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Comparison of the Effects of Different Lipopolysaccharides on the Serum Bactericidal Reactions of Two Strains of *Escherichia coli*

ROSEMARY J. ALLEN AND G. KENNETH SCOTT*

Department of Biochemistry, The University of Auckland, Auckland, New Zealand

The serum killing of *Escherichia coli* ML308 225 and PB94 was inhibited by lipopolysaccharide extracted from either organism, but not by lipopolysaccharide from three pathogenic *Enterobacteriaceae*.

We have recently reported that purified lipopolysaccharide (LPS) from *Escherichia coli* ML308 225 can protect this organism from the complement-dependent bactericidal action of human serum. The process does not affect complement activation and depends upon the binding of LPS to bacteria. Quantitation of LPS binding suggests that it may function by association with the bacterial outer membrane in areas not already covered by LPS (1). Supporting evidence for this view comes from the discovery that the LPS of a serum-resistant mutant of *E. coli* ML308 225 is qualitatively indistinguishable from that of the parent strain, but is present in twice the quantity. The LPS contents of mutant and LPS-treated parent cells are similar (4). It was considered likely that this phenomenon merely reflects the relatively low LPS content of the outer membrane of *E. coli* ML308 strains (1, 6). However, a cell-surface LPS-binding protein has been isolated from *Salmonella minnesota* R595 (2), which suggests that LPS binding to bacteria may be a more general phenomenon. Accordingly, we have repeated our basic experiments with two strains of *E. coli* and with LPS preparations from five different sources.

E. coli ML308 225 was grown as previously described (1). *E. coli* PB94, a prototrophic mutant derived from *E. coli* K-12, was a gift from P. Bergquist of the Department of Cell Biology, University of Auckland, and was grown under the same conditions. LPS from the ML308 225 and PB94 strains was extracted as previously described (1). LPS from *E. coli* O111:34, *E. coli* O55:B5 and *S. minnesota* R595 was purchased from Calbiochem.

For bactericidal assays, the bacteria were harvested by centrifugation in the early exponential growth phase of batch culture (absorbance at 650 nm of 0.15). Sensitivity to serum is high for both strains under these conditions and the reaction is complement-dependent in each case (1, 3). Assay mixtures were made to a final volume of 1.0 ml, using 30 mM tris(hydroxymethyl)-aminomethane-hydrochloride buffer (pH 7.5) containing 0.15 mM calcium chloride and 0.02 mM magnesium sulfate. Approximately 2.5×10^8 bacteria were present in each assay, and up to 0.4 ml (200 μg) of LPS dissolved in assay buffer was included. Serum (0.2 ml) was always added, and assay mixtures were incubated at 37°C for 60 min. Samples were taken at intervals for serial dilution and viable counting, and the results are summarized in Table 1.

Under the assay conditions used, the viable count for *E. coli* ML308 225 incubated with serum was reduced by at least 95% in the absence of LPS. In the presence of a final concentration of 200 μg of LPS from the same strain per ml, the viable count was 97% after a 60-min incubation. Similar results were recorded with *E. coli* ML308 225 and LPS from *E. coli* PB94; the bactericidal reaction was completely inhibited by a final concentration of 100 μg of the PB94 LPS per ml. In contrast, LPS from *E. coli* strains O111:B4 and O55:B5 and from *S. minnesota* R595 did not inhibit the bactericidal reaction even slightly at final concentrations of up to 200 μg/ml.

Bactericidal assays with *E. coli* PB94 were inhibited by LPS from this strain and from *E. coli* ML308 225. Complete inhibition of the bactericidal reaction was caused by 200 μg of the former per ml and 100 μg of the latter per ml. The three other LPS samples did not inhibit the serum killing of *E. coli* PB94 at the highest concentrations.

Protection from the lethal effects of serum complement action by isolated LPS is not confined to *E. coli* ML308 225. A strain derived from *E. coli* K-12 responds to added LPS in the same way, and each strain is protected by the LPS isolated from the other. We have previously shown with *E. coli* ML308 225 that this phenomenon correlates with LPS binding to the bacteria and is not due to an anticomplementary effect of LPS (1). It seems reasonable to postulate that the effect of LPS on *E. coli* K-12 occurs by the

TABLE 1. *Effect of various LPS on serum bactericidal reactions*

E. coli strain	Source of LPS	Concn of LPS (μg/ml)	Viability of test strain (%)
ML308 225	—[a]	—	3
	E. coli ML308 225	200	97
	E. coli PB94	100	104
	E. coli O111:B4	200	3
	E. coli O55:B5	200	0
	S. minnesota R595	200	7
PB94	—	—	1
	E. coli ML308 225	200	100
	E. coli PB94	100	106
	E. coli O111:B4	200	2
	E. coli O55:B5	200	0
	S. minnesota R595	200	0

[a] —, No LPS added.

same mechanism as for *E. coli* ML308 225, although we have not attempted to measure LPS binding to the K-12-derived strain.

The other *E. coli* LPS types used in these experiments were derived from so-called smooth pathogenic strains which are relatively serum resistant, although *S. minnesota* R595 is a rough mutant (7). The fact that they did not protect the test strains from serum killing suggests that they did not bind to these organisms. LPS uptake from vesicles into the outer membrane of *S. typhimurium* has been demonstrated by Jones and Osborn (5). This phenomenon only occurred with LPS-defective mutants and was most efficient when the outer polysaccharide chains were absent. These workers concluded that a steric constraint prevented LPS uptake by wild-type *Salmonella typhimurium*. It is possible that similar steric problems might prevent significant uptake of the large LPS molecules derived from *E. coli* O111:34, *E. coli* O55:B5, and *S. minnesota* R595, although the limited specificity of LPS-binding proteins on the cell surfaces of *E. coli* ML 308 225 and PB94 could also account for these results.

The similarity between *E. coli* K-12 and ML308 strains with respect to protection from complement action by added LPS suggests that it may be possible to isolate a serum-resistant mutant of K-12 with increased LPS concentrations, analogous to the *E. coli* ML308 GLT strain described by Guan and Scott (4). Such a K-12-derived strain would have obvious advantages for the genetic analysis of LPS synthesis and membrane incorporation and of serum resistance.

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LITERATURE CITED

1. **Allen, R. J., and G. K. Scott.** 1980. The effect of purified lipopolysaccharide on the bactericidal reaction of human serum complement. J. Gen. Microbiol. **117**:65–72.
2. **Geyer, R., C. Galanos, O. Westphal, and J. R. Golecki.** 1979. A lipopolysaccharide-binding cell-surface protein from *Salmonella minnesota* R595. Eur. J. Biochem. **98**:27–38.
3. **Goldman, J. N., S. Ruddy, K. F. Austen, and D. S. Feingold.** 1969. The serum bactericidal reaction. III. Antibody and complement requirements for killing a rough *Escherichia coli*. J. Immunol. **102**:1379–1387.
4. **Guan, L. T., and G. K. Scott.** 1980. Analysis of the outer membrane components of *Escherichia coli* ML308 225 and of a serum-resistant mutant. Infect. Immun. **28**: 387–392.
5. **Jones, N. C., and M. J. Osborn.** 1977. Interaction of *Salmonella typhimurium* with phospholipid vesicles. Incorporation of exogenous lipids into intact cells. J. Biol. Chem. **252**:7398–7404.
6. **Kaback, H. R.** 1971. Bacterial membranes. Methods Enzymol. **22**:108–113.
7. **Luderitz, O., O. Westphal, A. M. Staub, and H. Nikaido.** 1971. Isolation and chemical and immunological characterization of bacterial lipopolysaccharides, p. 145–233. *In* G. Weinbaum, S. Kadis, and S. J. Ajl (ed.), Microbial toxins: a comprehensive treatise, vol. 4. Academic Press, Inc., New York.