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Opsonization of various capsular (K) *E. Coli* by the alternative complement pathway

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Summary. A virulence factor of *E. coli* K-1 is its capacity to avoid opsonization via the alternative complement pathway (ACP). Since it is not known if *E. coli* with other capsular (K) antigens have similar properties we examined various capsular *E. coli* for opsonization by the ACP. To assess opsonization we used whole blood luminol-dependent chemiluminescence (CL) and the magnesium salt of ethyleneglycol tetraacetic acid to block the classical pathway. *E. coli* K-types 6, 7, 27, 30, 42, 53, 57 and 75 were effectively opsonized via the ACP (>65% of CL obtained with unchelated normal serum). K types 2 and 13 were opsonized by the ACP in both the high range >65% and intermediate range 36–65%. Only K-types 1, 3, 5, 12 and 92 were poorly opsonized (<35%) by the ACP. The data demonstrate that most *E. coli* K-types were opsonized via the ACP. The poor opsonization of *E. coli* K3, 5, 12 and 92 by the ACP may be a virulence factor for these bacteria.

INTRODUCTION

Blood culture isolates of *E. coli* K-1 are not opsonized via the alternative complement pathway (ACP) but are restricted to opsonization via the classical pathway (Stevens *et al.*, 1978a). Since the ACP serves as a natural non-immune defence system, poor opsonization of bacteria via this pathway may be a potential virulence factor (Fine, 1975; Stevens *et al.*, 1978a). Because *E. coli* antigens may serve as virulence factors and since there is no information about the complement requirements of other specific K-types we initiated this study to assess the complement pathway involved in the opsonization of 16 different K-types of *E. coli*.

To determine the specific complement pathway for opsonization, we pre-opsonized the *E. coli* with normal pooled human serum and serum chelated with the magnesium salt of ethyleneglycol tetraacetic acid (MgEGTA), which blocks the classical complement pathway (Fine *et al.*, 1972). We assessed opsonization using the modified luminol-amplified chemiluminescence (CL) technique with whole blood instead of isolated granulocytes. Whole blood CL was used because of its sensitivity, relative simplicity and demonstrated utility in measuring the opsonophagocytic CL response of human blood phagocytes (Allen, 1977; Cohen, Shirley & DeChatelet, 1983; DeChatelet & Shirley, 1981; Faden & Maciejewski, 1981; Selvaraj *et al.*, 1982; Stevens & Young, 1977) and specific opsonins (Anderson, Edwards & Baker, 1980; Welch & Stevens, 1979).

This is the first report to demonstrate that the presence of a capsular antigen does not preclude an *E. coli* isolate from being opsonized by the ACP. Additionally, only K-types 1, 3, 5, 12 and 92 were poorly opsonized by the ACP.

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MATERIALS AND METHODS

Preparation of E. coli *isolates*

The *E. coli* were blood culture isolates identified by standard laboratory criteria (Edwards & Ewing, 1972) and obtained from individual patients at the UCLA Center for the Health Sciences, identified by an isolate number and from isolates provided by the Centers for Disease Control (CDC), Atlanta, GA. In some cases only single isolates of a particular K-type were available for testing. The UCLA isolates were serotyped by Dr Bertil Kaijser, Institute of Medical Microbiology, University of Goteborg, Sweden. For testing, all isolates were subcultured into brain heart infusion broth for $2\frac{1}{2}$ hr at 37° to log phase, washed twice in normal saline and adjusted to a concentration of $1{\cdot}5 \times 10^9$ colony forming units (CFU)/ml.

Preparation of blood and serum

Heparinized venous blood was obtained from normal, healthy volunteers at the UCLA Blood Donor Center. The total leucocyte concentration was determined using a Coulter counter model ZBZ. Differential counts were done on blood smears stained with Wright's stain. For use in CL a 10-fold dilution of the blood in Hanks's balanced salt buffer (HBSS; Stevens & Young, 1977) provided suitable concentration of polymorphonuclear cells (PMN). Fresh pooled normal human serum was obtained from eight normal laboratory personnel and stored at −80°.

Preopsonization of bacterial isolates

E. coli isolates or zymosan were pre-opsonized for 10 min at 37° by incubation of 0·7 ml of either $1{\cdot}5 \times 10^7$ CFU/ml or $1{\cdot}5 \times 10^7$ zymosan particles/ml (10 mg zymosan approximates $5{\cdot}2 \times 10^8$ particles/ml; Rosen & Klebanoff, 1976) in 0·3 ml of either pooled human serum, ethylenediamine tetraacetic acid (EDTA)-chelated serum at a final concentration of EDTA 80 mM or MgEGTA-chelated serum at a final concentration of 10 mM MgEGTA. After pre-opsonization the bacteria or zymosan were washed once and reconstituted to 1 ml with a calcium free HBSS.

Whole blood luminol-chemiluminescence

Chemiluminescence was measured at ambient room temperature in polypropylene vials using a Beckman LS230 liquid scintillation spectrophotometer in the out-of-coincidence mode. For measurement of resting values of heparinized whole blood, vials containing a mixture of 0·9 ml calcium free HBSS, 75 μl of heparinized blood diluted 10-fold in HBSS and 0·1 ml of 2×10^{-5} M luminol (Stevens, Winston & Van Dyke, 1978b) were counted for two consecutive cycles. Phagocytosis and CL were initiated by addition of 1 ml of pre-opsonized *E. coli* or zymosan (1×10^7 CFU or particles/ml) to yield a bacteria or zymosan to PMN ratio of approximately 200:1. All assays were run in duplicate. The vials were hand-shaken and counted for 0·1 min with repetitive counting at various intervals for a minimum of 1 hr. Controls included diluted whole blood alone, diluted whole blood plus unopsonized bacteria, or pre-opsonized bacteria or pre-opsonized zymosan alone.

Calculations

The chemiluminescence data was calculated and expressed as integral, i.e. the total amount of CL elicited within the first 60 min. Because the amount of chemiluminescence can vary dependent on the particular isolate and source of PMN, we determined the percent opsonization of each isolate via the ACP by dividing the mean integral value of duplicate assays obtained when using MgEGTA-chelated serum as the opsonin by the mean integral of duplicate assays obtained using normal serum for opsonization.

RESULTS

Kinetics of chemiluminescence

Figure 1 illustrates the kinetics of the luminol-dependent CL of PMN undergoing phagocytosis of three different K-types of *E. coli* after opsonization with either normal serum, MgEGTA-chelated serum or EDTA-chelated serum. *E. coli* 021:K2 represents an isolate that was a good activator of the ACP and effectively opsonized by that pathway, since the PMN-CL when using MgEGTA serum was 83% of that when normal serum was used as an opsonin. *E. coli* 06:K13 was opsonized at an intermediate rate with an approximate 45% PMN-CL response when using MgEGTA-serum as an opsonin. *E. coli* 01:K1 illustrates a poor activator of the ACP and an isolate that evaded opsonization by the ACP since the integral was only 11% of normal when MgEGTA serum was used as an opsonin.

Percent opsonization of various *E. coli* types of the ACP

For all isolates and zymosan there was no PMN-CL when using EDTA-chelated serum. When zymosan

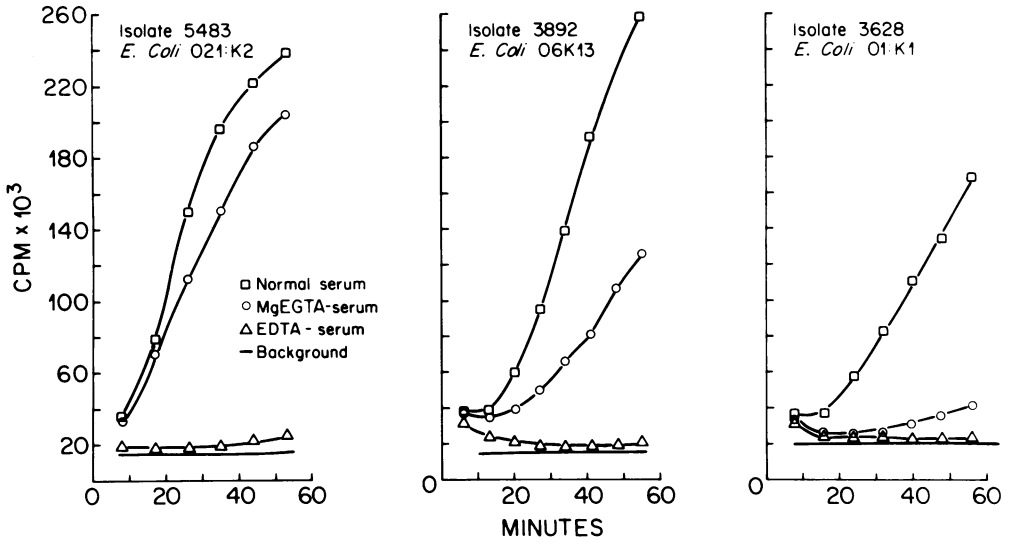

Figure 1. Opsonization of various capsular (K) *E. coli* as measured by whole blood luminol-dependent chemiluminescence of PMN undergoing phagocytosis. The whole blood was obtained from a single donor. CFU ($1{\cdot}05 \times 10^7$) of *E. coli* isolates 5483(O21:K2), 3892(O6:K13) and 3628 (O1:K1) were preincubated in pooled human serum as described in 'Materials and Methods' in the presence or absence of the chelators MgEGTA or EDTA at a final concentration of 10 mM or 80 mM respectively.

was pre-opsonized with MgEGTA-serum CL was essentially 100% of the response when using normal serum. In the absence of serum there was no isolate that interacted with the PMN to elicit CL.

Table 1 summarizes the *E. coli* types that were opsonized by the ACP greater than 60% and considered to be strong activators of the ACP. These included various isolates of K2 and K13 and single isolates of the K-types 6, 7, 27, 30, 42, 53, 57, and 75.

Table 2 lists those *E. coli* types that were opsonized by the ACP in an intermediate range of 30 to 60% and included various isolates of K2 and K13 and individual isolates of K52 and 53. All the isolates of *E. coli* with K2 and K13 were opsonized by the ACP at a high or intermediate rate.

Table 3 illustrates those *E. coli* types that were

Table 1. Percent (%) opsonization of various *E. coli* O and K-types by the alternative complement pathway as assessed by the integral of luminol-dependent chemiluminescence

UCLA No. or CDC isolate	O and K-type	Mean %±SD
5483	O21:K2	83·9±5·4
5436	O25:K2	78·4±1·3
5428	O6:K2	73·8±2·1
5830	O2:K2	70·2±1·8
4795	O11:K2	66·3±1·6
CDC	O4:K6	63·4±1·0
CDC	O7:K7	83·8±3·8
4625	O6:K13	63·3±1·7
5362	O22:K13	61·5±2·2
CDC	O8:K27	100·0±2·4
CDC	O9:K30	73·5±4·6
CDC	O8:K42	100·0±15·0
4953	O8:K53	63·4±2·3
CDC	O9:K57	66·4±1·3
CDC	O113:K75	67·2±1·5

Table 2. Percent (%) opsonization of various *E. coli* O and K-types by the alternative complement pathway as assessed by the integral of luminol-dependent chemiluminescence

UCLA No. or CDC isolate	O and K-type	Mean %±SD
4801	O6:K2	44·9±1·2
3096	O6:K2	36·2±2·4
4480	O6:K13	56·5±1·5
5108	O2:K13	47·7±1·7
3892	O6:K13	44·9±1·6
CDC	O4:K52	51·3±2·8
5206	O4:K53	56·6±1·8

Table 3. Percent (%) opsonization of various *E. coli* O and K-types by the alternative complement pathway as assessed by the integral of luminol-dependent chemiluminescence

UCLA No. or CDC isolate	O and K-type	Mean %±SD
3628	O1:K1	9·2±0·6
CDC	O7:K1	27·2±0·8
4092	O4:K3	20·7±1·0
CDC	O4:K3	29·6±2·9
4522	O75:K5	7·9±0·7
CDC	ON:K5	9·0±0·2
5116	O4:K12	24·7±1·0
CDC	O73:K92	8·5±0·6

poorly opsonized via the ACP (<30%) and included the K-types 1, 3, 5, 12 and 92.

In order to correlate and associate a particular K-type with its possible role in preventing opsonization by the ACP we compared the opsonization of isolates with similar O types, O7 and O4, but with different K-types. Table 4 summarizes this comparison. The presence of either K1 with O7 or K3 or K12 with O4 was associated with a significant reduction in opsonization of these isolates by the ACP as compared to isolates of O7 with the K7-type and isolates of O4 with the K-types 52 or 53 ($P<0·01$ two-sample two-tailed t test).

DISCUSSION

Initial studies using laboratory isolates of *E. coli* demonstrated that these bacteria activate (Fine *et al.*, 1972) and are opsonized (Forsgren & Quie, 1974; Jasin, 1972) by the ACP. However, recent reports using clinical isolates of encapsulated *E. coli* K-1 or unidentified non-K-1 encapsulated isolates demonstrated that *E. coli* can evade opsonization by the ACP (Stevens *et al.*, 1978a; Van Dijk, 1979). Based on these reports, it appeared that the presence of a capsule would provide *E. coli* with a mechanism to evade activation of the ACP. Surprisingly, 11 of 16 encapsulated blood culture isolates of *E. coli* were efficiently opsonized via the ACP demonstrating for the first time that the presence of a specific capsular polysaccharide does not necessarily prevent opsonization of *E. coli* by the ACP. While this was the first demonstration that blood isolates of encapsulated *E. coli* could be opsonized by the ACP and were heterogenous in their opsonic complement requirements, this heterogeneity among encapsulated bacteria within the same species is not without precedent. Previous studies have shown that different serotypes of *Streptococcus pneumoniae* can activate (Fine, 1975; Stevens, William & Reed, 1977) or be opsonized (Giebink *et al.*, 1977) by either the ACP or classical pathway of complement. Why certain K-types of *E. coli* were opsonized via the ACP is presently unknown. Similar to that reported for *S. pneumoniae* there may be surface directed antibody that mediates ACP activation (Fine, 1975) or perhaps certain polysaccharide capsular antigen may directly activate the ACP (Giebink *et al.*, 1978). In addition, the amount of capsular surface antigen on certain isolates may not be sufficient to mask or sterically hinder inner lipopolysaccharide from activation of the ACP. The specific manner by which certain encapsulated types of *E. coli* are opsonized via the ACP awaits further investigation. Because K-types 2 and 13 are frequently associated with urinary tract infection (Hanson *et al.*, 1977; Kaijser *et al.*, 1977) and were shown to be moderately opsonized via the ACP it would appear there are other contributing factors involved in their virulence.

Table 4. Percent (%) opsonization of *E. coli* isolates of O groups 7 and 4 by the alternative complement pathway as assessed by the integral of luminol-dependent chemiluminescence

UCLA No. or CDC isolate	O and K-type	Mean %±SD
CDC	O7:K1	27·2±0·8
CDC	O7:K7	83·8±3·8
4029	O4:K3	20·7±1·0
CDC	O4:K3	29·6±2·9
5116	O4:K12	24·7±1·0
CDC	O4:K6	63·9±1·0
5206	O4:K53	56·6±1·8
CDC	O4:K52	51·3±2·2

Confirming our previous study (Stevens *et al.*, 1978a) in which we used a phagocytic and killing assay to monitor the opsonization of *E. coli* K-1 by the ACP we found little opsonization of *E. coli* K-1 by the ACP as measured by whole blood luminol-CL. This finding substantiates the validity of this CL method to assess accurately the opsonic activity of specific complement pathways. The described whole blood CL technique eliminated the necessity of cell separation and provided an efficient and relatively simple assay to assess opsonization.

In addition to K-1, *E. coli* K-types 3, 5, 12, and 92 evaded opsonization by the ACP. Why these types were poorly opsonized by the ACP is presently not known and is under study. The reason why *E. coli* K-1 and 92 evaded opsonization by the ACP may be related to their sialic acid surface. Both K-1 and 92 are sialic acids being homopolysaccharides of *N*-acetylneuraminic acid (Glode *et al.*, 1977) differing only by their glycosidic linkage. K-1 is α 2, 8 (McGuire & Binkley, 1964) and K-92 α 2, 8 and 2, 9 linked (Glode *et al.*, 1977). Because the amounts of surface sialic acid on sheep erythrocytes can modulate the function of the ACP by increasing the affinity of cell bound C3b for β-1-H but not for B and thereby inhibit the activation of the ACP (Fearon, 1978, Kazatchkine *et al.*, 1979), it is possible the sialic acid surface of *E. coli* K-1 and 92 similarly prevented activation of the ACP.

Since *E. coli* K3, 5 and 12 are frequently found in chronic urinary tract infection (Hanson *et al.*, 1977; Kaijser *et al.*, 1977) and because some surface K antigens are poor immunogens (Jann & Westphal, 1975; Kaijser & Olling, 1973) evasion of opsonization by the ACP may play a role in their virulence.

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