ACTIVATION OF MACROPHAGES BY POLYSACCHARIDES FROM YEAST-LIKE FUNGI

M. A. Kashkina and I. S. Freidlin

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Reports have recently been published on the adjuvant activity of certain polysaccharides obtained from yeasts and other species of fungi [1, 2, 4] and on their ability to stimulate granulocytopoiesis and monocytopoiesis and to activate macrophages [5, 7, 14]. In other publications on mechanisms of the immunopotentiating action of polysaccharides, attention has been concentrated on activation of T- and B-cells, and doubts have been expressed on stimulation of phagocytosis [6, 10, 12]. Some polysaccharides of yeast origin are regarded as potential substances for immunotherapy, and for that reason the precise mechanism of their action must be clarified.

The object of the present investigation was to study the effect of polysaccharides from yeast-like fungi on the various manifestations of functional activity of mouse peritoneal macrophages cultured in vitro.

EXPERIMENTAL METHOD

The following homopolysaccharides were used in the experiments: mannans—linear polysaccharides consisting of mannopyranose residues connected alternately by β-1,3- and β-1,4-bonds; dextrans, consisting to the extent of 95% of α-1,6- linked glucans and a glucan consisting of α-1,4-, α-1,6-, and β-1,3-glucopyranosylglucose (on average 50% of the structure of the glucan molecule is accounted for by β-1,3-bonds).

Macrophages were cultured in vitro by the method described previously [3]. To study the effect of polysaccharides on functional activity of the macrophages, the preparations were added to the culture medium in a final concentration of 200 μg/ml, which was nontoxic for the macrophages. The cells were incubated in the

TABLE 1. Activity of Adhesion of Macrophages under the Influence of Polysaccharides

Producer of polysaccharide	Polysaccharide	Working number of polysaccharide	Index of adhesion
Rhodotorula rubra	Mannan (65 000)	14	1,12±0,05
Sporobolomyces species	Mannan (120 000)	26	1,16±0,06
Aureobasidium pullulans	Glucan (9 000 000)	28	1,3±0,13
Leuconostoc mesenteroides	Dextran (75 000)	1	1,11±0,08
	Dextran (1 000 000)	2	1,12±0,05
	Dextran (100 000 000)	3	1,20±0,06

Legend. Mol. wt. given in parentheses.

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TABLE 2. Phagocytosis of E. coli Cells under the Influence of Polysaccharides (M ± m)

Working number of polysaccharide*	Stage of ingestion		Stage of digestion
	per cent of phagocytosing cells	phagocytic index	per cent of digesting macrophages
Control	54±1,6	1,1±0,08	70±2,1
14	70±2,1	2,4±0,2	70±2,9
26	75±1,4	2,1±0,15	85±1,4
28	81±1,9	1,8±0,1	89±2,0
1	57±3,1	1,06±0,05	71±1,9
2	52±2,5	0,9±0,18	70±1,5
3	60±4,1	1,2±0,14	68±2,8

*For names of polysaccharides see Table 1.

presence of polysaccharides for between 2 and 48 h.

The effect of polysaccharides on adhesion of the macrophages to glass [8], the phagocytic activity of the macrophages against latex particles 1.4 μ in diameter and killed Escherichia coli cells labeled with fluorescein isothiocyanate, and on the intensity of the process of digestion and the chemotaxic activity of the macrophages [13] was studied.

EXPERIMENTAL RESULTS

Quantitative estimation of adhesiveness of the macrophages to glass in the presence of polysaccharides compared with adhesion of the cells in control cultures showed that some of the preparations potentiated adhesion (Table 1).

Glucan and dextran with high molecular weight were most active in this respect, and mannans and dextrans with lower molecular weights were less active. The effect of potentiation of macrophage adhesion was most marked in the case of the mannan of Sporobolomyces sp. (No. 26), with mol. wt. 120,000. The ability of polysaccharides to potentiate macrophage adhesion to glass may perhaps increase with a rise in their molecular weight.

The ability of yeast polysaccharides to potentiate adhesion of macrophages to glass revealed by these experiments can be regarded as an indication of nonspecific activation of macrophages connected with a change in the properties of the plasma membrane of the cells and with activation of their metabolism [11].

Changes in the principal function of macrophages (phagocytosis) under the influence of yeast polysaccharides also demonstrated the activating action of the preparations. As Table 2 shows, the phagocytic part of the macrophage population increased by the greatest degree under the influence of the glucan and by a rather lesser degree under the influence of mannans. Dextrans had no effect on phagocytic activity.

Similar results were obtained when phagocytosis of latex particles under the influence of polysaccharides was studied.

The process of intracellular digestion of bacteria by the macrophages was stimulated by only two of the six preparations tested: glucan (No. 28) and a mannan (No. 26) with mol. wt. 120,000.

These results are evidence of the nonspecific activating action of β-structured polysaccharides on the phagocytic function of macrophages.

Using a comparative study of the activity of the polysaccharides listing factors promoting chemotaxis of macrophages, the highest activity was found in the mannans (index of chemotaxis 1.9 ± 0.1 to 2.0 ± 0.2). Dextrans Nos. 1 and 3 (index of chemotaxis 1.3 ± 0.25) had a weaker chemotaxic action. As regards glucan No. 28, it had no properties of chemical attraction for macrophages whatsoever (index of chemotaxis 1.0 ± 0.05).

The high-molecular-weight glucan, which had not only a chemotaxic action on the cells, had the most marked activating effect on all functions of the macrophages studied. Mannans, stimulating the function of ingestion, had a less marked activating effect. The degree of activity of these polysaccharides depended

directly on their molecular weight. The mannan of Sporobolomyces sp. (mol. wt. 120,000) stimulated the functions of ingestion and digestion of the macrophages to a greater degree than the mannan of Rhodotorula rubra, which has a molecular weight only half as large. Of all the polysaccharides tested, dextrans were the least active. Only the dextran with the highest molecular weight (100,000,000) stimulated adhesion of the macrophages to glass.

The results of this investigation thus shed light on some new aspects of the biological activity of yeast polysaccharides. This activity is manifested variously in experiments on cultures of macrophages and it depends not only on the molecular weight of the polysaccharides, but also on their structure. Mannans of Rh. rubra and Sporobolomyces sp., with β-bonds between their monosaccharide units, and also the glucan of Aureobasidium pullulans, in which β-bonds account for 50% of the total on average, are able to activate the functions of macrophages. The β-1,3-glucan of the cell wall of Saccharomyces cerevisiae has a similar action [9]. The α-structured dextrans which were studied do not possess these properties.

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