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A MACROPHAGE RECEPTOR FOR (MANNOSE/GLUCOSAMINE)-GLYCOPROTEINS OF POTENTIAL IMPORTANCE IN PHAGOCYTIC ACTIVITY

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SUMMARY: Lung macrophages, in the absence of serum factors in vitro, strongly bound and ingested yeast cells (Candida krusei and zymosan). Binding was temperature-and calcium-dependent, and was inhibited by the presence of D-mannose, D-glucosamine, horseradish peroxidase and beta-glucuronidase. Pretrypsinization of the macrophages also prevented binding of yeast cells. Binding was not affected by D-mannitol, D-glucose, D-galactose nor L-fucose. I suggest that macrophage binding of yeast cells is mediated by a mannose/glucosamine receptor on the cell membrane. This receptor may be responsible for opsosin-independent phagocytosis of activators of the alternative complement pathway and, as well, the phagocyte-dependent clearance of certain lysosomal enzymes.

The pulmonary alveolar macrophages have been described as the defenders of the lung against bacterial infection (1,2). To aid in the recognition and ingestion of foreign particles, the macrophages have membrane receptors which bind the Fc portion of IgG (3,4). However, to be operative against microorganisms, this mechanism requires the presence of immune IgG and therefore an immune host. In the nonimmune host, the phagocyte may recognize foreign particles via their complement receptors, provided the particle activates the alternative complement pathway (5,6). In addition, phagocytes have been demonstrated to recognize certain carbohydrates present on bacterial cell walls (7,8) or in the case of other bacteria, lectins present on the bacteria bind to carbohydrates on the surface of the host cells (9). Recently an opsonin-independent phagocytic-mechanism of activators of the alternative complement pathway by human monocytes has been described using yeast cells and rabbit erythrocytes (6). In regard to phagocyte recognition of yeast cells, I have further analysed this type of cell interaction and found that carbohydrate specific receptors on the phagocyte mediate adhesion

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of yeast cells. This binding is inhibited by beta-glucuronidase indicating that it may involve a common receptor mechanism responsible for phagocyte clearance of certain lysosomal enzymes.

MATERIALS AND METHODS

Yeast suspensions. Two yeast preparations were used: zymosan A (Sigma Chemical Co., St. Louis, Mo.)-a cell wall preparation obtained from Saccharomyces cerevisiae, and viable Candida krusei (a laboratory strain). C. krusei was grown in Trypticase soy broth or a phosphate-depeleted broth supplemented with 1 mCi carrier-free ^{32}P as orthophophoric acid (New England Nuclear Corporation, Boston, MA). The candida cultures were incubated with constant shaking for 18 h at 37°C. The yeast were washed three times with phosphate buffered saline (0.1M PO_4, 0.15M NaCl, pH 7.4) and resuspended in tissue culture medium 199[1] (TCM 199, GIBCO Laboratories, Grand Island, N.Y.) buffered to pH 7.4 with 25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Sigma Chem. Co.). In some studies to determine the role of the divalent cations, calcium and magnesium, in macrophage binding of yeast cells, the yeast were suspended in Dulbecco's PBS (calcium, magnesium-free) to which various amounts of calcium and magnesium had been added. All yeast suspensions were diluted to a concentration of 10^7 cells/ml (based on spectrophotometric absorbance at 520 nm).

Lung macrophages. Macrophages from the lungs of female outbred Swiss mice, 18 to 21 g (Microbiological Associates, Bethesda, MD.) were obtained by pulmonary lavage as previously described (4). The cells were suspended in TCM 199. Direct cell counts were performed in a hemocytometer, and differential cell counts were performed on Wright-stained cell monolayers. Greater than 98% of the lung cells recovered were identified as macrophage and greater than 98% were viable as determined by trypan blue exclusion.

Macrophage-yeast binding assays. Monolayers of the macrophages were prepared by incubating 0.2 ml portions of the lung cell suspensions (5 x 10^5 macrophages/ml TCM 199) on albumin-coated 22mm^2 cover glasses (placed in the bottoms of 35mm plastic petri dishes) for 30 min at 37°C. This allowed the cells to adhere and spread on the glass surfaces. To coat the cover glasses, they were dipped into a solution of 1% bovine serum albumin and allowed to air dry. The albumin helped to reduce nonspecific binding of the yeast to the glass surface and thus facilitated removal of the free particles. Six macrophage monolayers were prepared for each experimental variable and each assay was repeated at least three times.

The fluid covering the macrophages was removed and 0.2 ml of the appropriate yeast suspension was layered over the macrophages (2.0 x 10^6 yeast/10^5 macrophages) and allowed to incubate for 30 min at 37°C. To determine temperature dependence of binding an incubation period of 1 h at 4°,25°, and 37°C was used. To remove free yeast, each mixed cell culture was then washed five times with 1 ml volumes of phosphate buffered (0.04 M PO_4, pH 7.4) isotonic saline solution supplemented with 0.1% gelatin. For direct cell counts of macrophages-yeast binding, the monolayers were rapidly air dried, fixed with absolute methanol and processed with Wright stain. A minimum of 100 macrophage on each cover glass was randomly selected and read microscopically (1000x magnification) to quantitate the number of yeast asso-

1). Abbreviations used in this paper: PBS, phosphate buffered saline; TCM 199, tissue culture medium 199; HRP, horseradish peroxidase; EDTA, ethylenediaminetetraacetic acid; and EDTA, ethyleneglycoltetraacetic acid.

ciated with the phagocytes. The values for a given experimental group were combined and a mean value calculated and expressed as the binding index (Total number of yeast/100 macrophages). In those studies employing [^{32}P] C. krusei, the cover glasses were washed five times, removed from the petri dishes and dipped into a series of three beakers containing wash fluid, broken in half and both pieces placed in a scintillation counting vial containing 0.5 ml of 0.5% Trition X-100. Scintillation cocktail was added and the ^{32}P-counts measured in a Beckman liquid spectrophotometer. The counts were corrected for quench and background counts.

Enzyme and binding antagonist studies. To examine the sensitivity of binding to enzyme treatment, the macrophages were pre-treated with neuraminidase (2 units/ml, derived from Vibrio comma) or 0.01% trypsin (diphenyl carbamyl chloride treated to inactivate chymotrypsin) for 15 min at 37°C. The macrophages were then washed five times with TCM 199 and challenged with the appropriate yeast suspension as described above. To determine the antagonistic behavior of horseradish peroxidase, beta-glucuronidase (derived from Escherichic coli) and the various carbohydrates tested, the antagonist was mixed with the yeast suspensions which were then used to challenge the macrophages. All of the reagents were obtained from the Sigma Chemical Company.

Macrophage Fc-receptor binding assays. The binding of IgG-coated sheep erythrocytes to macrophages was determined as previously described (4). Competition between the Fc receptor for IgG and the nonimmunologic receptor for candida was determined in co-incubation studies.

RESULTS

Lung macrophages were capable of binding yeast cells in the absence of serum factors; however, the binding activity was temperature dependent (Table 1). At the optimum temperature, 37°C, the majority (94.2±1.2%, mean ± standard error) of the macrophages were active with a mean of 4.5±0.1 bound yeast particles. In addition, the binding of candida was also dependent upon the presence of calcium (Table 2). Those macrophages incubated in TCM 199 depleted of calcium by EGTA-Mg or calcium and magnesium by EDTA demonstrated a significant reduction in binding of yeast cells. The addition of calcium to

TABLE 1

Effect of Temperature on Macrophage-Candida Binding[1)]

Incubation Temperature	% Macrophages Active	Binding Index
4°C	22.0±1.7	32±3
25°C	58.3±2.4	162±11
37°C	94.2±1.2	426±14

1) Macrophage-candida binding determined in TCM 199 using a 1 h incubation period. Values expressed as mean ± standard error.

TABLE 2

Role of Divalent Cations in Macrophages-[^{32}P] Candida Binding

Challenge Medium	[^{32}P] Candida Bound (cpm)[1]	% Control
TCM 199 control	3,172 ± 429	100%
TCM 199 + EDTA[2]	76 ± 20	2%
TCM 199 + EGTA-Mg[3]	578 ± 84	18%
TCM 199 + EGTA-Ca[3]	2,855 ± 380	90%
PBS (Ca^{++}, Mg^{++} free) control	805 ± 135	100%
PBS + Mg^{++}[4]	2,130 ± 121	264%
PBS + Ca^{++}[4]	14,500 ± 1,630	1801%
PBS + Ca^{++}-Mg^{++}	9,930 ± 1,144	1234%

1) Values expressed as mean ± standard error.
2) Ethylenediaminetetraacetate present at a concentration of 10mM.
3) Ethyleneglycoltetraacetic acid complexed with an equimolar concentration of $MgCl_2$ or $CaCl_2$ (final concentration of 10mM).
4) Dulbecco's phosphate buffered saline supplemented with either 1mM $MgCl_2$ or $CaCl_2$.

EGTA removed the suppressive action of this chelator. Additional studies employing Dulbecco's PBS (supplemented with magnesium and/or calcium) further supported the evidence indicating a specific requirement for calcium in the binding of candida to macrophages. In studies examining the stability of the macrophage yeast-receptor, trypsinization of the macrophages resulted in a significant reduction in their candida-binding index (5±3% control binding index). In contrast, neuraminidase treatment resulted in an enhancement of the ability of the macrophages to bind candida (135±5% control binding index). Trypsin or neuraminidase treatment of candida did not alter the binding index. Since the receptor was pH sensitive (unpublished observations), studies with acidic glycosidases - mannosidase - were not possible.

Mannan (a mannose polymer) is the major soluble component of the yeast cell wall (10). Candida are agglutinated by concanavalin A which binds to mannose units (11), indicating that the mannose is exposed on the yeast cell wall. Therefore it seemed highly probable that the macrophages may utilize a binding mechanism which reacts with this component of the yeast cell wall.

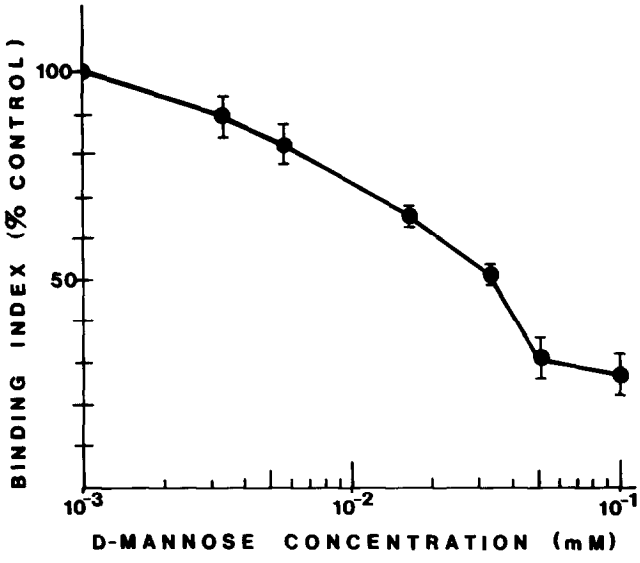

Fig. 1. Concentration-dependent inhibition curve of macrophage-candida binding by D-mannose. Binding index is the total number of candida bound/100 macrophages (30 min at 37°C).

In competition studies using various concentrations of D-mannose (Fig. 1), it was determined that mannose was indeed capable of suppressing the ability of the macrophages to bind candida. A D-mannose concentration of 33mM resulted in a 50% inhibition of macrophage-candida binding (I_{50} = 3.3 x 10^{-2}M). At this concentration, D-glucose, D-galactose, L-fucose and the alcohol derivative of mannose, D-mannitol, did not affect binding of candida to the macro-

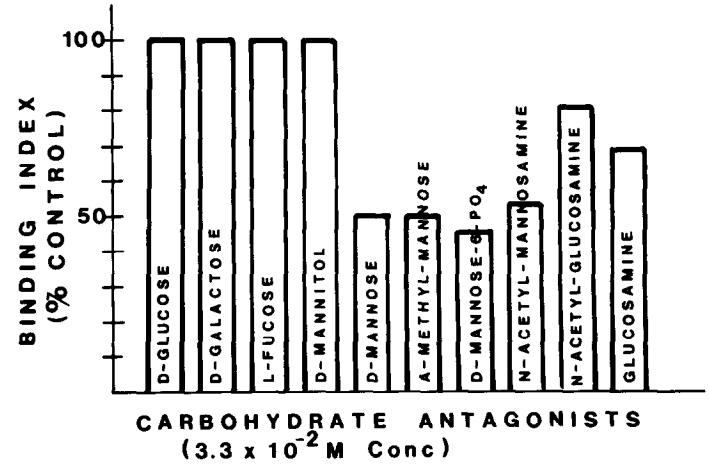

Fig. 2. Effects of specific monosaccharides and amino-sugars on macrophage-candida binding. Binding index is the total number of candida bound/100 macrophages (30 min at 37°C).

phages (Fig. 2). In contrast, α-methyl-mannose, mannose-6-phosphate, N-acetyl-mannosamine, glucosamine and N-acetyl-glucosamine all reduced the binding index.

Yeast mannan has been shown to compete with the reticuloendothelial dependent clearance of horseradish peroxidase (HRP) and beta-glucuronidase (12). In my assay system, the ability of lung macrophages to bind yeast cells was very sensitive to the presence of HRP with binding almost completely blocked at a HRP concentration of 0.01mM (Fig. 3, $I_{50} = 5 \times 10^{-7}$M). Beta-glucuronidase also reduced the binding index ($I_{50} = 2.3 \times 10^{-5}$M) although not as avidly as did HRP. Pretreatment of candida with D-mannose, D-glucosamine, or HRP did not suppress the macrophage binding-index.

The binding and ingestion of zymosan particles by mononuclear cells is often used by immunologists to identify macrophages and their functional activity. To determine if this macrophage activity had characteristics similar to that involving candida-binding, zymosan particles were substituted for the candida in my assay model. Macrophage binding of zymosan demonstrated the same characteristics as did candida binding (Table 3). Thus a common receptor-mechanism appeared to be operative for both yeast particles.

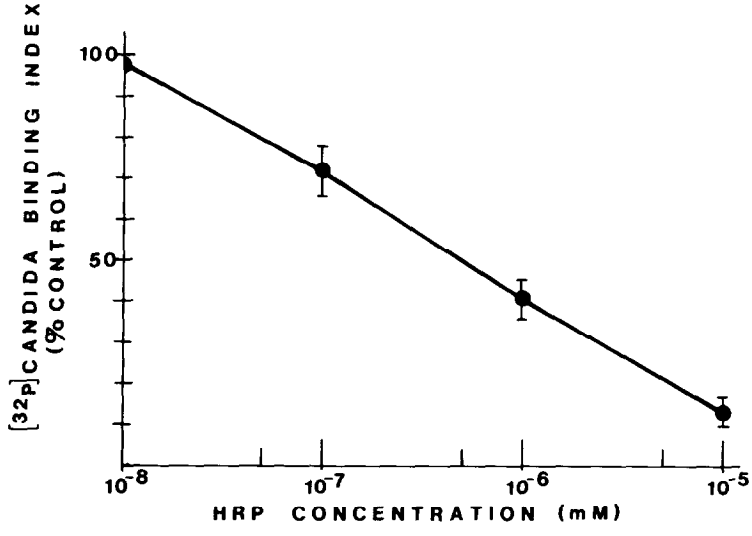

Fig. 3. Concentration-dependent inhibition curve of macrophage-candida binding by horseradish peroxidase. Binding index is the total ^{32}P candida cpm/10^5 macrophages (30 min at 37°C).

TABLE 3

Antagonists for Macrophage-Zymosan Binding

Treatment[1)]	Binding Index[2)]	% Control
None (control)	457 ± 16	100%
D-mannose (30mM)	292 ± 12	63.9%
D-glucosamine (30mM)	321 ± 14	70.2%
Horseradish peroxidase (0.01mMO	201 ± 15	44.0%
Trypsin (0.01%)	146 ± 8	31.9%

1) In those assays involving D-mannose, D-glucosamine and horseradish peroxidase, the antagonist was present during the incubation of the macrophages with the zymosan particles. The effects of trypsin were determined following a 15 min pre-incubation of the macrophages with a 0.01% trypsin solution. The trypsin was removed from the cultures before the addition of the zymosan particles. Preincubation of the zymosan particles with D-mannose or horseradish peroxidase, with subsequent removal of the antagonist during incubation of the particles with the macrophages, did not suppress the Binding Index.

2) The total number of zymosan particles bound to 100 macrophages (Binding Index) was determined by direct microscopic examination as described under Materials and Methods. Values expressed as mean ± standard error.

The macrophage's Fc receptor for IgG also appears to be dependent upon the N-acetyl-glucosamine-mannose region of IgG carbohydrate (13). To determine whether or not the Fc receptor was also responsible for macrophages binding of yeast cells, competition studies between suspensions of 0.5% (v/v) IgG-coated erythrocytes, candida and HRP (0.01mM) were performed. Neither the candida nor the HRP reduced macrophage binding of IgG, 115±5% and 90±5% control Fc-IgG binding, respectively. Thus, macrophages binding of yeast cells and IgG-coated particles involved two separate mechanisms.

DISCUSSION

Carbohydrates have an active role in cell-cell interactions where they are involved in phagocyte recognition of galactose units on asialo-erythrocytes (14), glucose-galactose units on bacteria (7,8), or phagocyte membrane-mannose units for lectins on bacteria (9). In addition, carbohydrates also actively participate in phagocytic recognition of lymphokines (15), lysosomal glycosidases (16), and IgG (13). In the latter case, the N-acetylglucosamine/mannose residues of IgG are also required for complement activation (13).

The results of this study have demonstrated that alveolar macrophages have a receptor system that mediates the binding of (mannose/glucosamine)-glycoproteins whether these glycoproteins are present on microorganisms or components of enzymes. This was evident from the fact that macrophage binding of yeast cells was inhibited by the free saccharides and amino-sugars or by horseradish peroxidase, a glycoprotein rich in mannose and glucosamine (17), and beta-glucuronidase, a glycoprotein containing mannose, glucosamine and N-acetylglucosamine (18). Kaplan and Nielsen (19) have recently demonstrated that alveolar macrophages actively absorb horseradish peroxidase by a temperature-energy dependent process.

I propose that the recognition of mannose/glucosamine)-glycoproteins by alveolar macrophages involves a common receptor mechanism responsible for the phagocytic defense capacity of binding and ingestion of opsonin-independent activators of the alternate complement pathway and clearance of specific enzymes present in the phagocyte's microenvironment.

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