SHORT COMMUNICATION

Human Serum Complement Requirements for Bacterial Killing and Protoplast Lysis of *Escherichia coli* ML308 225

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Normal human serum kills *Escherichia coli* ML308 225 and lyses protoplasts derived from this organism. Human serum which is depleted of complement component C9 or deficient in component C8 is not bactericidal, but C9-depleted serum will lyse protoplasts whereas C8-deficient serum will not. Bacterial lipopolysaccharide, which can protect bacteria from the serum bactericidal reaction, does not protect protoplasts from lysis by serum.

INTRODUCTION

The mechanism of action of the late-acting serum complement components in erythrocyte lysis is being resolved at the molecular level. In particular, the observation that erythrocyte lysis occurs even in the absence of component C9 (Stolfi, 1968) has recently been confirmed by the work of Müller-Eberhard and his collaborators (Biesecker & Müller-Eberhard, 1980). This phenomenon presents a marked contrast to observations on the bactericidal and bacteriolytic action of human complement on various strains of *Escherichia coli*; component C9 is essential for both bacterial killing and lysis (Goldman *et al.*, 1969; Inoue *et al.*, 1969; Schreiber *et al.*, 1979; Allen & Scott, 1980). It is likely that the apparent discrepancy may be due to the bacterial outer membrane. This structure consists of a matrix of proteins in a partial phospholipid bilayer with the lipid portions of lipopolysaccharide (LPS) molecules integrated into the outer layer so that the polysaccharide chains project into the extracellular environment (Di Rienzo *et al.*, 1978). These polysaccharide chains are particularly important in determining the degree of bacterial sensitivity to complement action. Both qualitative and quantitative changes in LPS composition affect complement sensitivity; the well-known 'smooth to rough' transition is a mutation which shortens the polysaccharide chains and leads to increased sensitivity (Rowley, 1954). Increased incorporation of lipopolysaccharide into the bacterial outer membrane, whether artificial or caused by mutation, reduces complement sensitivity. We have shown that purified LPS from *E. coli* ML308 225 can protect this bacterium from complement action. The protective effect does not involve the inhibition or stimulation of complement activation; it is proportional to the amount of LPS bound to the bacteria. Complete protection correlates with the binding of sufficient LPS to cover the bacterial surface completely (Allen & Scott, 1980). We suggested that LPS binding probably involved the incorporation of the lipid portion of the molecules into the phospholipid bilayer of the outer membrane, as has been claimed for the uptake of LPS from vesicles by *Salmonella typhimurium* (Jones & Osborn, 1977). The suggestion was supported by our observation that the LPS of a serum-resistant mutant of *E. coli* ML308 225 contained twice as much of a qualitatively indistinguishable LPS fraction as the parent strain (Guan & Scott, 1980).

A technique for the production of protoplasts from *E. coli* lacking visible remnants of the outer membrane and cell wall has been developed (Weiss, 1976). Using such protoplasts, we examined the effect of complement on the bacterial cytoplasmic membrane in the absence of the outer membrane. The effect of purified LPS on complement-mediated protoplast lysis was also studied, as a further test of our hypothesis that the protective effect of LPS on whole bacteria depends on its insertion in the outer membrane.

METHODS

The conditions for growth of *E. coli* ML308 225, for the human serum complement bactericidal assay, the preparation of C9-depleted human serum and LPS purification have been described previously (Allen & Scott, 1980). Human C8-deficient serum was obtained through the good offices of Drs R. Ellis-Pegler and R. K. C. Sharpin, and the Auckland Regional Blood Transfusion Centre. This serum failed to give a precipitin line with anti-human C8 serum (Cordis Laboratories, Miami, Fla., U.S.A.) in double gel diffusion tests.

Bacterial protoplasts were made by the method of Weiss (1976). To measure protoplast lysis, protoplasts were suspended in 30 mM-Tris/HCl buffer pH 7.5 containing 0.15 mM-CaCl₂, 0.02 mM-MgSO₄ and 0.6 M-sucrose to give an A_{650} of 1.0, and 0.8 ml portions of this suspension were mixed with 0.2 ml human serum and incubated at 37 °C; the A_{650} values of these mixtures were regularly measured. Control mixtures contained no serum, or 0.2 ml human serum that had been heated to 56 °C for 30 min. The effect of LPS was tested by incorporating up to 0.8 mg LPS ml⁻¹ into 1.0 ml mixtures of protoplasts and normal human serum.

RESULTS AND DISCUSSION

Incubation of 2.5×10^8 bacteria with 0.2 ml normal human serum for 60 min resulted in a reduction in the viable count of at least 95 %. As previously reported (Allen & Scott, 1980), with C9-depleted serum the viable count rose slightly over this period to 108 %. Similar results were obtained with C8-deficient human serum, with a final viable count of 118 %.

Typical results of protoplast lysis experiments are summarized in Fig. 1. Each experiment was performed at least three times, with similar results. It can be seen that both normal and C9-depleted sera promoted a slow but definite lysis of protoplasts. Control protoplasts and those incubated with heated serum or C8-deficient serum were stable for up to 60 min under the conditions used. Protoplast lysis is much slower than the complement-induced lysis of bacteria in hypotonic buffer (Allen & Scott, 1980). A reduced rate of lysis of intact bacteria in isotonic buffer has been reported for *E. coli* 200 P (Feingold *et al.*, 1968) and was attributed to the greater distance between the outer and cytoplasmic membranes caused by plasmolysis. In the present case, the slow rate of lysis may be a direct result of osmotic stabilization of membrane-damaged protoplasts, or it may be due to slower complement activation in the absence of most of the outer membrane components. Intact bacteria stimulate the alternative pathway of complement activation in the absence of added or natural antibodies (Allen & Scott, 1980), presumably due to some component(s) of the outer membrane. Similarly, residual outer membrane components on the protoplast surface could cause direct activation of the alternative pathway or classical pathway activation by interaction with natural antibodies.

Protoplast lysis was neither stimulated nor inhibited by LPS up to a final concentration of 0.8 mg ml⁻¹ (the results with lower concentrations are not shown in Fig. 1). We have previously shown that at these concentrations and with similar numbers of bacteria, LPS completely abolished the bactericidal activity of complement (Allen & Scott, 1980). It would appear that an intact outer membrane is necessary for LPS to exert its protective effect.

It is clear that complement action on the bacterial cytoplasmic membrane is analogous to its action on erythrocyte membranes, in that C8 is essential to both processes whereas C9 is not. This conclusion suggests that C9 may have evolved specifically to promote complement-mediated penetration of the bacterial outer membrane, in which role it is an essential component.

Fig. 1. Protoplast lysis by serum samples. Protoplast suspensions were mixed with serum, and the A_{650} values were measured at intervals and expressed as percentages of the initial values. Normal human serum, O; protoplasts without serum, ●; serum heated to 56 °C for 30 min, □; serum depleted of component C9, ■; C8-deficient serum, △; normal serum and LPS, ▲.

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