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K Antigen and Serum Sensitivity of Rough *Escherichia coli*

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We prepared bacterial hybrids which express both K1 and K27 antigens and examined the relative contributions of these capsules to serum resistance. *Escherichia coli* Hfr strain F639 (rough, $K27^+$, serum sensitive) was conjugated with *E. coli* recipient strain E412 (rough, $K1^+$, His^- Trp^- Str^r, serum resistant). Transconjugants which inherited both the *his* and *trp* linked genes for K27 antigen synthesis were analyzed. These hybrids retained and expressed the K1 antigen since the K1 locus is nonallelic with K27 gene loci. Hybrid strains which express both K1 and K27 antigens exhibit serum resistance, but not at the level of the $K1^+$ parental strain. An isogenic K1 derivative of a hybrid which expressed only K27 antigen was serum sensitive (>99% kill, 60 min). These findings indicate that the presence of the K1 capsular antigen can protect some rough strains of *E. coli* from serum bactericidal activity, whereas K27 and perhaps other K antigens fail to provide such a protective effect.

Although there is increasing evidence that K1 acidic polysaccharide capsular antigen is associated with the pathogenicity of *Escherichia coli* (6, 8, 11, 12, 25; A. S. Cross, J. C. Sadoff, and P. Gemski, Clin. Res. 27:342a, 1979), the precise mechanism(s) by which this antigen contributes to virulence remains unknown. Recent studies in our laboratory have demonstrated that, in some bacteremic *E. coli* isolates of the rough phenotype (3), the K1 antigen can confer resistance to the bactericidal effect of normal human serum. Resistance to the bactericidal effect of normal human serum appears to be an important factor in pathogenesis. Serum-resistant isolates are felt to have a selective advantage over serum-sensitive strains, since survival in serum enhances the capability of these isolates to disseminate within the human host. Previous studies have revealed that multiple components of the cell wall complex participate in affording protection from serum bactericidal activity. Such elements include production of a complete lipopolysaccharide layer (2, 19), variations in the amount of acidic exopolysaccharide capsular antigen (4, 5), and expression of some outer membrane proteins (21). In addition, the presence of certain plasmids in *E. coli* have also been associated with serum resistance (1, 10, 20). These plasmids may code for the synthesis of outer membrane proteins which are associated with resistance to the bactericidal effects of serum. One such protein, determined by the *traT* surface exclusion gene of plasmid R5-6, has recently been implicated as contributing to serum resistance (7).

Even though the basis for serum resistance in *E. coli* is complex and multifactorial, we have demonstrated that rough, serum-resistant, K1-positive strains were rendered extremely sensitive to serum killing as a consequence of single-step mutations resulting in K1-negative derivatives (3). Taylor and Robinson (22), however, have provided evidence which suggests that the K antigens supply little, if any, protection against serum bactericidal activity. They constructed hybrids between a serum-sensitive, rough, unencapsulated *E. coli* recipient and a K27-positive Hfr donor *E. coli* strain which contained the genes for K27 antigen. Recombinants which expressed K27 capsule remained serum sensitive, suggesting that K antigens are not important determinants of serum resistance. As a consequence of these apparently conflicting observations, we undertook the present study on the relative roles of K1 and K27 antigens in conferring resistance to the bactericidal effects of normal human serum. Our approach was to prepare hybrids in a rough strain of *E. coli* which express both the K27 and K1 determinants and then to examine the serum sensitivity of derivatives that have lost either or both of these K antigens.

MATERIALS AND METHODS

Bacterial strains. The pertinent characteristics of the *E. coli* strains employed in this study are summarized in Table 1. *E. coli* E412, used as a recipient in genetic crosses, has been previously characterized as a rough K1-positive strain isolated from a case of adult bacteremia (3). *E. coli* Hfr donor strain F639 (Fig. 1),

TABLE 1. *Escherichia coli* strains

Strain	Characteristics												
	Serotype	Phenotype[a]			Phage sensitivity								Agglutination K27 antiserum
					K1 specific					R specific			
		His	Trp	Str	A	B	C	D	E	BR60	Ffm	BR2	
F639 (Hfr donor)	Rough K27	+	+	s	−	−	−	−	−	NT[b]	NT	NT	+[c]
E412	Rough K1	+	+	s	+	+	+	+	+	+	+	+	−
E412 aux recipient	Rough K1	−	−	r	+	+	+	+	+	+	+	+	−
E412 × K27i (hybrid)	Rough K1; K27 (intermediate)	+	−	r	+	+	+	+	+	+	+	+	+[d]
E412 × K27c (hybrid)	Rough K1; K27 (complete)	+	+	r	+	+	+	+	+	+	+	+	+[c]
E412 × K27cAR (hybrid)	Rough K27	+	+	r	−	−	−	−	−	+	+	+	+[c]

[a] Abbreviations: His, histidine; Trp, tryptophan, Str, streptomycin; r, resistant; s, sensitive.
[b] NT, Not tested.
[c] Rapid; large clumps.
[d] Slow; very fine-grained clumps.

originally described by Schmidt et al. (15), was employed to transfer the genes for K27 antigen to recipient *E. coli* derivatives.

Media. Trypticase soy broth (BBL Microbiology Systems), Penassay broth (Difco Laboratories), and Trypticase soy agar (BBL) were used for the routine cultivation of these organisms. The composition of minimal medium used for selection and scoring recombinants has been described previously (16). When required, amino acids and other growth factors were added to the minimal medium at a final concentration of 25 μg/ml. Streptomycin, employed as a counterselective agent against donor cells in some of the matings, was incorporated into selective media at a concentration of 100 μg/ml.

Phage sensitivity tests for K1 antigen and rough lipopolysaccharide. The "rough-specific" phages Ffm, BR60, and BR2, previously described by Wilkinson et al. (24), were propagated on *E. coli* E412. The same host was used in the propagation of the K1-specific phages (A, B, C, D, and E) which were originally isolated and characterized by Gross et al. (4). The procedures for propagation and titration of phages have been previously described fully (24).

For phage sensitivity studies, Trypticase soy agar plates were surface inoculated with bacteria by flooding with broth cultures, allowed to dry, and then spotted with drops of phage suspension (about 10^8 PFU/ml). Lytic sensitivity to at least one of the rough-specific phages was taken to represent expression of a rough lipopolysaccharide. The presence of K1 antigen was similarly demonstrated by lytic sensitivity to K1-specific phages A, B, C, D, and E.

Detection of K27 antigen. The presence of K27 antigen was established by slide agglutination tests. Antisera against K27 antigen was generated in rabbits with strain F639 as previously described (13).

Mutagenesis. Auxotrophic mutants of *E. coli* E412 were generated by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Organisms grown overnight at 37°C in 10 ml of Penassay broth were centrifuged and resuspended in 0.5 ml of fresh Penassay broth. A 0.1-ml amount of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine solution (4.0 mg/ml) was then added to the cell suspension. After 20 min of incubation at 37°C, 4.5 ml of additional Penassay broth was added to the cell suspension. Incubation at 37°C was allowed to continue for another 5 h, at which time the bacterial suspensions were diluted and plated on Trypticase soy agar. Isolates which survived this treatment were subsequently analyzed to define their specific auxotropic characteristics.

Mating experiments. The mating procedure used was

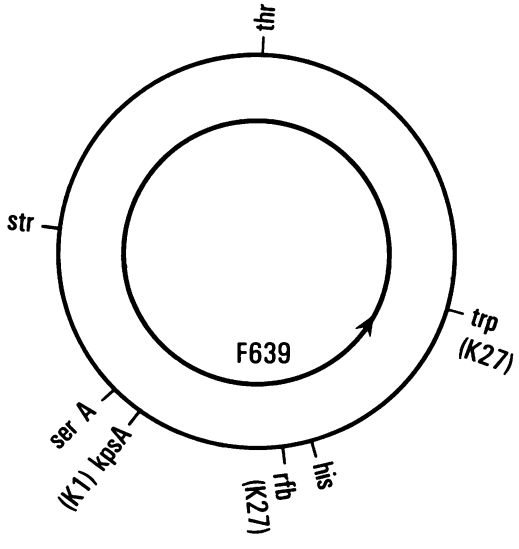

FIG. 1. Chromosome of *E. coli*.

that of Schmidt et al. (14) as previously described. The mating mixture was diluted in buffered saline and spread onto appropriate minimal selection agar with 100 μg of streptomycin to inhibit Str^s donor organisms. After incubation for 2 days at 37°C, isolated recombinants were picked and purified twice on the original selective medium.

Serum bactericidal test. For routine tests, about 10^6 bacteria, grown in Trypticase soy broth at 37°C and removed during log-phase growth, were added to 10 to 20% fresh, pooled human serum from normal volunteers. The serum was diluted in Eagle medium (Microbiological Associates, Walkersville, Md.) in a total volume of 100 μl. The reaction mixtures were incubated for 60 min at 37°C with shaking. Ten-microliter samples were removed at 0, 30, and 60 min, diluted, and plated on Trypticase soy agar for colony count determinations. Serum resistance was defined as less than a 50% reduction of the original bacterial inoculum within 60 min.

RESULTS

Isolation and characterization of *E. coli* hybrids that express both K27 and K1 antigens. As has been shown by previous studies by Schmidt et al. (16), two chromosomal loci are required for synthesis and expression of complete K27 antigen. One locus is closely linked to the *his* operon, whereas the other is near the *trp* genes. These K27 genes are widely separated from the genes controlling K1 antigen which have been positioned by Orskov et al. (9) near *serA* (Fig. 1). Because K1 and K27 genes are not allelic, we reasoned that it would be possible to construct hybrids which contain both of these K antigens. His^- and Trp^- mutations were introduced into a Str^r derivative of strain E412 to yield the recipient strain used in our crosses. This auxotrophic derivative of strain E412 was mated with $K27^+$ donor F639 (Table 1), and recombinants were selected for inheritance of the donor *his* allele. The majority of the his^+ recombinants agglutinated in K27 antiserum. However, two types of agglutination reactions were evident. Hybrids which had inherited only the *his* donor region yielded very fine-grained but unquestionably positive agglutination in K27 antiserum. These hybrids, therefore, represent the K27i form previously described by Schmidt et al. (16) as being an intermediate K27 form. K27i hybrids have been shown to have a reduced expression of K27 antigen owing to the lack of the *trp*-linked locus. One such hybrid, designated E412 × K27i, was chosen for further study.

In addition to the K27i type of hybrid, two of the *his*-selected recombinants appeared to have full expression of K27 antigen. Both had also gained the distal nonselected trp^+ donor gene and yielded rapid agglutination reactions, with large clumps in K27 antiserum. These hybrids had presumably inherited both K27 loci responsible for complete K27 antigen expression. Further characterization of these two recombinants showed that they retained the recipient properties of sensitivity to K1-specific phages and sensitivity to rough-specific phages Ffm, BR60, and Br2. In addition, immunization of rabbits with cells from one of these hybrids, designated E412 × K27c, evoked production of specific antibodies against the K27 antigen. Hybrid strain E412 × K27c was chosen for further studies of serum resistance.

Sensitivity of hybrids to the bactericidal effect of normal human serum. Our previous findings with strain E412 indicated that K1-positive clones were serum resistant, whereas K1-negative derivatives were uniformly sensitive to the bactericidal action of normal serum. To establish whether K27 antigen could also confer such protection on strain E412, we next isolated from hybrid strain E412 × K27c isogenic derivatives which retain K27 antigen but fail to produce K1 capsule. Such a derivative, designated E412 × K27cAR, was readily isolated as a clone resistant to lysis by K1-specific phages (3).

The results of serum sensitivity tests on the parent and hybrid strains are shown in Fig. 2. Donor strain F639 and *E. coli* K-12, used as a

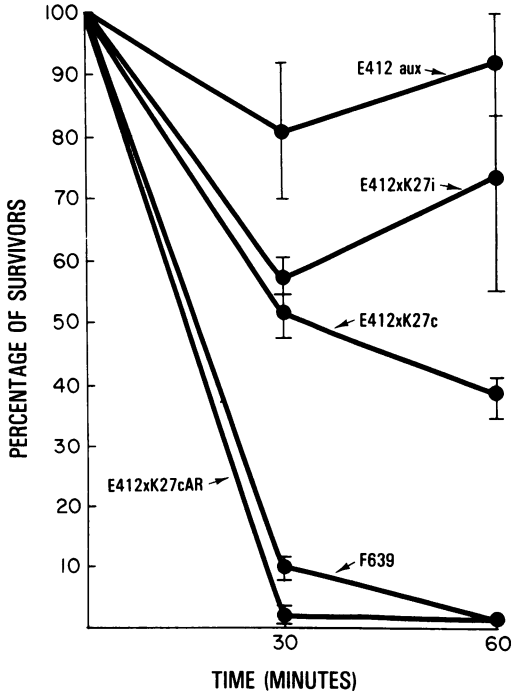

FIG. 2. Bactericidal effect of serum against *E. coli* E412 and its hybrid derivatives. E412 aux, $K1^+$ recipient; E412 × K27i, $K1^+$ and $K27^+$ (intermediate) hybrid; E412 × K27c, $K1^+$ and $K27^+$c (complete) hybrid; E412 × K27cAR, $K27^+$ hybrid which has lost K1;F639, *E. coli* Hfr donor.

control in this experiment, are extremely sensitive to the bactericidal activity of serum, with less than 1% survival at 60 min of incubation. The recipient strain *E. coli* E412 His^- Trp^- Str^r, which expresses the K1 antigen, is resistant to serum bactericidal activity. Hybrids E412 × K27i and E412 × K27c (complete K27), which express antigenic determinants of both K1 and K27 antigens, exhibit an intermediate sensitivity to serum. Yet the recipient strain E412 × 27c-AR, a derivative which had inherited both *his*- and *trp*-linked genes for complete K27 expression but is K1 negative, was found to be markedly susceptible to serum bactericidal activity.

DISCUSSION

Our previous studies of naturally occurring, bacteremic, K1-positive rough *E. coli* isolates indicated that K1 antigen can provide resistance to the bactericidal action of normal human serum. Such strains were not killed in serum bactericidal tests, whereas their isogenic K1-negative derivatives were very sensitive, being killed at a level similar to rough *E. coli* K-12 (3). In the present study, we have extended our observation with one of these strains (E412) and have addressed the question of whether K27 antigen can also confer resistance to serum bactericidal activity. By means of genetic crosses in which an auxotrophic E412 K1-positive rough derivative was mated with a rough, K27-positive *E. coli* Hfr donor, we prepared hybrids which simultaneously expressed both their native K1 antigen and the newly acquired K27 antigen. Both K27 intermediate forms (hybrid E412 × K27i), which produce reduced levels of K27 antigen, and K27 complete forms (hybrid E412 × K27c), which yield wild-type expression of K27 antigen, were recovered from these crosses. With respect to the serological properties, both K27 forms behaved in a manner similar to that described by Schmidt et al. (16).

The results of serum bactericidal tests on these hybrids and other appropriate strains (Fig. 2) indicate that K27 antigen, unlike K1 antigen, fails to provide any significant resistance against the bactericidal effects of normal human serum. Both the donor K27-positive strain (F639) and the K27-positive, K1-negative hybrid (E412 × K27cAR) were rapidly killed by normal human serum (Fig. 2). These findings support the observations of Taylor and Robinson (22), who also showed that K27 antigen had no effect on survival in serum. The response of hybrid E412 × K27c to serum remains unexplained. Unlike the $K1^+$ recipient strain, which was fully resistant, this hybrid, which produces both the K27 and K1 antigen, was found to be partially sensitive to normal human serum. It is conceivable that expression of K27 may interfere with the effectiveness of K1 capsule in providing protection to the bactericidal effects of serum.

It is evident from these as well as other (17, 18, 23) studies that the K1 capsular antigen contributes to serum resistance in some *E. coli* strains of rough phenotype. The K27 antigen, and perhaps other K antigens, does not provide a similar protective effect to *E. coli* upon exposure to human serum. The significance of this effect of the K1 antigen on the pathogenicity of *E. coli* awaits further investigation.

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