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Ingestion of Yeast Forms of *Sporothrix schenckii* by Mouse Peritoneal Macrophages

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The ingestion by thioglycolate-elicited mouse peritoneal macrophages of yeast forms of two strains of *Sporothrix schenckii* was studied. Yeast forms opsonized with concanavalin A (ConA) were extensively phagocytized, and the phagocytic indexes depended on the concentration of ConA and apparently on the number of lectin receptors at the yeast surface as well. Neuraminidase treatment of *S. schenckii* increased the ingestion of unopsonized yeasts 7.7-fold. The addition of monosaccharides and derivatives partially inhibited phagocytosis. Mannose, rhamnose, and galactose, which are major constituents of *S. schenckii* surface antigens, reduced the phagocytic indexes by 40 to 50%. Glucosamine, *N*-acetylglucosamine, and *N*-acetylneuraminic acid were equally effective as inhibitors of phagocytosis. A mixture of five neutral sugars and glucosamine inhibited phagocytosis by 73%. The inhibitory effect of simple sugars could be amplified by using neuraminidase-treated yeast cells. Pentoses and glucose were inactive or slightly inhibitory. A purified rhamnomannan inhibited phagocytosis of the homologous strain, whereas partially purified peptidopolysaccharides were toxic to peritoneal macrophages. A partially purified galactomannan from *S. schenckii* was inhibitory (62% inhibition), and a peptidopolysaccharide fraction in which the *O*-linked carbohydrate chains had been removed neither was toxic to macrophages nor inhibited phagocytosis. Pretreatment of macrophages with simple sugars under conditions inhibiting ingestion or binding of *S. schenckii* did not affect phagocytosis of latex particles or sensitized sheep erythrocytes. The presence of receptors at the peritoneal macrophages which bind *S. schenckii* cell surface components is suggested.

Macrophages are able to ingest particles and soluble substances. Mechanisms of endocytosis have been extensively studied (19, 21, 26). Ingestion of particles by macrophages is preceded by their attachment to the macrophage membrane. In several instances, however, attachment of ligands to phagocytic cells is not followed by endocytosis (16, 17, 20, 30).

Treatment of macrophages with lectins (13) such as concanavalin A (ConA) can result in cells able to bind bacteria (1), yeasts (3), and erythrocytes (8) without ingestion. Conversely, the attachment to macrophages of yeast cells coated with ConA is followed by extensive phagocytosis (3).

Binding of unopsonized microorganisms to macrophages can be inhibited by carbohydrate units which are also cell wall constituents. Galactose partially inhibits binding to macrophages of a noncapsulated *Klebsiella aerogenes* mutant known to have this sugar as a cell wall component (7). Lipopolysaccharide sugar components also inhibit binding of *Salmonella typhimurium* to macrophages. These results suggest that the macrophage membrane has receptors that recognize a variety of carbohydrate-containing determinants present at the cell surface of microorganisms. These receptors may be glycoproteins in view of their susceptibility to proteolytic enzymes, β-galactosidase, and periodate (7, 12).

In the present study, we investigated the phagocytosis of *Sporothrix schenckii*, a hyphomycete with several recognized cell surface constituents, by thioglycolate-elicited mouse peritoneal macrophages. Both untreated or ConA-opsonized cells of the *S. schenckii* yeast phase were used. Details of the fine structures of constituents of the cell walls from yeast forms of *S. schenckii* are described elsewhere (4, 10, 14, 15, 22-24).

In vitro determination of phagocytosis of unopsonized *S. schenckii* yeast forms may contribute to the understanding of the early infection by this fungus and also its dissemination in states of

immunosuppression or decreased immunological responsiveness. Shedding of antigenic material by yeast cells (4) and the eventual trapping of antibodies at a distance from the infecting cell is another way of keeping yeasts unopsonized and thereby less susceptible to phagocytosis.

MATERIALS AND METHODS

Microorganisms. Yeast forms of two strains of *S. schenckii* (1099.18 and 1099.24) grown for 7 days in brain heart infusion (BHI) at 25°C were used. Cells were harvested by centrifugation and washed four times in 0.01 M phosphate-buffered saline (PBS). Suspensions of 5×10^7 cells per ml were prepared in Hanks solution containing 0.1% glucose.

ConA-treated cells. *S. schenckii* yeast forms agglutinate in the presence of ConA at 125 μg/ml (minimum concentration). ConA-treated cells for phagocytosis experiments were prepared with lectin concentrations (66 and 100 μg/ml) unable to cause agglutination.

Macrophages. Peritoneal macrophages were obtained from 20 to 25-g mice inoculated (1 ml per mouse) with Brewer thioglycolate medium (Difco Laboratories) 3 days before the collection of cells in Hanks solution. Animals were killed with ether, and their peritoneal cavities were washed with 5 ml of Hanks solution containing 0.1% glucose and 10-U/ml heparin. Approximately 0.3 ml of the peritoneal exudate was dispensed on appropriate microscope slides as described before (9). Macrophages were left to adhere onto the microslides for 1 h at 37°C. Slides were then washed with 0.01 M PBS, pH 7.2.

Phagocytosis of yeast cells. Slides with adhered macrophages (1.5×10^4 cells) were washed in PBS, and 0.3 ml of the suspension of *S. schenckii* yeast forms was added. Phagocytosis was observed during 90 min at 37°C. After this period, the slides were washed with PBS containing 0.2 M mannose to remove yeast cells adhered to the outside surface of the macrophage as well as unbound yeasts. Cells were fixed with 2.5% glutaraldehyde in 0.1 M PBS, pH 7.2, for 30 to 60 min and by brief heating. Cells were stained by the Gram method. An average of 200 macrophages were counted on several microscope fields to determine the percentage of macrophages phagocytizing at least one yeast cell (P) and the average number of yeasts in these macrophages (F). The phagocytic index (I) was determined by multiplying P × F (5).

Phagocytosis of unopsonized yeasts. Unopsonized yeasts of one strain (1099.24) were treated with neuraminidase (10 U/ml, Sigma Chemical Co. type X) before their contact with macrophages. Sialic acid liberated by neuraminidase was assayed by the thiobarbituric acid method (27). For phagocytosis, 100 μl of a suspension of cells treated with neuraminidase (0.5 ml of cells plus 5 U of enzyme for 30 min at 37°C in 0.05 M acetate buffer, 0.13 M NaCl, pH 6.5) was used, and ingestion was determined after 90 min.

Inhibition of phagocytosis. For the inhibition experiments, monosaccharides or derivatives at 10 mg/ml in Hanks solution were added to the slide-adhered macrophages (0.1 ml). After 20 min of exposure, cells were gently washed (7), and 0.25 ml of a yeast suspension of 2.5×10^7 to 5.0×10^7 cells per ml was added. Procedures involving gentle washing or no washing after preincubation with sugars were compared by testing the inhibitory activities on phagocytosis of D-galactose, *N*-acetylglucosamine, and L-rhamnose. Slides were incubated for 90 min at 37°C, washed, fixed, and Gram stained as described above. Polysaccharides tested as inhibitors were used in the range of 5 to 20 mg/ml. They were extracted in hot dilute alkali and were either precipitable by Fehling solution or unprecipitable as described before (23). Fehling-precipitable fractions contain rhamnomannans, whereas the unprecipitable polysaccharide consists of galactomannan and some glucan. Peptidopolysaccharides were isolated from the supernatants of *S. schenckii* cultures by ethanol precipitation, dialysis against distilled water of the aqueous solution of polymers, and precipitation of their borate complexes at pH 8.5 by hexadecyltrimethylammonium bromide (Cetavlon) (23). Acidic molecules were neutralized with 10% $(NH_4)_2CO_3$ and diluted in Hanks solution without glucose before being tested as inhibitors of phagocytosis.

Ingestion of neuraminidase-treated *S. schenckii* and inhibition by simple sugars. Strain 1099.24 was used at a concentration of 5×10^7 cells per ml. Yeasts were treated with 10 U of neuraminidase for 30 min at 37°C in acetate buffer, pH 6.6. Cells were then centrifuged at 4°C, washed four times with Hanks solution, and resuspended in 1 ml of the same solution containing rhamnose, galactose, and *N*-acetylglucosamine at 10 mg/ml. Incubation of macrophages with sugars was for 20 min, and phagocytosis of neuraminidase-treated cells was measured after 90 min.

Binding of yeast cells to macrophages. For binding experiments, macrophages were incubated with 5×10^7 cells of strains 1099.18 and 1099.24 per ml at 4°C for 30 min. Slides were then washed five times in PBS and fixed as before. Macrophages having at least one surface-bound yeast cell were counted; 200 macrophages were inspected. For the inhibition experiment, macrophages were preincubated with *N*-acetylglucosamine at 10 mg/ml for 20 min and then put in contact with the yeast suspension at 4°C.

Phagocytosis of latex particles and sensitized sheep erythrocytes. Percentages of macrophages phagocytizing latex particles (1/100) and the phagocytic indexes of ingestion of antibody-sensitized sheep erythrocytes (2%) were determined in macrophages treated with monosaccharides at 10 mg/ml for 20 min as compared with untreated controls.

RESULTS

Both *S. schenckii* strains agglutinated with ConA at 500 μg/ml. Under these conditions, inhibition of agglutination was tested with D-mannose. The minimum inhibitory concentrations of mannose were 19 mg/ml and 2 mg/ml for strains 1099.24 and 1099.18, respectively. This suggests that strain 1099.24 has more receptors for ConA than does strain 1099.18. As expected, yeasts coated with ConA at subagglutinating lectin concentrations were more efficiently phagocytized by thioglycolate-induced macrophages than by untreated cells. The phagocytic index was influenced by increasing the concentration of ConA from 66 to 100 μg/ml, suggesting that a saturation of receptors was not achieved

TABLE 1. Phagocytosis of lectin-coated and uncoated yeast forms of two *S. schenckii* strains[a]

Con A concn (μg/ml)	*S. schenckii* 1099.18			*S. schenckii* 1099.24		
	P	F	I	P	F	I
0	14 ± 10	2.0 ± 0.4	26 ± 16 (5)	38 ± 4	2.4 ± 0.3	89 ± 12 (5)
66	62 ± 10	2.8 ± 0.3	173 ± 38 (4)	92 ± 2	5.1 ± 0.8	465 ± 72 (4)
100	85 ± 0	4.0 ± 0.8	341 ± 64 (3)	100 ± 0	10.3 ± 2.2	1033 ± 216 (3)

[a] Definitions of P and F are in the text; I is the phagocytic index. Values ± standard deviations are averages of independent experiments with macrophages obtained from different animals (numbers in parentheses).

TABLE 2. Phagocytosis of neuraminidase-treated *S. schenckii* 1099.24[a]

Yeast	P	F	I
Untreated	28.8 ± 1.5	1.7 ± 0.2	48.9 ± 8.4 (3)
Neuraminidase treated (10 U/ml)	82.3 ± 3.7	4.6 ± 0.4	378.5 ± 22.1 (3)

[a] P, F, and I ± standard deviations are as in Table 1, footnote *a*; number of animals used is shown parenthetically.

at the lower concentration. The phagocytic indexes of lectin-coated and untreated cells of strain 1099.24 were, in this experiment, three times higher than those for strain 1099.18 (Table 1).

S. schenckii (1099.24) yeast cells treated with neuraminidase (10 U/ml) were taken up by activated macrophages much more efficiently than by untreated cells (Table 2). No significant reduction of the phagocytic indexes with both strains was observed at 0.2 U of neuraminidase per ml. Neuraminidase (10 U/ml) released 3.6 μg of sialic acid per ml of packed yeast cells as assayed by the thiobarbituric acid method (27).

The effect of several mono- and polysaccharides on the phagocytosis of *S. schenckii* is shown on Table 3. Phagocytic indexes with values of less than 70% of that of the control were considered as evidence of partial inhibition of phagocytosis. Among monosaccharides, mannose, rhamnose, galactose, glucosamine, *N*-acetylglucosamine, *N*-acetylneuraminic acid, and a mixture of five neutral sugars plus glucosamine

TABLE 3. Partial inhibition of *S. schenckii*[a] phagocytosis by monosaccharides and polysaccharides[b]

Addition	P	F	I	% of control
No addition	27.0 ± 5	1.9 ± 0.2	52.0 ± 8	100
No addition[c]	29.0 ± 1.5	1.7 ± 0.2	49.2 ± 8.4	100
Ribose	27.3 ± 2.0	1.8 ± 0.2	49.1 ± 7.8	94.4
Ribose[c]	26.0 ± 1	2.1 ± 0.2	54.7 ± 5.2	111.1
Xylose	27.0 ± 3	1.6 ± 0.2	43.0 ± 8	82.6
Arabinose	25.0 ± 1.5	1.9 ± 0.1	47.5 ± 6.5	91.3
Arabinose[c]	24.2 ± 2.7	1.9 ± 0.1	46.1 ± 8.7	93.7
Glucose	26.3 ± 4.5	1.4 ± 0.1	36.8 ± 3.4	70.7
Mannose	17.0 ± 4	1.8 ± 0.2	30.0 ± 8	57.6
Rhamnose	19.1 ± 1	1.6 ± 0.1	29.0 ± 2	55.7
Galactose	17.0 ± 2	1.6 ± 0	27.0 ± 3	52
Glucosamine	14.6 ± 3.7	1.5 ± 0.1	22.1 ± 6.2	42.5
N-Acetylneuraminic acid[c]	16.8 ± 2.5	1.3 ± 0.1	22.1 ± 5	44.9
N-Acetylglucosamine[c]	13.0 ± 2	1.2 ± 0.1	16.0 ± 4.4	32.5
Rha + Man + Xyl + Gal + Glc + $GlcNH_2$[e]	11.8 ± 1.5	1.2 ± 0.1	13.8 ± 3.2	26.5
Galactomannan[d]	14.3 ± 2.2	1.4 ± 0.1	19.8 ± 5.2	38
Peptidopolysaccharide derivative[f]	31.2 ± 4.6	1.7 ± 0.1	53.3 ± 10.8	102.5

[a] Except where indicated, experiments were made with *S. schenckii* 1099.18. P, F, and I ± standard deviations are as in Table 1, footnote *a*.

[b] Monosaccharides were added at 10 mg/ml, and polysaccharides were added at 5 mg/ml. Values are averages of three experiments with macrophages from three different mice.

[c] *S. schenckii* 1099.24 was used.

[d] Crude polysaccharide isolated from strain 1099.18.

[e] Individual sugars added to the mixture at 2 mg/ml each.

[f] Peptidopolysaccharide from strain 1099.18 after removal of *O*-linked carbohydrate chains by β-elimination (see the text).

TABLE 4. Ingestion of *S. schenckii* 1099.18 by macrophages pretreated with purified rhamnomannan[a]

Rhamnomannan concn (mg/ml)	P	F	I	% of control
0	29.1 ± 1.5	1.8 ± 0.1	52.5 ± 5.1	100
10	17.8 ± 1.5	1.5 ± 0.1	28.0 ± 4.3	53.3
15	18.8 ± 1.5	1.5 ± 0.0	28.8 ± 2.3	54.8
20	14.3 ± 1.0	1.5 ± 0.1	21.5 ± 3.0	40.9

[a] Values ± standard deviations are averages of results of three experiments with macrophages from three different mice. P, F, and I are as in Table 1, footnote *a*.

were inhibitors. Pentoses, glucose, and a peptidopolysaccharide derivative of strain 1099.18 were not inhibitory by the criteria used. In both strains, the partially purified peptidopolysaccharides were toxic for macrophages and could not be tested as inhibitors. By removing *O*-linked carbohydrate chains by β-elimination (0.05 N KOH plus 2 M KBH_4 at 45°C for 16 h), the resulting derivative was not inhibitory of phagocytosis.

A preparation of a galactomannan from *S. schenckii* (1099.18) which was not precipitated by Fehling reagent was inhibitory of phagocytosis. The purified rhamnomannan from *S. schenckii* strain 1099.18 was also inhibitory at 10 mg/ml with no significant increase of this effect at higher concentrations of the polysaccharide (Table 4). The procedure, including gentle washing of macrophages after incubation with sugars as generally used in this work, gave phagocytic indexes similar to those of unwashed systems (Table 5).

The inhibitory activity of monosaccharides on phagocytosis was also studied in neuraminidase-treated yeasts. The inhibitory effect was much enhanced with rhamnose, galactose, and *N*-acetylglucosamine (Table 6), the aminosugar being the most effective (83.3% inhibition). *N*-Acetylglucosamine was then used in experiments to determine the effects on phagocytosis of the time of incubation of macrophages with sugars (Table 7), the sugar concentration (Table 8), and its effect on the binding of yeasts to macrophages at 4°C. Best inhibition of phagocytosis was observed when macrophages were preincubated for 20 min with *N*-acetylglucosamine (Table 7). Incubation with *N*-acetylglucosamine for 30 min without washing and without addition of the yeast suspension could result in vacuolized macrophages. The best concentration of *N*-acetylglucosamine for maximal inhibition was 10 mg/ml (Table 8); hence, this concentration was used for the other potential inhibitors. *N*-Acetylglucosamine also inhibited binding of *S. schenckii* (1099.18 and 1099.24) yeast cells to macrophages at 4°C, suggesting a surface recognition mechanism as a first step for phagocytosis (Table 9). To determine whether sugars at the concentration generally used (10 mg/ml) were toxic to macrophages, a control was made by measuring the ingestion of latex particles (1/100). Percentages of phagocytosis in macrophages preincubated with *N*-acetylglucosamine, glucosamine, galactose, and ribose at 10 mg/ml were 38.5, 42.0, 37.8, and 39.3, respectively, as compared with 37.6% of the control without sugar addition. Phagocytosis of sensitized sheep erythrocytes was also determined with macro-

TABLE 5. Effect on phagocytosis of pretreatment of macrophages with monosaccharides (10 mg/ml) with and without washing before addition of *S. schenckii* yeast suspensions[a]

Pretreatment	*S. schenckii* 1099.18		*S. schenckii* 1099.24	
	I	% of control	I	% of control
Without washing				
No addition	56.7 ± 6.4	100	60.5 ± 12.5	100
D-Galactose	25.5 ± 3.9	45	28.5 ± 6.1	47.1
N-Acetylglucosamine	20.4 ± 3.6	36	20.4 ± 4.6	33.7
L-Rhamnose	26.7 ± 0.8	47	34.2 ± 6.4	56.5
With washing				
No addition	44.5 ± 1.1	100	55.5 ± 8.3	100
D-Galactose	22.1 ± 4.8	49.6	22.4 ± 2.3	40.3
N-Acetylglucosamine	14.2 ± 3.0	31.9	18.8 ± 5.9	33.8
L-Rhamnose	26.1 ± 4.2	58.6	26.6 ± 3.9	47.9

[a] I, Phagocytic index.

TABLE 6. Inhibition of phagocytosis of neuraminidase-treated yeasts strain 1099.24 by simple sugars[a]

Addition	P	F	I	% of control
No addition	80.3 ± 2.0	2.4 ± 0.1	192.8 ± 10.7	100
L-Rhamnose	29.6 ± 1.7	2.1 ± 0.1	61.4 ± 8.1	31.8
D-Galactose	24.0 ± 1.8	2.1 ± 0.1	50.5 ± 6.2	26.1
N-Acetylglucosamine	16.5 ± 2.3	1.9 ± 0.1	32.3 ± 3.9	16.7

[a] Sugars added at 10 mg/ml. P, F, and I ± standard deviations are as in Table 1, footnote *a*.

phages pretreated with D-galactose, *N*-acetylglucosamine, or L-rhamnose at 10 mg/ml for 20 min (Table 10). No inhibitory effect was observed.

DISCUSSION

Correlation of results of the present work and the host defense mechanisms in vivo against *S. schenckii* infection seems possible in some aspects. Clearly, opsonization of yeast cells with antibodies as with hydrophobic lectins greatly increases phagocytosis of *S. schenckii*. The phagocytosis of unopsonized yeast forms depends on the cell surface constituents as shown by neuraminidase treatment, by inhibition of phagocytosis by competitive low-molecular-weight ligands, and by isolated antigenic polymers. Peptidopolysaccharides which are released extracellularly can neutralize humoral antibodies and can be toxic to macrophages, as observed in vitro, and thus represent an important factor of pathogenicity of *S. schenckii*. *S. schenckii* surface rhamnomannans and galactomannans probably have a role in the binding of yeasts to macrophages, but these molecules are also excreted extracellularly. They are thus potential inhibitors of the phagocytosis of unopsonized *S. schenckii* as also observed in vitro.

As expected, ConA-opsonized cells were extensively phagocytized by thioglycolate-elicited mouse peritoneal macrophages. Receptors for ConA on *S. schenckii* cells were detected by a cytochemical method (24) and characterized as peptidorhamnomannan complexes and galactomannans (22–24). The ingestion by macrophages of *S. schenckii* yeast cells pretreated with ConA is similar to that of ConA-treated *Saccharomyces cerevisiae* (3). With *S. cerevisiae*, the attachment to macrophages of ConA-coated cells results in 80% ingestion of interacting yeasts. A maximum of 12 yeast cells per macrophage is reached at 200 μg/ml of ConA. Phagocytosis of *S. schenckii* yeast cells depended on the concentration of ConA used to opsonize cells as well as on the number of ConA-reactive receptors exposed at the cell surface. Such a difference was suggested by using two strains of *S. schenckii* which required different concentrations of mannose to inhibit their agglutination by the same concentration of ConA. Cells of one of these strains (1099.18) have a tendency to lose the outer layers of the cell wall during growth in a liquid medium or wash (4), resulting in forms with fewer surface receptors for ConA. The phagocytic index of ConA-opsonized cells of strain 1099.24 was approximately threefold more than that with strain 1099.18. The average yield obtained for the ingestion of ConA-treated yeast cells of *S. schenckii* (1099.24) was 10.3 ± 2.2 yeasts per macrophage, which is comparable to the value obtained for the attachment of *S. cerevisiae* to macrophages though at higher lectin concentrations (3).

Sialic acid residues present at the cell surface of *S. schenckii* yeast cells are important constituents protecting unopsonized fungal cells from

TABLE 7. Effect of preincubation of macrophages with *N*-acetylglucosamine on their capacity to ingest *S. schenckii* yeast cells[a]

Addition and preincubation time (min)	*S. schenckii* 1099.18		*S. schenckii* 1099.24	
	I	% of control	I	% of control
No addition	67.1 ± 4.8	100	64.4 ± 10.2	100
N-Acetylglucosamine (10 mg/ml)				
5	69.6 ± 11.9	103.7	62.3 ± 5.6	96.7
10	59.1 ± 5.0	88.0	56.5 ± 10.3	87.7
15	44.9 ± 4.4	66.9	44.6 ± 3.0	69.2
20	21.0 ± 0.6	31.2	16.3 ± 3.8	25.3

[a] I, Phagocytic index.

TABLE 8. Effect of *N*-acetylglucosamine concentration on the phagocytosis of yeast cells[a]

N-Acetylglucosamine concn (mg/ml)	*S. schenckii* 1099.18		*S. schenckii* 1099.24	
	I	% of control	I	% of control
0	74 ± 7.4	100	63.5 ± 10.6	100
1	69.6 ± 18.6	94	71.6 ± 8.3	112.7
2	49.4 ± 10.5	66.7	56.2 ± 9.7	88.5
5	36.5 ± 1.9	49.3	54.3 ± 9.9	85.5
10	18.7 ± 3.7	25.2	18.8 ± 5.1	29.6

[a] I, Phagocytic index.

TABLE 9. Percentage of macrophages binding yeast cells at 4°C with and without preincubation with *N*-acetylglucosamine

N-Acetylglucosamine concn (mg/ml)	% binding ± SD	
	S. schenckii 1099.18	*S. schenckii* 1099.24
0	35.0 ± 7.4	37.8 ± 9.8
10	5.3 ± 3.0	3.1 ± 2.1

phagocytosis. Acidic residues, detected by reaction with colloidal iron hydroxyde at low pH (4, 23) have been characterized as *N*-glycolylneuraminic acid units in a glycolipid fraction (2). Treatment of *S. schenckii* yeast cells with neuraminidase increased the phagocytic index 7.7-fold over that of untreated cells. It seems possible that removal of acidic groups from the cell surface may have increased its hydrophobicity, thus promoting the necessary interfacial tension (26) important for phagocytosis.

Receptors on macrophages for cell surface constituents of *S. schenckii* could be suggested by inhibition experiments with simple sugars and polymers. Previously, binding of *Propionibacterium acnes* and *S. typhimurium* to macrophages was inhibited by sugars present as constituents of the cell wall envelopes (7, 11, 12). Rhamnose, undetected in the lipopolysaccharide of an *S. typhimurium* mutant or in the wall of *P. acnes* was nevertheless inhibitory in both cases, suggesting lack of specificity (29). In *S. schenckii*, mannose, rhamnose, galactose, glucosamine, *N*-acetylglucosamine, and *N*-acetylneuraminic acid partially inhibited phagocytosis (less than 70% the phagoctic index of the control with no sugar added). Pentoses and glucose were inactive or slightly inhibitory. A mixture of some of the above sugars was more effective than individual substances as an inhibitor of phagocytosis. The inhibitory sugars are constituents of *S. schenckii* cell surface components (23). Isolated polysaccharides from *S. schenckii* including a purified rhamnomannan and a partially purified galactomannan were also inhibitors.

Interestingly, removal of sugar residues by β-elimination from a peptidopolysaccharide preparation abolished its toxicity, but the resulting compound did not inhibit phagocytosis.

The inhibitory effect of monosaccharides on the phagocytosis of *S. schenckii* could be amplified by using yeast cells which have been treated with neuraminidase. Since *N*-acetylglucosamine was the best inhibitor, it was used to standardize the condition for the inhibitory tests. At a 10-mg/ml sugar concentration, macrophages kept unaltered their capacity to phagocytize latex particles or sensitized sheep erythrocytes. The inhibitory activity of simple sugars on the phagocytosis of *S. schenckii* cannot thus be attributed to functional alterations of macrophages as suggested by others with high levels of sucrose and hyperglycemic concentrations of glucose (18, 25). The exposure of macrophage cultures for 24 h to several neutral monosaccharides at 10 to 20 mg/ml, including pentoses, glucose, and galac-

TABLE 10. Phagocytosis of sensitized erythrocytes (2%) by mouse peritoneal macrophages pretreated with monosaccharides[a]

Pretreatment	P	F	I
None	75.8 ± 3.8	12.5 ± 2.0	946 ± 139.7 (3)[b]
D-Galactose	73.5 ± 5.4	11.5 ± 1.3	843.2 ± 88.8 (3)
N-Acetylglucosamine	72 ± 3.1	13.4 ± 0.8	968.6 ± 99.2 (3)
L-Rhamnose	74.3 ± 6.2	14.5 ± 2.1	953.9 ± 181.2 (3)

[a] Monosaccharides added at 10 mg/ml for 20 min. P, F, and I ± standard deviations are as in Table 1, footnote *a*.

[b] Number of animals used is shown parenthetically.

tose, irrespective of whether they were metabolizable by the cells, was not followed by vacuolization. Cells were morphologically identical to control macrophages cultivated in unsupplemented serum (6). In our experience, *N*-acetylglucosamine induces vacuolization after 30 min of incubation with macrophages alone. Since macrophages used in phagocytosis experiments were gently washed (7) after 20 min of preincubation with sugars, direct effects of the latter on macrophage physiology were minimized during the phagocytosis period. It seems therefore that the inhibition of phagocytosis by sugars reflects the existence of receptors on macrophages for *S. schenckii* surface components which are indispensable for the cell-binding initial step. We do not know whether the receptor(s) of mouse peritoneal macrophages responsible for binding mannose-containing cell surface components of *S. schenckii* are similar to the common receptor in alveolar macrophages which recognizes mannose/glucosamine-glycoproteins and binds *Candida krusei* (27). However, since *N*-acetylglucosamine is more efficient than mannose as an inhibitor of *S. schenckii* binding and ingestion and is not a prominent constituent of the yeast cell wall (15) it seems that the receptor in peritoneal macrophages has a different specificity. A relatively high affinity for monosaccharides is also suggested for the receptors of mouse peritoneal macrophages since the inhibitory activity of phagocytosis and particle binding by simple sugars is not affected by washing.

The above results point to the existence of multiple carbohydrate receptors on the macrophage membrane which specifically recognize *S. schenckii* cell surface components. Another alternative is the occurrence of carbohydrate-binding receptors of broader specificity. The *N*-acetylglucosamine-binding receptor of macrophages suggested in the present work seems to fall in the second category.

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