EVIDENCE FOR THE INVOLVEMENT OF HUMAN POLYMORPHONUCLEAR LEUCOCYTE MANNOSE-LIKE RECEPTORS IN THE PHAGOCYTOSIS OF *ESCHERICHIA COLI*

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Received 6 March 1979
Revised version received 23 July 1979

1. Introduction

Several bacterial species are endowed with surface structures which are able to bind to complementary receptors on the surface of mammalian cells. This specific adhesion may be an essential step at the initiation of infection [1]. In several enterobacteria the molecular structures responsible for this specific binding are borne by non-flagellar filamentous appendages called fimbriae, which are classified into several types, according to morphology and adhesive property [2]. The active sites of *Escherichia coli* fimbriae are heat-labile proteins which can hardly be eluted and are able to agglutinate erythrocytes and to bind to the surface of epithelial cells [3,4]. Both the agglutinating activity of *E. coli* and its ability to adhere to epithelial cells are inhibited by the monosaccharide D-mannose, indicating that the receptors present on the erythrocyte and on the epithelial cell surface have a mannose-like structure [3,4].

It has also been reported that mouse peritoneal macrophages and human polymorphonuclear leucocytes contain surface mannose residues which are involved in the binding of certain Gram-negative bacteria [5,6].

It is known that the surface receptors of phagocytes for immunological reactants, such as the C3 fragment of complement and the immunoglobulins, have separate roles in the phagocytic process. Namely the receptor for C3 is mainly involved in the binding of the particle whereas the receptor for IgG directly stimulates the ingestion [7–12].

The question then arises as to whether the binding of bacteria to the phagocytes through the mannose residues is followed by internalization. By using an electron microscopic assay for phagocytosis we show here that mannose-like structures are involved in the ingestion of *E. coli* by human polymorphonuclear leucocytes in suspension.

2. Materials and methods

2.1. *Leucocytes*

Human leucocytes were obtained from heparinized venous blood by dextran sedimentation. Dextran (1 ml, 4.5%) (mol. wt 150 000–200 000) solution in isotonic saline was added to 5 ml blood and the erythrocytes were let to sediment at room temperature. Leucocytes were harvested by centrifugation at 250 × *g* for 7 min, were washed 3 times and were exposed to hypotonic saline for 30–90 s in order to lyse the contaminating red cells. Leucocytes were centrifuged again at 250 × *g* for 7 min and were finally resuspended in Krebs-Ringer-phosphate buffer (KRP) without Ca^{2+}. The percentage of neutrophils (PMN) in the preparations used ranged from 75–85%. It was not necessary to further separate PMN from lymphocytes and monocytes since these 2 types of cells did not appreciably affect the determinations carried out. In fact lymphocytes do not phagocytose nor exhibit a respiratory burst. Monocytes have a slower rate of phagocytosis than neutrophils and therefore their contribution to the total extent of phagocytosis would be irrelevant due to the small percentage of monocytes. Monocytes do have a respiratory burst but this is 70% mitochondrial and is therefore inhibitable by mitochondrial inhibitors [13]. For this reason

we have used 2 mM KCN in the assays of oxygen consumption.

2.2. *Phagocytosable particles*

The following particles were employed: (i) *E. coli* J53, a *pro-, met-* auxotrophic strain derived from K-12, employed [14] as a representative of phagocytosable *E. coli;* (ii) a strain of *β-hemolyticus Streptococcus* group A; (iii) polystyrene beads, 0.81 μM diam. (Latex, Difco, Detroit). *E. coli* was grown for 48 h in nutrient broth (Difco) at 37°C; *β-hemolyticus Streptococcus* was grown in BHI broth (Difco) at 37°C overnight. Bacteria were washed 3 times in KRP without Ca^{2+} and suspensions of the desired concentration were prepared in the same buffer.

2.3. *Oxygen consumption*

Oxygen uptake by intact cells was determined polarographically with a Clark oxygen electrode attached to a recorder, as reported [15,16]. The assay medium contained 2 mM KCN, 2×10^7 PMN, and the sugar when required. The temperature was 37°C and the final volume was 2 ml. Phagocytosis was initiated by adding 2×10^9 bacteria or 3 mg Latex.

2.4. *Measurement of phagocytosis*

Aliquots of PMN suspensions incubated with particles, in presence or in absence of the sugars, were drawn directly from the electrode chamber 3 min after addition of the particles to be phagocytosed, and were fixed by addition of an equal volume of a 3% solution of purified glutaraldehyde in 0.2 M cacodilate buffer (pH 7.2) containing 2% sucrose. After 1 h the suspension was centrifuged, and the pellet was resuspended in 1% osmium tetroxide solution in 0.1 M cacodilate buffer (pH 7.2). After dehydration in graded ethanols, the cell pellet was embedded in Dow epoxy resin 332 [17]. Ultrathin sections, prepared by an ultrotome III (LKB), were doubly stained with an aqueous solution of 2% uranyl acetate for 30 min and lead citrate [18] and were examined in a Philips EM 300 electron microscope. For each preparation about 500 PMN were examined on the micrographs, and the number of bacteria-containing leucocytes and the number of bacteria contained within individual leucocyte were recorded. In some experiments, the number of bacteria adherent to the leucocyte surface was also recorded.

3. Results

Figure 1 is an electron micrograph of human PMN exposed to *E. coli* J53 in the presence or absence of α-MMP. Numerous bacteria were phagocytosed by each polymorph in the absence of the sugar while in its presence phagocytosis was strikingly inhibited. The number of bacteria adhering to the leucocyte surface was also markedly reduced in the presence of α-MMP. From a survey of a series of electron micrographs it was found that a mean of 5.75 bacteria were adherent to each polymorph in the absence of α-MMP and only 0.28 in its presence.

A quantitative assessment of the effect of α-MMP and other sugars on the interaction between human polymorphs and *E. coli* is given in table 1. Mannose and α-MMP drastically reduced both the number of bacteria-containing cells and the number of bacteria inside each cell. The other sugars tested were ineffective. The same table also shows the oxygen consumption of leucocytes at rest or phagocytosing *E. coli* J53 in the presence or absence of the various sugars. The cells phagocytosing in the presence of D-mannose or α-MMP showed a markedly lowered oxygen consumption as compared to the cells phagocytosing in the absence of each of the two sugars. The other sugars tested had no significant effect on the oxygen uptake of phagocytosing cells. None of the sugars tested, including D-mannose and α-MMP had an appreciable inhibitory effect on the respiration of resting cells. It appears therefore that the decreased oxygen uptake of phagocytosing leucocytes in the presence of either D-mannose or α-MMP merely reflects the impaired uptake of particles (as one would reasonably expect) and not a toxic effect of the sugars on the cell metabolism. Table 2 shows that while leucocytes exposed to *E. coli* J53 in the presence of α-MMP had both a lower respiration and a lower phagocytosing than that of control cells, as already shown above, the leucocytes challenged with two other phagocytosable particles, namely *Streptococcus* and polystyrene beads, in the presence of α-MMP, had an oxygen consumption and a phagocytic activity similar to that of the cells in the absence of the sugar. This suggests that mannose-like residues are not involved in the phagocytosis of Streptococci and latex beads. Figure 2 is an electron micrograph showing phagocytosis of Streptococci by leucocytes in the presence and absence of α-MMP.

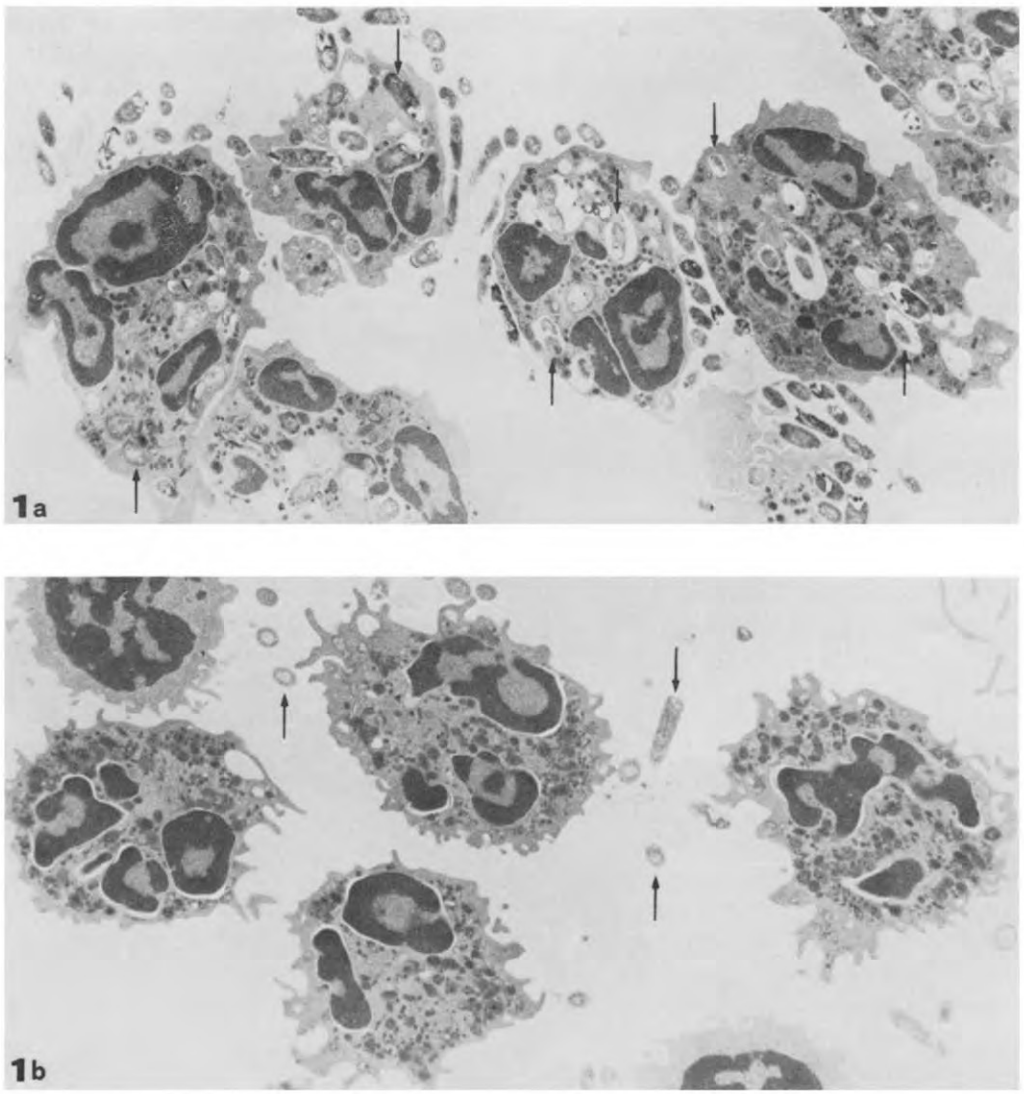

Fig.1. Electron micrographs of human polymorphonuclear leucocytes exposed to *E. coli* J53 in the absence (1a) and presence (1b) of 25 mM α-methylmannopyranoside. Examples of intracellular (1a) or extracellular (1b) bacteria are indicated by the arrows. In (1a) several bacteria adherent to the cell surface can be seen. In (1b) neither ingested bacteria nor adherent bacteria are seen. Magnification 5270 ×.

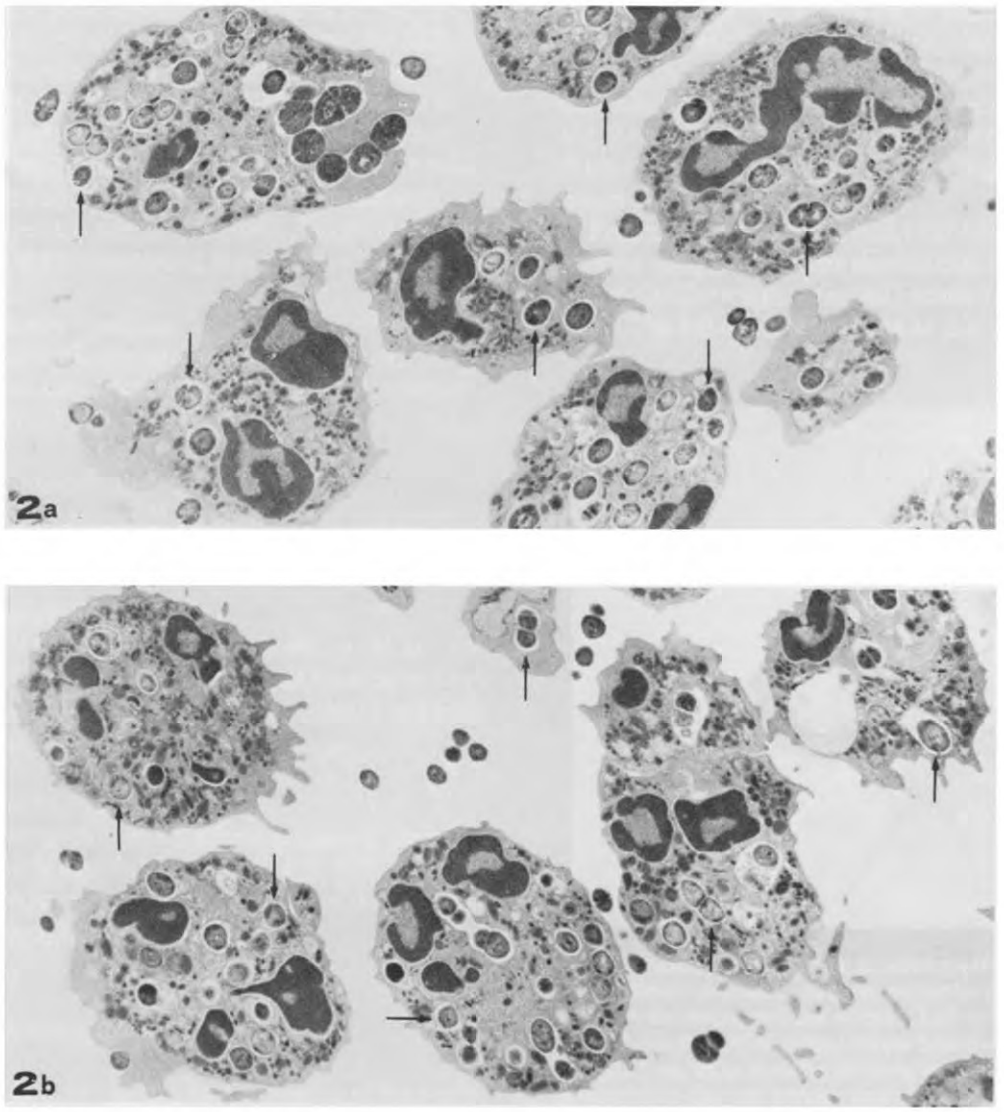

Fig.2. Electron micrographs of human polymorphonuclear leucocytes exposed to *β-hemolyticus Streptococcus* in the absence (2a) and presence (2b) of 50 mM α-methylmannopyranoside. Examples of intracellular bacteria are indicated by the arrows. The extent of phagocytosis was not appreciably affected by the monosaccharide. Magnification 5270 ×.

Table 1
Effect of several monosaccharides on the phagocytosis of *E. coli* J53 by human polymorphonuclear leucocytes (PMN) and on the stimulated oxygen consumption associated with phagocytosis

Monosaccharide (25 mM)	Phagocytosis		Oxygen consumption (natoms oxygen .min^{-1} .2 × 10^7 PMN^{-1})	
	% PMN containing bacteria	Mean number of bacteria/ PMN observed	Resting cells	Phagocytosing cells
None	98.44	7.03	16.5	99.5
α-MGP	98.17	6.73	14.0	92.0
D-mannose	27.0	0.70	15.8	33.7
α-MMP	25.0	0.51	14.6	26.9
D-galactose	99.42	6.99	20.2	97.2
L-fucose	99.48	7.04	18.0	89.6
GalNAc	98.20	7.23	18.0	94.0
GlcNAc	99.16	7.37	22.5	103.2

Abbreviations: α-MGP, α-methylglucopyranoside; α-MMP, α-methylmannopyranoside; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine

4. Discussion

Phagocytosis is a process which takes place at least in two distinct steps: (i) the attachment of the particle to the phagocyte surface; (ii) the engulfment or ingestion of the particle by the phagocyte. It is generally believed that a prominent feature of the surface interaction between the phagocyte and the particle to be phagocytosed, after the two have arrived in close proximity to each other, is its discriminatory character [19]. There is no doubt that binding of C3b or IgG to the particles makes them much better accepted by the polymorphonuclear phagocytes than the uncoated particle, permitting attachment and/or engulfment. This phenomenon, which is called opsonization, is due to the presence of specific receptors for these two immune reactants on the polymorphonuclear leucocyte surface. Although phagocytosis is strikingly enhanced by opsonins, and sometimes depends entirely on them, there are several

Table 2
Effect of α-methylmannopyranoside (α-MMP) on the phagocytosis of *E. coli* J53, *β-hemolyticus Streptococcus* and latex particles by human polymorphonuclear leucocytes (PMN) and on the stimulated oxygen consumption associated with phagocytosis of these particles

Phagocytosable particle	Concentration of α-MMP	Phagocytosis		Oxygen consumption natoms oxygen .min .2 × 10^7 PMN
		% PMN containing particles	Mean number particle/ PMN observed	
E. coli J53	–	98.44	7.14	83.0
(2 × 10^9/2 × 10^7 PMN)	25 mM	25.00	0.51	12.3
β-hemolyticus Streptococcus	–	57.0	4.11	86.0
(2 × 10^9/2 × 10^7 PMN)	50 mM	45.1	3.62	72.0
Polystyrene beads	–	71.8	4.56	45.0
(latex) (3 mg/2 × 10^7 PMN)	50 mM	67.2	4.22	33.7

Phagocytosis was evaluated on electron micrographs. Values of the oxygen consumption are the differences between those of phagocytosing cells and those of resting cells

particles, including bacteria, which are phagocytosed without apparent need for serum factors. In such instances the properties or the structures of both the particles and the phagocyte responsible for the ingestion are not defined.

We report here that D-mannose and α-methyl-mannopyranoside inhibited the ingestion of *E. coli* by polymorphonuclear leucocytes. Neither D-mannose or α-MMP had any effect on the phagocytosis of other particles, such as Streptococci and latex. These findings confirm data concerning the involvement of mannose residues in the binding of *E. coli* to phagocytes [5] and show in addition that such residues also trigger the ingestion of *E. coli* J53 by human polymorphonuclear leucocytes. The presence of D-mannose specific lectins on the surface of Gram-negative bacteria has been suggested on the basis of the inhibition of hemagglutination [3] or of the binding of *E. coli* to epithelial cells [4] by D-mannose. An extract from a strain of *E. coli* K-12 has been obtained [4] which showed lectin-like properties. By analogy with [4] it is tempting to speculate that the strain used in our experiments may also have lectin-like structures, and that it is the masking of these structures by the mannose or the α-MMP added which inhibits phagocytosis. This hypothesis needs further investigation. It is interesting, however, that neither mannose nor α-MMP which successfully inhibit phagocytosis of *E. coli*, have any effect on the phagocytosis of *β-hemolyticus Streptococcus*, indicating that the surface structures of these two bacteria which interact with the phagocytes are quite different.

It is therefore conceivable to conclude that phagocytosis of *E. coli* J53 by human polymorphonuclear leucocytes may be triggered by a D-mannose-specific lectin-like substance present on the surface of the microorganism which binds to D-mannose-like receptors on the phagocyte surface.

Acknowledgements

This work was supported by grant no. 77.01481.04 from the National Research Council of Italy (CNR).

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