selection agar plates containing 100 μg of streptomycin per ml to inhibit Str[s] donor cells. Antisera were prepared in rabbits by the method of Schlecht and Westphal (10). Serological types of recombinants were identified by slide agglutination in O8 (anti-F459) antisera, K27 (anti-F782) antisera (14), and R1 (anti-F470) antisera. A total of 120 recombinants were analyzed.

[b] As expected from the direction of transfer of chromosomal markers by F459 (17), this group of recombinants has inherited both functional *trp*-linked and *his*-linked loci encoding production of K27 antigen.

[c] F445 transfers chromosomal markers in the opposite direction to F459 (14), and the majority of the K27-producing recombinants have presumably inherited only the *his*-linked locus.

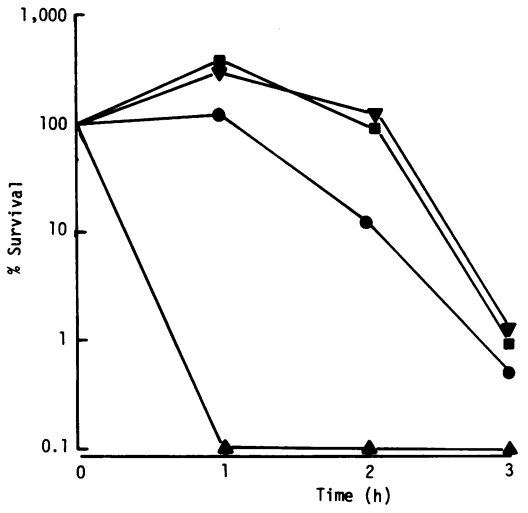

FIG. 1. *Interaction of E. coli F470 (▲), O8:K⁻ his⁺ recombinant UL 10 from the cross F459 × F470 (●), and UL 10 carrying plasmids R1 (■) and NR1 (▼) with human serum. An early exponential-phase broth culture of each strain was washed in 0.06 M NaCl and suspended in 0.05 M tris(hydroxymethyl)-aminomethane-hydrochloride buffer (pH 8.4) to a concentration of 10⁶ cells per ml; 0.5 ml of this suspension was added to 1.5 ml of serum, and viable counts were obtained at the beginning of the test and after 1, 2, and 3 h of incubation 37°C. Matings were conducted as previously described (15, 17). Differences in the serum responses of the various strains were assessed using the Wilcoxon sum of ranks (T) test (21). The differences between the responses of UL 10 and each of the plasmid-carrying strains were significant (n = 5; T = 15; P = 0.01, for each time interval). Plasmids R1 and NR1 were also transferred to two other his⁺ O8:K27⁺ recombinants (UL 11 and UL 87) from the cross F445 × F470; significant differences between these recombinants, with and without each plasmid, were also found (n = 5). Surface properties of all strains were tested using anti-O and anti-K antisera before and after transfer of plasmid markers; inheritance of plasmids did not affect the agglutination characteristics of the strains in these antisera.*

brids had not inherited *trp*-linked genes essential for the production of enough K antigen to confer O-inagglutinability. These intermediate hybrids have been termed K27i forms by Schmidt and co-workers (14). A selection of these hybrids and the three recombinants (Table 1) that had presumably inherited the *trp*-linked locus and were thus O-inagglutinable (UL 11, UL 87, and UL 96) displayed the delayed serum killing effect and were indistinguishable in this respect from the O8:K⁻ recombinants from the F459 × F470 cross. The K27 antigen does not, therefore, influence the serum sensitivity of F470 derivatives, regardless of lipopolysaccharide O-side-chain carriage.

We have found previously that the plasmids R1 and NR1, both belonging to incompatibility group FII, are able to confer significantly higher degrees of serum resistance on enterobacterial river isolates displaying the delayed sensitive response to serum (7). These plasmids were therefore transferred to a number of the recombinants derived from *E. coli* F470. Plasmids R1 and NR1 were transferred individually to a number of *his*⁺ recombinants from the cross F639 × F470 (O⁻:K27) and to F470; all plasmid-carrying progeny were as serum sensitive as the parental types. However, when these plasmids were transferred to recombinants carrying the O8 antigen or both the O8 and K27 antigens, significant increases in the serum resistance of the progeny were observed. The responses to serum of UL 10 (O8:K⁻), UL 10 R1, and UL 10 NR1 are shown in Fig. 1. Plasmids belonging to incompatibility groups Ia (plasmid R483), N (R46), P (RP1), and W (R388) had no effect on the serum reactivity of UL 10.

These results strongly suggest that the R1- and NR1-determined factors that increase serum resistance, presumably protein components of the outer membrane, are only functional when superimposed upon a full complement of lipopolysaccharide O-side chains. The presence of O-side chains and the carriage of either R1 or NR1 appear to considerably enhance the ability of *E. coli* strains to survive in systems containing large amounts of human serum.

Many naturally occurring strains, however, are able to grow rapidly in high concentrations of serum (16), and other as yet undetermined factors must contribute to the survival of these strains. Envelope proteins, presumably chromosomally determined, appear able to significantly protect gram-negative cells against serum bactericidal activity (19). It is hoped that a better understanding of the genetics of outer membrane protein biosynthesis will eventually lead to the identification of those factors responsible for complete serum resistance.

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