Bactericidal, bacteriolytic and opsonic activity of human serum against *Escherichia coli*

MAJA ROZENBERG-ARSKA, J. C. PORSIUS, EMMY Y. JAARSMA and J. VERHOEF

Department of Clinical Bacteriology and Laboratory of Microbiology, University Hospital, University of Utrecht, Catharijnesingel 101, 3511 GV Utrecht, The Netherlands

Summary. The effect of human serum on *Escherichia coli* was studied with serum-sensitive and serum-resistant strains. The bactericidal effect of human serum on serum-sensitive strains of *E. coli* depended on the activation of the classical complement pathway. The role of activation of the alternative pathway was less important. After incubation in sub-bactericidal concentrations of serum these strains were also easily phagocytosed by polymorphonuclear leukocytes (PMNL). Strains of *E. coli* of certain O-types required not only an intact classical pathway but also the presence of specific antibodies for effective killing by serum and effective phagocytosis by PMNL, despite rapid activation of complement and rapid deposition of C3 on the bacterial surface in the absence of antibody. Capsulate strains O1K1 and O78K80 resisted the bactericidal effect of serum even in the presence of specific antibodies; phagocytosis by PMNL only occurred after opsonisation with specific antibodies.

Introduction

The bacteriolytic activity of serum and the phagocytic capacity of leukocytes constitute important host defences against bacterial infections (Glynn and Howard, 1970; Klebanoff, 1975). Some gram-negative bacteria are sensitive to the lytic action of serum, whereas others are resistant (Taylor, 1983). The bactericidal effects of serum are mediated by the activated components of either the classical or the alternative complement pathway (Inoue *et al.*, 1968; Forsgren *et al.*, 1975; Fine, 1981). Activation of either pathway results in generation of the membrane attack complex (C5b-9), which is responsible for irreversible alterations in the structure of bacterial membranes leading to cell death (Goldman *et al.*, 1969; Schreiber *et al.*, 1979). Killing by serum is usually accompanied by bacteriolysis, which depends on adequate amounts of lysozyme (Inoue *et al.*, 1959). Rough strains without O antigens are usually lysed in serum, smooth strains are often resistant (Joiner *et al.*, 1982*a* and *b*) and capsulate strains usually highly resistant (Howard and Glynn, 1971). The exact mechanism of resistance to serum killing of some gram-negative bacteria in the presence of adequate amounts of antibodies is still not well understood. In this study we compared the consumption and fixation of complement by different serum-sensitive and

serum-resistant strains of *E. coli* and the rate of phagocytosis by polymorphonuclear leukocytes (PMNL) after opsonisation.

Materials and methods

Bacterial strains

Five non-capsulate strains—*E. coli* O111 and its uridine 5'-diphosphate(UDP)-galactose 4-epimerase-deficient mutant *E. coli* J5 (Rc), *E. coli* O158 (isolated from a stool culture), *E. coli* K12:PC2166, *E. coli* O1:K⁻:H7 (a spontaneous non-K1 mutant of *E. coli* O1:K1:H7 grown at 22°C)—and two capsulate strains—*E. coli* O78:K80 (isolated from a blood culture) and *E. coli* O1:K1:H7 (isolated from a stool culture and kindly provided by Dr R. Bartolussi, Dalhousie University, Halifax, Nova Scotia) were used. K antigens were determined by the haemagglutination-inhibition technique of Glynn and Howard (1970). The K1 antigen was also detected by countercurrent immuno-electrophoresis (Robbins *et al.*, 1974) with meningococcal group b antiserum (The Wellcome Research Laboratories, Beckenham, Kent).

Radioactive labelling

Strains of *E. coli* grown on a blood agar plate were seeded into 5 ml of Mueller-Hinton Broth (Difco) containing 0.02 mCi thymidine-methyl-³H (5 Ci/mmol, sp.act; The Radiochemical Centre, Amersham, Bucks) and 1.25 mg of deoxyadenosine (BDH Chemicals Ltd, Poole, Dorset). After incubation for 18 h at 37°C, the bacteria were washed thrice with phosphate-buffered

saline (PBS; pH 7.4) and adjusted to a final concentration of 10^9 cfu/ml with a Klett-Summerson photoelectric colorimeter standardised by a pour-plate method (Verhoef *et al.*, 1977).

Sera

Normal human serum (NHS) from 10 healthy donors was pooled and stored in small portions at -70°C . These sera were used as a source of human complement. Serum samples were thawed, only once, shortly before use. Inactivated serum (INHS) was obtained by heating NHS at 56°C for 30 min. In some experiments NHS was chelated with 10 mM ethylene glycol-bis (β -aminoethyl ether)-*N,N*-tetra-acetic acid (EGTA; Sigma Chemical Co., St Louis, MO.) containing 10 mM MgCl_2 (Mg EGTA serum) (Forsgren *et al.*, 1975).

For opsonisation of *E. coli* strain O111 and strain O78:K80 antisera were prepared in rabbits with heat killed *E. coli* strain O111 and formalin-killed *E. coli* strain O78:K80 (to preserve the K antigen) according to a method described by Van Dijk *et al.* (1981). Rabbit serum containing anti-human C3b was obtained from the Central Laboratory for Blood Transfusion (Amsterdam, The Netherlands). After isolation of immunoglobulins by $(\text{NH}_4)_2\text{SO}_4$ precipitation followed by separation on Sephadex G-25 fine (Pharmacia Fine Chemicals, Uppsala, Sweden), these antibodies were radiolabelled with Na^{125}I by using Jodogen (Pierce Chemical Co., Rockford, IL) according to Fraker and Speck (1978). Briefly, 2 mg Jodogen (1,3,4,6-tetra-chloro-3a,6a-diphenyl-glycoluril; Pierce Chemical Co.) was dissolved in 20 ml of chloroform, and 100 μl of suspension was vapourised with dry nitrogen. Thereafter, 100 μl of human anti C3b (10 mg/ml PBS) was added to the thin layer of the Jodogen, followed by addition of 500 μCi Na^{125}I (13–17 mCi/ μg sp.act; The Radiochemical Centre, Amersham). Labelling was performed at room temperature for 15 min and Na^{125}I bound to anti C3b was separated from unbound Na^{125}I on Sephadex G-25 fine (Pharmacia Fine Chemicals) in PBS containing 1% BSA as eluent. Portions of 500 μl of ^{125}I -antihuman C3b containing approximately ^{125}I 10^7 cpm/ μg were kept frozen at -20°C .

Isolation of PMNL

PMNL were isolated as previously described (Verhoef *et al.*, 1977). Briefly, venous blood samples from healthy adult donors were drawn into heparinised syringes and allowed to settle by gravity in dextran (mol. wt 70,000; Pharmacia Fine Chemicals AB, Uppsala, Sweden) 6% in normal saline (blood 10 ml, saline 3 ml). The leukocyte-rich plasma was withdrawn and centrifuged at 160 g for 10 min. The pellet was resuspended in Eagle's Minimal Medium; 16 ml of the cell suspension was carefully layered on to 3 ml of Ficoll-Paque (Pharmacia Fine Chemicals AB) and centrifuged at 160 g for 35 min. Mononuclear cells at the interface were removed. Residual erythrocytes in the pellet were lysed with ice-cold NH_4Cl 0.87% in sterile water. After centrifugation at 160

g for 15 min, PMNL were washed twice in Hanks's Balanced Salts Solution with gelatin (Gel-HBSS). Leukocytes were adjusted to a concentration of 10^7 PMNL/ml of Gel-HBSS (the percentage of monocytes in this suspension was always less than 3%).

Serum bactericidal assay

Bacterial viability after incubation in various concentrations of NHS for various time intervals was compared with bacterial viability after incubation in INHS in HBSS. Briefly, 0.3 ml of different strains of *E. coli* (2×10^8 cfu/ml) were mixed with 0.7 ml of different concentrations of either NHS, MgEGTA-serum or INHS (in HBSS containing BSA 1%). At various times thereafter, 100- μl samples were removed from the mixtures. Portions were serially diluted in HBSS with BSA 1% and plated on Brain Heart Infusion Agar (Difco) plates. After incubation for 18 h at 37°C , the number of colonies was counted and extent of killing was expressed as the percentage of decrease in colony count compared with bacteria incubated in the same dilutions of INHS. Serum resistance was arbitrarily defined as < 20% reduction of the original bacterial inoculum after incubation for 60 min in NHS.

Serum bacteriolytic assay

Serum bacteriolysis was measured by determination of the release of ^3H -thymidine from radiolabelled bacteria. Release of ^3H -thymidine was measured by the method previously described (Rozenberg-Arska *et al.*, 1984). Briefly, 0.3 ml of ^3H -thymidine-labelled strains of *E. coli* (2×10^8 cfu/ml) was mixed either with 0.7 ml of NHS (10%) or with 0.7 ml of MgEGTA-serum (10% and 50%). After incubation for 15, 30, 60 and 90 min, tubes were removed from the water bath and placed on ice, followed by centrifugation at 12 000 g for 10 min. Radioactivity in supernate and sediment was measured in a liquid scintillation counter (Mark II Nuclear, Chicago, IL) after addition of 2.5 ml scintillation fluid (Aqualuma Plus, Lumac Systems Inc., Titusville, FL). The percentage lysis was expressed as the percentage of radioactivity released from labelled bacteria into the supernate. ^3H -thymidine labelled *E. coli* incubated in INHS was used as a control.

Measurement of complement consumption

To 0.1 ml of bacterial suspension containing 5×10^8 cfu/ml, 0.4 ml of NHS or 10 mM MgEGTA-chelated serum (10%) was added and incubated for various time intervals (0, 30, 60 and 90 min). After incubation the total haemolytic complement remaining in the mixture was titrated with optimally sensitised sheep erythrocytes by the method of Mayer (1961). The consumption of complement in each test sample was expressed as a percentage of the haemolytic complement remaining in a control sample of serum incubated with buffer only.

Fixation of C3b to strains of *E. coli*

Binding of C3b to the bacterial surface was measured indirectly with ^{125}I -labelled anti-C3b. Different strains of *E. coli* were grown overnight in Mueller-Hinton Broth at 37°C, centrifuged at 1400 *g* for 15 min and thereafter washed thrice in PBS. Sediments were resuspended in Gel-HBSS and adjusted to a concentration of 2×10^8 cfu/ml with a Klett-Summerson photoelectric colorimeter. Mixtures of serum and bacteria were prepared as described above for the serum bactericidal assay and incubated for various time intervals. After centrifugation at 850 *g* for 15 min at 4°C bacteria were washed in Gel-HBSS. ^{125}I -labelled anti-C3b (sp.act. 10^7 cpm/ μg) prepared in Gel-HBSS was added to the bacterial sediments and incubated further for 30 min at room temperature. Mixtures were centrifuged and sediments were washed thrice with cold HBSS. The amounts of radioactivity associated with the pellets were counted in a PW 4800 automatic counter (Philips, Eindhoven, The Netherlands). Bacteria incubated in INHS were used as a control for nonspecific binding. The amount of C3b bound to bacterial surfaces was calculated as the percentage of maximally bound ^{125}I .

Opsonisation of strains of *E. coli*

Bacteria were opsonised by incubating 10^9 cfu/ml in various concentrations of serum for 30 min at 37°C. Serum was removed by centrifugation (15 min at 1600 *g*) and bacteria were resuspended in Gel-HBSS to a final concentration of 5×10^8 cfu/ml.

Bacterial uptake

Phagocytosis was assayed as previously described (Verhoef *et al.*, 1977). Briefly, 0.3 ml of the suspension of opsonised [^3H]-thymidine-labelled bacteria (5×10^8 cfu/ml) was added to 0.3 ml of PMNL (10^7 cells/ml) in polypropylene vials (Biovials, Beckman Instruments Inc., Fullerton, CA). For each bacterial strain 2 vials were used. Phagocytosis mixtures were incubated in a shaking waterbath at 37°C. After 15 min phagocytosis was stopped by adding 3 ml of ice-cold PBS to each of the vials. To determine leukocyte-associated radioactivity, the first vials containing different bacterial strains were centrifuged (160 *g* for 5 min. at 4°C), and leukocyte pellets were washed thrice with ice-cold PBS to remove non-leukocyte-associated bacteria and were suspended in 2.5 ml of scintillation fluid (Aqualuma Plus). To determine total radioactivity (representing leukocyte-associated plus non-leukocyte-associated bacteria), the remaining vials were centrifuged at 1600 *g* for 15 min and the pellets were resuspended in 2.5 ml of scintillation liquid. Radioactivity was measured in a Mark II liquid scintillation counter (Nuclear Chicago Corp., Des Plaines, IL). Phagocytosis was expressed as the percentage uptake (after 15 min) of total added radioactivity.

Results

Susceptibility to normal human serum and MgEGTA-chelated serum

The susceptibilities of *E. coli* strains PC2166, J5, O111, O158, O78K80, O1K1 and its mutant O1K⁻ were determined by incubating each strain for various times at 37°C in the presence of 10% NHS; bactericidal as well as bacteriolytic effect was studied. More than 96% of *E. coli* strain PC2166 and *E. coli* strain J5 were killed by 10% NHS after incubation for 30 min (table I). There was little killing of *E. coli* strain O111 until it had been incubated for 30 min (<20%); however, after incubation for 60 and 90 min, 52% and 66% of the bacteria, respectively, were killed. In the presence of 10% serum containing specific antibodies to strain O111, 74% of bacteria were killed after incubation

Table I. Killing of non-capsulate and capsulate strains of *E. coli* by normal human serum (NHS) and by MgEGTA-chelated serum

Strain	Incubation time (min)	Percentage killing* in		
		NHS 10%	MgEGTA	
			10%	50%
PC2166	30	>99.9	4	23
	60	>99.9	9	96.1
	90	>99.9	15	>99.9
J5	30	96.7	3	48.7
	60	99	5	74.5
	90	99.8	8	97
O111†	30	18	0	0
	60	52.4	0	0
	90	66.7	0	0
O158	30	59.9	0	0
	60	55.9	0	0
	90	41.9	0	0
O1K	30	83	0	23.4
	60	82	0	44.5
	90	81.5	0	87.1
O1K1	30	5	0	0
	60	7	0	0
	90	7	0	0
O78K80	30	0	0	0
	60	0	0	0
	90	0	0	0

* Percentage reduction of initial viable count; results shown are mean of three independent experiments.

† Incubation in 10% antiserum for 30 and 90 min resulted in 74% and 90% killing respectively.

for 30 min and 85% after 90 min. *E. coli* strain O158 was killed by 10% NHS during incubation for 30 min, but during further incubation bacterial growth was observed. In contrast, both capsulate *E. coli* strains O1K1 and O78K80 were resistant to the bactericidal activity of 10% NHS and appeared to multiply after incubation for 60–90 min. The non-capsulate mutant O1K⁻ appeared to be serum-sensitive and was killed readily after incubation for 30 min.

The addition of 10 mM MgEGTA to 10% NHS, a procedure that renders the classical pathway non-functional, inhibited the bactericidal activity (table I). However, 50% chelated NHS was bactericidal against *E. coli* strains PC2166, J5 and O1K⁻. The rates of killing after incubation for 30 min were much slower than with 10% NHS. After 90 min there was practically no difference between 10% NHS and 50% MgEGTA-chelated serum (table I). *E. coli* strains O111 and O158 as well as both capsulate strains were not affected by chelated serum. Heating of NHS at 56°C for 30 min or treatment with EDTA completely abolished bactericidal activity of serum.

To study the bacteriolytic effect of serum, the

percentage of radioactivity released from radio-labelled bacteria after exposure to 10% NHS or MgEGTA-chelated serum for various times was measured. Incubation of [³H]-thymidine labelled *E. coli* strains PC2166 and J5 in NHS for 90 min resulted in release of 90% of the radioactivity from the bacteria into the medium (fig. 1). After incubation for 90 min, only 35% and 20% radioactivity were released from *E. coli* strains O111 and O158 respectively. It is interesting that *E. coli* O158, which was more rapidly killed in serum than *E. coli* O111, released less radioactivity into the medium. This difference can probably be explained by growth of this strain in serum and it is possible that *E. coli* O158 incorporated thymidine during the growth. Both capsulate strains showed practically no release of radioactivity; however, the non-capsulate mutant O1K⁻ was lysed in 10% serum, resulting in 78% release of [³H]-thymidine into the medium. Incubation of *E. coli* strains PC2166 and J5 in 10% serum chelated with MgEGTA led to much lower release of radioactivity, which did not exceed 30% (fig. 1). The same results were obtained with *E. coli* O1K⁻ (data not shown). Other strains of *E. coli* (O111, O158, O1K1 and O78K80) did not show any substantial release of radioactivity (fig. 1). Heated and EDTA-treated serum caused no release of radioactivity from radiolabelled bacteria.

Fig. 1. Consumption of haemolytic complement by strains of *E. coli* and release of radioactivity from [³H]-thymidine-labelled strains incubated in 10% NHS and 10% MgEGTA-chelated serum for various times. □ represent consumption of complement in NHS; ■ represent consumption of complement in MgEGTA serum; ○—○ represent radioactivity released into medium after incubation in NHS; and ●—● represent radioactivity released into medium after incubation in MgEGTA-treated serum. Results are expressed as percentages.

Consumption of complement

Consumption of C3 was examined in kinetic experiments in 10% NHS and 10% MgEGTA-chelated serum (fig. 1). After 15, 30, 60 and 90 min the total haemolytic complement remaining in the mixtures was determined. All non-capsulate strains consumed complement rapidly and essentially reached an endpoint at 90 min with 85–95% consumption of C3 (fig. 1). Thus, these differences in killing of non-capsulate strains were not a consequence of inefficient complement activation. After incubation for 90 min both capsulate strains consumed only 35–50% of complement (fig. 1). When strains of *E. coli* were incubated in 10% MgEGTA-chelated serum, complement consumption was reduced (fig. 1).

Binding of C3b to strains of *E. coli*

Binding of C3b to the surface of different strains of *E. coli* during the incubation in NHS and MgEGTA-chelated serum was studied. Radio-iodinated anti-human C3b was added to bacteria previously incubated in 10% NHS or 10% MgEGTA-chelated serum for various times and the

Fig. 2. Fixation of complement (C3) by strains of *E. coli* after incubation in 10% NHS (A) and 10% MgEGTA-chelated serum (B). Points represent the mean of three independent experiments.

percentage of bound C3b calculated. With all non-capsulate strains more than 60% of C3b was bound after incubation for 15 min, reaching an endpoint at 60 min with binding of 80–90% (fig. 2A). In contrast incubation of the capsulate *E. coli* strains O1K1 and O78K80 in 10% NHS serum resulted in only 30 and 40% binding after 60 min. Incubation of strains of *E. coli* in MgEGTA-chelated serum led to a slower rate of C3b binding than in normal human serum (fig. 2B).

Opsonisation of strains of *E. coli* in NHS and in MgEGTA-chelated serum

To study the opsonic requirement of strains PC2166, J5, O111, O158, O1K1, O1K⁻ and O78K80 the uptake of these strains by PMNL after incubation for 30 min in different concentrations of NHS and MgEGTA-chelated serum was measured. *E. coli* strains PC2166, J5 and O158 were easily opsonised in 5% NHS showing 90, 90 and 84% uptake respectively (table II). *E. coli* strain O111 differed from other non-capsulate strains as opsonisation in 5% NHS did not lead to phagocytosis (table II), whilst incubation in 5% specific antiserum resulted in 56% uptake. Capsulate strains O1K1 and O78K80 were not efficiently opsonised in NHS; after opsonisation in 20% NHS uptake by PMNL was only 25% and 9% respectively (table II). Opsonisation of *E. coli* strain O78K80 in 20% specific antiserum resulted in 55% phagocytosis by PMNL. To determine if K antigen reduced the ability of *E. coli* to be opsonised in NHS, *E. coli* strain O78K80 was heated for 15 min at 80°C. After the destruction of K antigen *E. coli* O78K⁻ was readily opsonised in 5% NHS and phagocytosed at

Table II. Phagocytosis by PMNL of strains of *E. coli* opsonised in NHS or MgEGTA-chelated serum†

Strains	Percentage phagocytosis* of <i>E. coli</i> opsonised in					
	NHS			MgEGTA		
	2.5%	5%	20%	2.5%	5%	20%
PC2166	80	90	ND	40	65	ND
J5	85	90	ND	45	60	ND
O111‡	0	5	ND	0	0	ND
O158	78	84	ND	5	10	ND
O1K ⁻	85	97	ND	50	72	ND
O78K ⁻	35	78	ND	24	35	ND
O1K1	0	17	25	0	9	15
O78K80§	0	4	9	0	0	4

* Mean of three determinations.

† Preparation consisted of 10^7 PMN/ml and 5×10^8 bacteria/ml previously opsonised in serum for 30 min. Phagocytosis mixtures were incubated for 15 min at 37°C.

‡ Opsonisation in 5% antiserum resulted in 56% phagocytosis.

§ Opsonisation in 20% antiserum resulted in 55% phagocytosis.

a rate of 78%. Also the non-capsulate mutant O1K⁻ was very well opsonised in 5% NHS, which resulted in 97% uptake by PMNL.

Opsonisation in 5% MgEGTA-chelated serum led to 65 and 60% uptake of *E. coli* strains PC2166 and J5. *E. coli* strain O158 showed only 10% uptake when opsonised in 5% MgEGTA serum, but opsonisation in 5% NHS led to 84% uptake; 5% MgEGTA serum did not opsonise *E. coli* O111. Both encapsulated strains showed minimal uptake when opsonised even in 20% MgEGTA serum.

Discussion

Previously we have shown that some strains of *E. coli* lose viability and often become degraded when exposed to NHS (Van Dijk *et al.*, 1981; Rozenberg-Arska *et al.*, 1984). Capsulate strains and also strains of certain O-types appeared resistant to the bactericidal and bacteriolytic action of serum. Complement was required for killing by NHS, since treatment of NHS at 56°C for 30 min abolished all bactericidal activity. Optimal serum bactericidal activity required an intact classical pathway of complement and the presence of antibodies. However, 50% MgEGTA-chelated serum also lysed and killed *E. coli* strains PC2166 and J5 and the non-capsulate mutant O1K⁻. This indicates that certain

strains of *E. coli* can also be killed by the alternative pathway of complement activation. However, the rate of killing in 50% MgEGTA serum was very slow despite the fact that these gram-negative rods were able to activate the alternative complement pathway rapidly. In contrast, activation of the classical pathway of complement by these strains correlated very well with killing and fixation of C3b by the bacterial surface. Similar results were obtained by Roberts and Phillips (1981, 1983) who showed that strains of *E. coli* of the common urinary O-serogroups differ widely in their sensitivity to NHS.

It is possible that this slow rate of killing or lysis in MgEGTA serum is due to some remaining activity of the classical pathway. Strains of certain O-types such as *E. coli* O111 and *E. coli* O158 were killed very slowly when incubated in NHS, and this effect was completely abolished by MgEGTA-chelated serum. These strains required an intact classical pathway and the presence of antibodies for killing by serum. The presence of specific antibodies directed against O antigens enhanced the bactericidal effect of NHS. It has been suggested that long O-antigenic polysaccharide chains associated with lipopolysaccharides cause antibody-mediated activation of complement too far from the target site (Rowley, 1973). There were no essential differences in complement consumption and complement fixation between serum-sensitive strains of *E. coli* killed quickly and those killed only slowly. Joiner *et al.* (1982a and b) showed that serum-sensitive and serum-resistant strains of *Salmonella minnesota* consumed complement during incubation in serum and deposition of membrane-attack complex on the surfaces of both strains was observed. However, serum-resistant *S. minnesota* was not killed by serum despite rapid complement consumption and rapid deposition of membrane-attack complex on the bacterial surface. The deposition of C5b-9 on the cell surface was not stable; it was released from the bacterial surface without causing lethal outer membrane damage (Joiner *et al.*, 1982a and b). It was also shown by these authors that addition of bactericidal antibodies did not change the rate of complement deposition on the cell surface but increased the bactericidal efficiency of complement factors (e.g., with *E. coli* O111). Thus, bactericidal

antibodies were not needed for complement consumption and fixation, but they were necessary for efficient killing of certain O types of *E. coli*, perhaps forming IgG-C3b complexes (Joiner *et al.*, 1983a and b). It is also possible that outer-membrane proteins are involved. Our results showed that both capsulate strains O1K1 and O78K80 resisted the bactericidal effect of serum as well as phagocytosis by PMNL in the absence of specific antibodies. This effect was attributed to the presence of a capsule, which blocked complement consumption and binding of C3b by masking surface components such as lipopolysaccharides capable of activating the complement pathway. To assess the role of the capsule, a spontaneous mutant without K1 capsular antigen and *E. coli* O78K80 after heat destruction of K antigen were studied. Both strains were able to activate complement and to bind C3 molecules (although, we cannot exclude the possibility that heating of *E. coli* O78K80 affects also other cell-envelope components). The non-capsulate O1K⁻ mutant was killed and lysed in NHS as well as in MgEGTA-chelated serum and both strains were readily phagocytosed. It was shown by Howard and Glynn (1971) that the state of capsulation of the bacteria and the kind of capsular antigens are important determinants of their capacity to bind complement. The amount of capsular material may vary in different strains and may also vary with different culture conditions. In general, when non-capsulate strains of *E. coli* encounter serum, their lipopolysaccharide-rich outer-membrane promotes complement fixation to the bacterial surface. Consequently the bacteria are either lysed by serum or ingested and removed by PMNL. When some non-capsulate strains of *E. coli* of certain O-types and capsulate strains encounter serum, complement is not fixed to the bacterial surface in the absence of specific antibacterial antibodies. Consequently, these strains resist lysis by complement and resist phagocytosis; this may account for enhanced pathogenicity of those bacteria. When specific antibodies are available, complement is bound and the resistant bacteria are either lysed or phagocytosed.

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