MANNOSE RESIDUES ON PHAGOCYTES AS RECEPTORS FOR THE ATTACHMENT OF *ESCHERICHIA COLI* AND *SALMONELLA TYPHI*

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SUMMARY

The attachment of *Escherichia coli* and *Salmonella typhi* to mouse peritoneal macrophages was inhibited by D-mannose, methyl α-D-mannopyranoside and yeast mannan, but not by any other sugar tested. D-Mannose and its derivatives also inhibited the attachment of *E. coli* to human polymorphonuclear leucocytes. Mannan inhibited phagocytosis when preincubated with *E. coli*, but not when preincubated with leucocytes. Attachment of opsonized bacteria to leucocytes was not inhibited by D-mannose or methyl α-D-mannopyranoside nor by any other sugar tested. Our results suggest that the surface of phagocytes, like that of epithelial cells, contains D-mannose residues which serve for the attachment of certain Gram negative bacteria.

INTRODUCTION

Recognition between the surfaces of foreign particles and phagocytes is considered to be a prerequisite for elimination of such particles by phagocytosis and intracellular digestion (1,2). In the case of bacterial invasion of an immune host, recognition is mediated by antibodies against the bacteria with or without the cooperation of the complement system (3). These serum components, collectively known as opsonins, interact specifically with the bacterial surface which is then recognized by the surface of the leucocytes resulting in attachment and ingestion of the microorganisms. Nothing is known, however, about the mechanism of bacterial recognition which is not mediated by serum components. We have recently reported (4) that adherence of *Escherichia coli* to human epithelial cells is mediated by a mannose-specific, lectin-like substance

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present on the surface of the bacterial cells, which binds to mannose residues on the mucosal cells. In the present study we provide evidence that mannose residues on the surface of phagocytes serve for the attachment to these cells of *E. coli* and *Salmonella typhi*.

MATERIALS AND METHODS

The following bacteria were used: *E. coli* strain 3092, a K_{12} derivative (5) and *S. typhi*. The cells were grown in brain heart broth (Difco Laboratories, Detroit, Mich.) for 48 hours, washed twice with phosphate buffered saline (0.1M PO_4, 0.15M NaCl, pH 7.2-7.4) (PBS) (4) and resuspended in the same buffer at a standard concentration of 5×10^9 organisms/ml. The mannose-binding activity of the bacterial cells was routinely tested by assaying their yeast agglutinating activity (4).

Attachment to leucocytes was examined as described previously on monolayers either of 24 hour cultures of mouse peritoneal phagocytes (6), or of fresh human polymorphonuclear leucocytes (7) using 1 ml of the standard bacterial suspension, with or without different sugars. In some of the experiments *E. coli* (1 ml standard suspension) or leucocytes in monolayers were preincubated for 30 min in a solution of 20 mg/ml mannan and washed twice with PBS before being used in the attachment test.

Antibodies against *E. coli* were raised in rabbits by injecting 1 ml i.v. of the standard bacterial suspension three times a week for two weeks. Sera were collected at the fourth week after immunization. The titer of the immune sera as assayed by slide agglutination with *E. coli* suspensions was 1:3,000.

All sugars used in this study were products of Pfanstiehl. Mannan was from Sigma. Other materials were from commercial sources of the highest purity available.

RESULTS AND DISCUSSION

E. coli and *S. typhi*, harvested after 48 hours of growth, readily adhered to leucocytes (Fig. 1) and were subsequently phagocytised in the absence of serum components. D-Mannose, methyl α-D-mannopyranoside (αMM) and yeast mannan, at 20 mg/ml concentrations, inhibited almost completely the association between the bacteria and mouse phagocytes (Fig.1, Table 1). These sugars also inhibited the attachment of *E. coli* to human polymorphonuclear leucocytes. The inhibition by D-mannose of αMM of the attachment was dose related and was linear in the range of 0.1-2.5 mg/ml. There was no inhibition by the other sugars tested (Table 1). The same results were obtained whether the incubation temperature was 37°C or 4°C, indicating

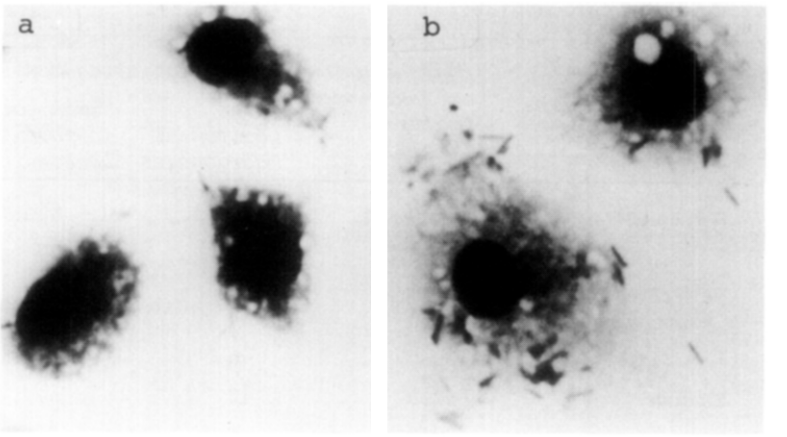

Figure 1. Attachment of *Escherichia coli* to mouse peritoneal macrophages in the presence (a) and absence (b) of methyl α-D-mannopyranoside.

that the sugars inhibited the attachment phase of phagocytosis since the engulfment phase does not take place at the lower temperature (8). Mannan inhibited phagocytosis when preincubated with *E. coli* (95% inhibition) but not when preincubated with leucocytes (∿5% inhibition), suggesting that the target cells for the inhibitory sugar were the bacteria and not the leucocytes.

In contrast to the non-opsonized bacteria, binding of opsonized bacteria to leucocytes was not inhibited by D-mannose or αMM nor by any other sugar tested (Table 2). The attachment of opsonized bacteria in the presence of mannose and its derivatives shows that these sugars do not alter the normal biological function of phagocytes and that the anti-*E. coli* mediated recognition between the surfaces of both cells does not involve mannose residues.

The results described above, together with our previous findings on the attachment of *E. coli* to the epithelial cells (4), strongly suggest that the surface of phagocytes, like that of the epithelial cells, contains mannose (or mannose-like) residues, which serve for the attachment of

Table 1: Inhibition of attachment of *E. coli* and *S. typhi* to human and mouse phagocytes by carbohydrates

Bacterial strain	*Inhibitor used*[a]	*Concentration in reaction mixture (mg/ml)*	*Percentage attachment*[b] *to*	
			mouse peritoneal macrophages	*human polymorphonuclear leucocytes*
E. coli	D-Mannose	2.0	22	n.d.[c]
		20.0	10	27
	Methyl α-D-mannopyranoside	2.0	14	n.d.
		20.0	6	29
	Mannan	2.0	12	n.d.
		20.0	8	27
	Methyl α-D-glucoside	2.0	74	n.d.
		20.0	n.d.	93
	L-Fucose	20.0	98	93
S. typhi	Methyl α-D-mannopyranoside	20.0	4	n.d.
	D-Mannose	20.0	6	n.d.
	L-Fucose	20.0	93	n.d.

a The percentage attachment in the presence of other sugars (20 mg/ml) was in all cases >90%. The sugars tested were: D-xylose, D-arabinose, D-galactose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine.

b Attachment experiments were done in triplicate by overlayering 1 ml of standard bacterial suspension containing the different sugars on the leucocyte monolayers, and incubating for 30 min at 4°C, followed by three washings with PBS to remove non-attached bacteria. The number of attached bacteria per leucocyte was obtained by counting 300 leucocytes at random. Values were calculated as percentage attachment in the presence of carbohydrates compared with control (without inhibitor) which was taken as 100% attachment.

c n.d. = not determined.

certain Gram negative bacteria. Indeed, specific inhibition by αMM and D-mannose of the binding of *Salmonella* to human epithelial cells has been observed by us (unpublished results), and to erythrocytes by Duguid *et al.* (9). It is very likely that the binding of these bacteria, like that of *E. coli* (4), is mediated by mannose-specific lectins present on the surface of *Salmonella* species.

Table 2: The effect of carbohydrates on the attachment of opsonized *E. coli* to mouse peritoneal macrophages

Carbohydrate used (20 mg/ml)	*Percentage attachment[a] of*	
	opsonized E. coli[b]	*non-opsonized E. coli[b]*
D-Mannose	85	8
Mannan	89	6
Methyl α-D-manno-pyranoside	91	6
D-Galactose	98	100
L-Fucose	94	97

a This was calculated as percentage attachment in the presence of carbohydrates compared with control (without inhibitor) which was taken as 100% attachment.

b *E. coli* was prepared by mixing 1 ml of the standard bacterial suspension with 0.1 ml of one-tenth dilution of anti-*E. coli* serum (opsonized) or of normal rabbit serum (non-opsonized) in PBS for 30 min at room temperature. The bacteria were washed twice with PBS and resuspended in the same buffer to the original volume before being used in the attachment test, as described in Table 1.

It appears that the mannose binding ability of certain Gram negative bacteria (4,9) plays a dual role in the host-parasite relationship: it enables the organism to establish colonization on mucosal surfaces by adherence to mannose residues on epithelial cells, as well as to be recognized by such residues on the phagocyte's membrane and thus to be ingested and digested by the latter cells.

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