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ANTIPHAGOCYTIC ACTIVITY OF THE CELL WALL POLYSACCHARIDE OF *ESCHERICHIA COLI*

P. DRI, G. D. ROTTINI, P. BELLAVITE and P. PATRIARCA

Istituto di Patologia Generale and Istituto di Microbiologia, University of Trieste, Trieste (Italy)

The hypothesis that a specific relationship exists in *Escherichia coli* among "O" antigenicity, virulence and capacity to resist phagocytosis has been supported by the studies of Medearis, et al. (3). They have shown that mutants of *E. coli* that are deficient in synthesis of a complete lipopolysaccharide are more susceptible to phagocytosis and less virulent than the wild strain.

A role in determining the virulence of *E. coli* has been also attributed to the surface polysaccharide of this microorganism, which bears the "K" antigenicity. In fact, Glynn (1) and Howard (2) have shown that the ability of *E. coli* crude extracts, which contain the polysaccharide, to inhibit agglutination of sheep erythrocytes by rabbit antibody (taken as a measure of the polysaccharide content) is in general directly proportional to the rate of clearance of the various strains from the mouse blood stream and to resistance to complement-mediated lysis.

We observed a similar relationship between the agglutination inhibition titer of crude extracts obtained from several *E. coli* strains and the susceptibility of the same strains to phagocytic killing by polymorphonuclear leukocytes (PMN) *in vitro* (6).

We report here the effects of partially purified polysaccharide of *E. coli* on the interaction between different strains of *E. coli* and PMN or macrophages *in vitro*.

MATERIALS AND METHODS

Both PMN and macrophages were obtained from guinea pig peritoneal exudates (5-6). The polysaccharide preparations were made from 3 strains: E. coli J53 (susceptible to phagocytosis), E. coli 044:K74 and E. coli 0111:K58 (resistant to phagocytosis). The last 2 strains differed in that a crude extract (containing both "K" antigen and "O" antigen) of E. coli 0111:K58 gave the same electrophoretic pattern when reacted with anti-O or anti-OK serum (K- strain), whereas the pattern obtained with a crude extract of E. coli 044:K74 was different with the 2 antisera (K+ strains). This indicated that the "K" antigen, at least when extracted from the bacterial body, was immunologically identical to the "O" antigen in E. coli 0111:K58 but different to that in E. coli 044:K74 (4).

The polysaccharide-containing extracts used in these studies were virtually free of protein and nucleic acid and contained from 15-20% of the hexoses in the form of lipopolysaccharide, as can be calculated from the data reported in Table I and from the equation shown in the footnote of the same table.

The conditions for determination of the effect of E. coli polysaccharides on the phagocytosis-induced respiratory burst in guinea pig PMN were as follows: The ratio of cells to bacteria was 1:40. The assays were done in the presence of 2mM KCN in order to inhibit oxygen consumption by live bacteria. The cells were preincubated in the electrode chamber with the polysacchardie (expressed as glucose equivalents as determined by the anthrone reaction). Heat treatment of E. coli was done at 100 C and suspensions of heat-killed organisms were then washed twice in KRP.

Adsorption of E. coli 044:K74 polysaccharide on live or heat-killed E. coli strains was carried out as follows: Aliquots of 1 ml of the polysaccharide-containing extract were incubated with 1 ml suspension (5×10^9 bacteria/ml) of each of the strains tested for 4 min at 37 C. After centrifugation, the supernatants were collected and two-fold dilutions from 1:8 to 1:1,024 were made of each of them and 0.5 ml of each dilution was incubated with 0.5 ml of a 5% sheep erythrocyte suspension for 30 min at 37 C. After 3 washings, the erythrocytes were tested for passive hemagglutination in a microtiter V-shaped plate (Cook Instruments, London, England) with homologous anti-OK serum.

RESULTS

As shown in Table I, crude extracts of both E. coli 0111:K58 and 044:K74 reacted with both anti-OK and anti-O homologous sera. The final extract of E. coli 0111:K58 still reacted with both antisera at the same titer, whereas in the final extract of E. coli 044:K74, the type O reactivity was much more reduced than the type K reactivity. This fact was due to the immunological identity between polysaccharide (K antigen) and lipopolysaccharide (O antigen) in E. coli 0111:K58, but not in E. coli 044:K74, thus indicating the final extract of E. coli 044:K74 had been selectively freed of the lipopolysaccharide (O antigen).

Fig. 1 shows the differential susceptibility of the 3 strains of E. coli used in this study to the phagocytic killing by PMN.

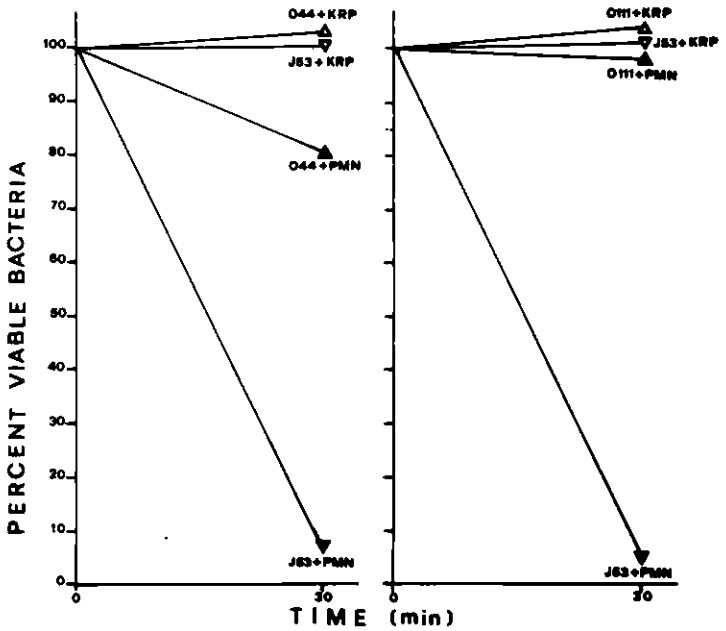

Fig. 1. Comparative susceptibility to phagocytic killing by guinea pig polymorphonuclear leukocytes (PMN) of E. coli J53, 0111:K58 and 044:K74. The bactericidal activity was assayed as described in a previous paper (6).

TABLE I

Hexose and KDO Content and Antigenic Properties of the Extracts of *E. coli* 044:K74 and 0111:K58 at Various Steps of Purification

Purification Step	044:K74 Extract				0111:K58 Extract			
	Hexose* (μg/ml)	KDO** (μ moles/ml)	P.H.T.*** anti-OK	anti-O	Hexose* (μg/ml)	KDO** (μ moles/ml)	P.H.T.*** anti-OK	anti-O
1. Crude Extract	454	1.181	1:256	1:32	346	0.577	1:256	1:256
2. After Centrifugation at 104,000 g for 4 hr	355	-	-	-	252	-	-	-
3. After RNAase Treatment and Phenol Extraction (Final Extract)	260	0.189	1:128	1:8	231	0.189	1:128	1:128

*Glucose equivalents as determined by the anthrone reaction.

**3-Deoxy D-Mannooctulosonic Acid.

***Passive hemagglutination titer with homologous anti-OK and anti-O sera.

$$\text{Free Hexose in final extract} = \text{Total Hexose in 3} - \left(\frac{\text{Hexose in 1} - \text{Hexose in 3}}{\text{KDO in 1} - \text{KDO in 3}} \times \text{KDO in 3}\right)$$

When the strains resistant to phagocytosis were freed of their polysaccharide by heat treatment at 100 C for 60 min, they became highly susceptible to phagocytosis. However, when the extracted polysaccharide was returned to the mixture of heat-treated bacteria and phagocytes, phagocytosis of heat-treated bacteria was almost completely inhibited. This is shown in Fig. 2 for E. coli 0111:K58. Similar results were obtained for E. coli 044:K74. Fig. 2 shows, in addition, that the polysaccharide extracted from E. coli 0111:K58 also inhibited phagocytosis of heat-treated J53. However, the polysaccharide was much less effective in inhibiting phagocytosis of live J53 (Fig. 3). The polysaccharide extracted from J53 did not inhibit phagocytosis of the 3 strains tested.

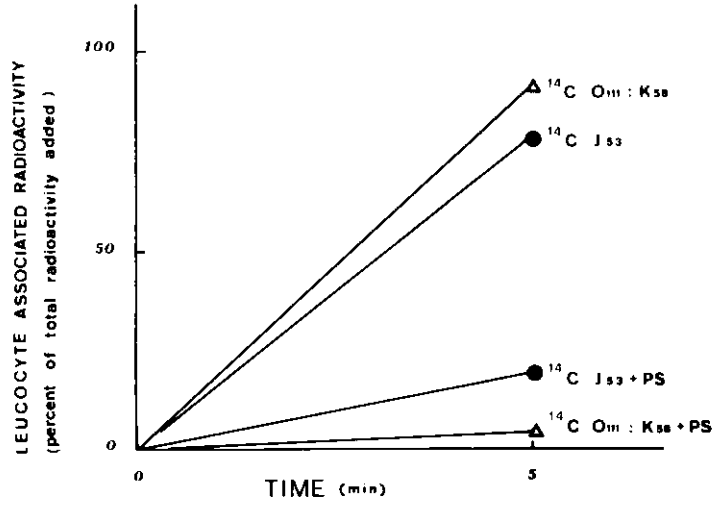

Fig. 2. Effect of the polysaccharide extracted from E. coli 0111:K58 on the phagocytosis of heat killed E. coli 0111:K58 and E. coli J53 labelled with 1-^{14}C-palmitate. Concentration of the polysaccharide, 36 µg of glucose equivalents per ml.

Table II shows the effect of the polysaccharide extracted from E. coli 0111:K58, 044:K74 or J53 on the phagocytosis-dependent oxygen uptake of PMN. As expected, neither of the 2 strains of E. coli resistant to phagocytosis stimulated the oxygen uptake, except after removal of the polysaccharide by heat treatment. When the polysaccharide extracted from a strain resistant to phagocytosis was added to PMN exposed to the homologous or a heterologous strain, made susceptible to phagocytosis by heat treatment, the oxygen uptake was completely inhibited.

TABLE II

Effect of E. coli 044:K74, 0111:K58 and J53 Polysaccharides (PS) on the Phagocytosis-induced Respiratory Burst in Guinea Pig Polymorphonuclear Leukocytes (PMN)

	Oxygen Consumption*							
	Experiment 1 044:K74 PS			Experiment 2 0111:K58 PS			Experiment 3 J53 PS	
PMN*** Plus	-PS	+10 µg/ml	+40 µg/ml	-PS	+10 µg/ml	+40 µg/ml	-PS	+30 µg/ml
-------	28.2	28.0	29.0	21.7	23.43	23.43	21.7	21.7
Live J53	93.7	-	85.1	121.7	-	99.8	-	-
Heat-Killed J53**	60.8	32.1	-	73.8	23.43	-	52.1	62.1
Live 0111:K58	28.0	-	-	21.7	-	-	-	-
Heat-Killed 0111:K58**	76.2	28.0	-	54.7	-	-	58.2	91.1
Live 044:K74	28.2	-	-	21.7	-	-	-	-
Heat-Killed 044:K74	82.3	35.1	-	52.1	26.6	-	-	-

*Figures represent nanoatoms oxygen consumed/min/3 x 10^7 PMN.
**At 100 C for 60 min and then centrifuged and washed twice in KRP.
***The ratio of cells to bacteria was 1:40.

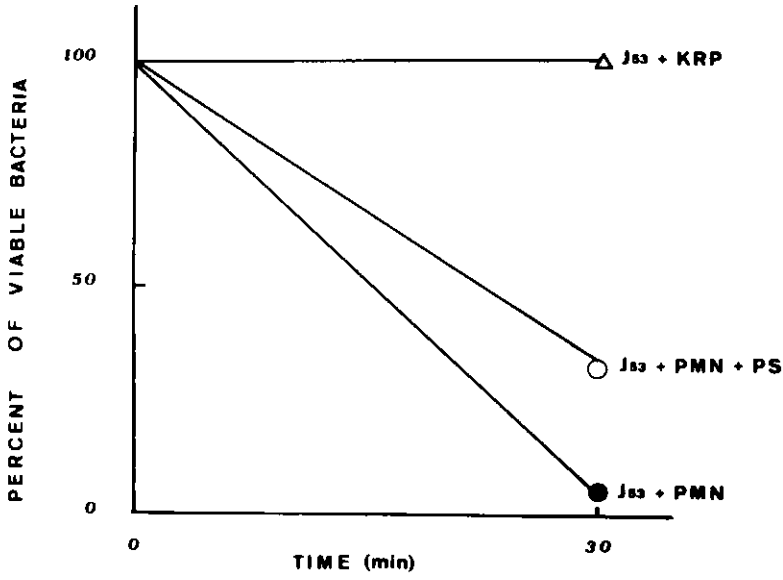

Fig. 3. Effect of the polysaccharide extracted from E. coli 0111:K58 on the phagocytic killing of E. coli J53. Concentration of the polysaccharide, 80 μg of glucose equivalents per ml.

The polysaccharide extracted from a strain resistant to phagocytosis also inhibited the oxygen uptake of PMN challenged with heat-treated J53 but not of PMN challenged with live J53. On the other hand, the polysaccharide extracted from strain J53 (susceptible to phagocytosis) did not inhibit the oxygen uptake of PMN exposed to a strain made susceptible to phagocytosis by heat treatment but rather it seemed to stimulate it.

The inhibitory effects of polysaccharide should not be due to a toxicity on the cell for the following reasons: a) the polysaccharide did not inhibit the oxygen uptake induced by live J53, b) cells pretreated with polysaccharide and then washed were still able to phagocytize a variety of particles, c) cells treated with polysaccharide responded with an increased oxygen uptake to stimuli other than particles such as phospholipase C and myristic acid (unpublished observations).

Therefore, it is likely that the polysaccharide modifies the surface properties of bacteria after binding to them. The binding of polysaccharide to the bacterial surface was studied by determining the passive hemagglutination titer of the polysaccharide-containing

extract before and after adsorption with each of the 3 strains tested. The results reported in Table III show that more polysaccharide bound to all the 3 heat-treated strains than to live J53. This correlated well with the data previously shown; that is, that the polysaccharide extracted from strains resistant to phagocytosis had only slight effect on the interaction between live J53 and PMN, whereas it deeply affected both phagocytosis of the 3 heat-treated strains and the biochemical response of leukocytes exposed to them.

TABLE III

Adsorption of E. coli 044:K74 Polysaccharide (PS) on Live or Heat Killed E. coli Strains

E. coli 044:K74 PS	Passive Hemagglutination Titer
Unadsorbed	1:512
Adsorbed with live J53	1:128
Adsorbed with heat-killed J53*	1:16
Adsorbed with heat-killed 044:K74*	1:64
Adsorbed with heat-killed 0111:K58*	1:16

*At 100 C for 60 min.

The effect of the polysaccharide-containing extracts was also studied on the interaction between E. coli 044:K74, 0111:K58, J53 and guinea pig peritoneal macrophages. The results paralleled those obtained with PMN (7).

DISCUSSION

These results support the role of the surface polysaccharide of E. coli as an anti-phagocytic agent and, therefore, as 1 of the determinants of the virulence of this microorganism. The inhibition of phagocytosis seemed to be related to an impaired recognition of the microorganism by phagocytes and its attachment to the phagocytic surface. In fact, electron microscopic observations revealed that when phagocytosis was inhibited by the polysaccharide, phagocytes did not show bacteria adherent to their surface. This impairment of recognition probably reflects some changes in bacterial surface properties, induced by the polysaccharide, rather than an interference of the polysaccharide with phagocytes.

The quality of polysaccharide seems crucial in determining its anti-phagocytic properties. In fact, the polysaccharide extracted from a strain susceptible to phagocytosis was much less effective as an anti-phagocytic agent than that extracted from a strain resistant to phagocytosis at the same concentration. It appears that whether the polysaccharide is immunologically identical with or different from the lipopolysaccharide is irrelevant in determining its anti-phagocytic activity, since an anti-phagocytic polysaccharide could be extracted from either type of E. coli. However, the question as to whether the anti-phagocytic activity of the polysaccharide is linked to a specific monosaccharide or oligosaccharide unit, or alternatively, to the overall structure of the molecule remains yet to be answered.

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