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Note

Protective effect of D-mannan of bakers' yeast against *Staphylococcus aureus* infection in mice

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Previously, we reported that D-mannan and D-glucan of *Saccharomyces cerevisiae* were growth-inhibitory against allogeneic tumor cells transplanted subcutaneously into mice[1,2], and that both polysaccharides displayed adjuvant action enhancing carbon-clearance activity of macrophage[3]. It is well known that macrophages are important effector elements in hosts' defense mechanisms, and lysosomal-enzyme activities in macrophages and lysozyme activity in serum have been regarded as indexes of the macrophage function[4,5]. DiLuzio and Williams[6], and Kokoshis *et al.*[7] have shown that prior treatment of mice with the D-glucan of *S. cerevisiae* significantly enhanced their survival when systematically challenged with *Staphylococcus aureus*. We therefore investigated the relationship between the protective effect of D-mannan of *S. cerevisiae* against *S. aureus* infection in mice, the phagocytosis and the change of activities of a few lysosomal enzymes of peritoneal macrophages, and serum lysozyme.

The effect of treatment with D-mannan for its protective action against *S. aureus* is shown in Table I. The mice were injected intraperitoneally with D-mannan

TABLE I

EFFECT OF D-MANNAN TREATMENT ON THE SURVIVAL OF ddY MICE WITH *S. aureus*

Addition	*Dose (mg/kg × 6)*	*Mortality (death/total)*	*Survival (%)*
None		16/21	24
D-Mannan	150	2/21	90

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TABLE II

EFFECT OF D-MANNAN TREATMENT ON PHAGOCYTIC ACTIVITY OF PERITONEAL MACROPHAGES[a]

Addition to S. aureus	*Average number of bacteria ingested by macrophages/ total number of bacteria*	*p*
None	0/9,400	
Macrophages of normal mice	398/9,400	<0.01[b]
Macrophages of D-mannan-treated mice	4,210/9,400	<0.01[c]

[a]See Experimental section for procedure. [b]Vs. the group of *S. aureus* without macrophages. [c]Vs. the group of *S. aureus* with macrophages of normal mice.

6 times in a dose of 150 mg/kg/day. The cells of *S. aureus* were injected intravenously one day after the last injection of D-mannan. On day 60, only 24% of the control mice survived as opposed to 90% survival in the D-mannan-treated group ($p < 0.01$). *In vitro* phagocytic activity of peritoneal macrophages of D-mannan-treated mice was measured by the method of Leijh *et al.*[8] and is reported in Table II. The phagocytic activity of peritoneal macrophages from the D-mannan-treated mice against *S. aureus* was about 10 times higher than that of the macrophages obtained from normal mice. The kinetics of the activity of the three lysosomal enzymes of peritoneal macrophages of ddY mice treated *in vivo* with D-mannan are shown in Figs. 1, 2,

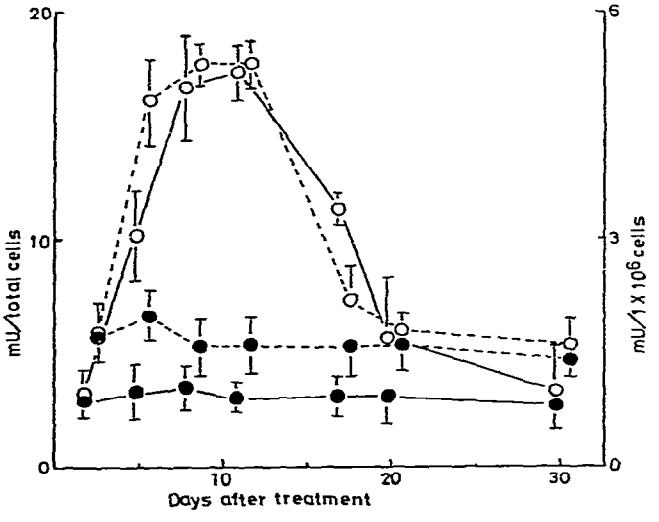

Fig. 1. Kinetics of acid phosphatase activity of peritoneal macrophages of mice treated with D-mannan. The mice were examined at day 2, 5, 8, 11, 17, 20, and 30 after administration of the D-mannan. Experimental conditions were as described in the text: (○) Mice treated with D-mannan at a dose of 150 mg/kg/day, 10 times; (●) mice left without a treatment as control; (——) mU/total cells; (---) mU/10^6 cells. Bar: mean value ± S.D.

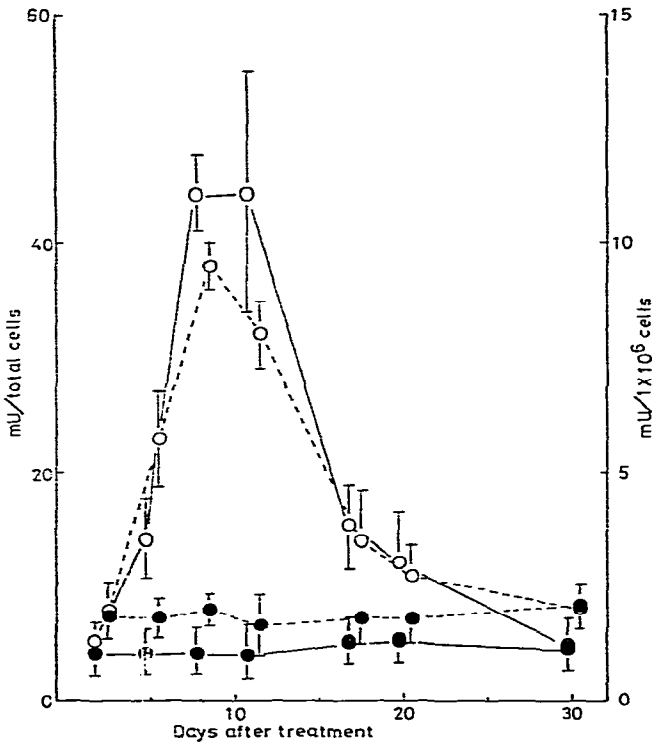

Fig. 2. Kinetics of β-D-glucuronidase activity of peritoneal macrophages of mice treated with D-mannan. Experimental conditions were as described in the text and legend to Fig. 1.

and 3. The total activity (per mouse) and that for 10^6 cells of both enzymes (acid phosphatase and β-D-glucuronidase) increased to 3–7 times and to 3–4 times against the control group in the initial stage (5–11 days) (Figs. 1 and 2). Total lysozyme activity also increased to 2–4 times and that for 10^6 cells to 1.5 times. The effect of D-mannan on enzyme activities of macrophages *in vitro* is shown in Fig. 4. The D-mannan in doses up to 4 mg/mL was found to enhance gradually the acid phosphatase activity of macrophages. β-D-Glucuronidase and lysozyme activity also increased to ~1.3 times against that of the control group for a dose of 4 mg/mL of the D-mannan. Figure 5 shows the results of kinetics of lysozyme activity in the serum of mice treated 10 times with D-mannan at a dose of 150 mg/kg/day. The curve reached a peak at 7–10 days after the beginning of D-mannan administration, and then the activity gradually decreased to the control level.

The D-mannan conferred protection against *S. aureus* infection in mice. Although the entire aspect of the protection mechanism against microbial infection is not yet clear, participation of lysosomal enzymes and release of active oxygen by the macrophages are considered very important factors in this mechanism. To clarify the mechanism of action of D-mannan, the effect on peritoneal macrophages was examined. The phagocytic activity of peritoneal macrophages for *S. aureus* increased

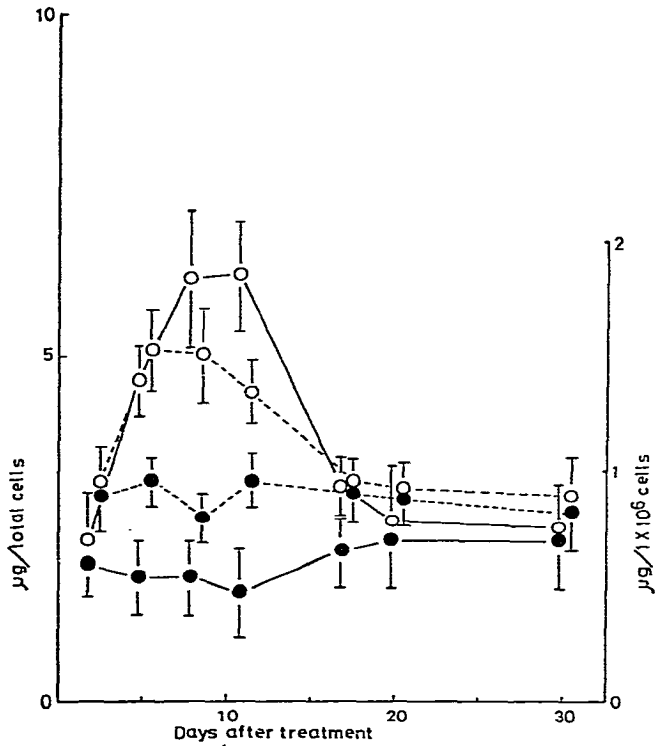

Fig. 3. Kinetics of lysozyme activity of peritoneal macrophages of mice treated with D-mannan. Experimental conditions were as described in the text and legend to Fig. 1.

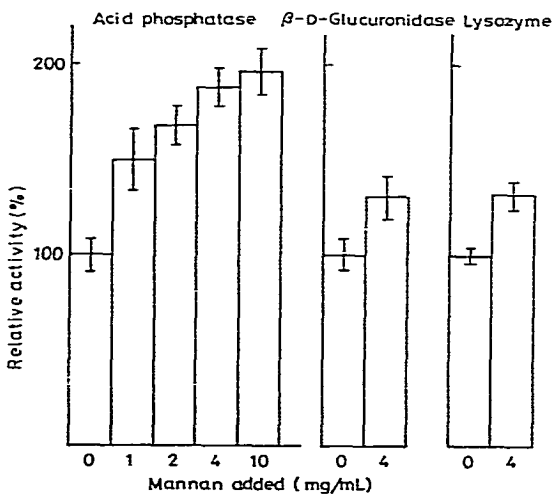

Fig. 4. Acid phosphatase, β-D-glucuronidase, and lysozyme activity of macrophage lyzates. The cultures of peritoneal macrophages (2×10^5) were challenged with various doses (mg/mL) of D-mannan. Enzyme activity was determined after 24 h of incubation. Bar: mean value ± S.D.

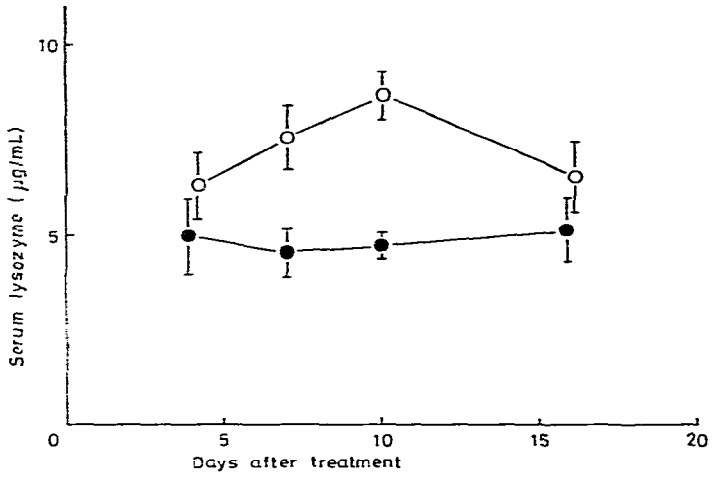

Fig. 5. Kinetics of serum lysozyme of mice treated with D-mannan. The mice were injected intraperitoneally with D-mannan at a dose of 150 mg/kg/day, 10 times. The mice were examined at day 4, 7, 10, and 16 after the beginning of D-mannan administration: (●) Control; (○) D-mannan-administered mice. Bar: mean value ± S.D.

much more in D-mannan-treated mice than in control mice, as shown in Table II. Furthermore, treatment with D-mannan resulted in a pronounced increase of the activity of lysosomal enzymes of the peritoneal macrophages (Figs. 1–4), and of the activity of serum lysozyme (Fig. 5) in mice, with results similar to those obtained with D-glucan[5,7]. Klockars and Roberts[9], and Namba *et al.*[10] also suggested that increased lysozyme concentration might contribute to bactericidal properties by providing the host with a nonspecific defense mechanism. Therefore, it is suggested that D-mannan treatment protects mice against experimental infection of *S. aureus* by stimulating macrophage activity. Recently, it has been reported that the D-mannan from *S. cerevisiae* inhibited, in neutrophiles, the release of myeloperoxidase, which was shown to be stimulated, *in vitro*[11], by phagocytosis of serum-opsonized zymosan.

EXPERIMENTAL

Materials. — Fresh, whole cells of bakers' yeast (*S. cerevisiae*) were supplied by the Oriental Yeast Co., Ltd., Tokyo, Japan. Male ddY mice 6- to 8-weeks old were used. D-Mannan of *S. cerevisiae* wild-type strain was prepared by the Fehling-solution method as described previously[1].

Protection assay against infectious challenge. — D-Mannan was administered intraperitoneally to the mice 6 times in a dose of 150 mg/kg/day[1–3]. The protection assay was performed as follows: *S. aureus* βH 248 strain was grown in Budokyukin (Staphylococci) medium (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) for 43 h at 37°, and the harvested cells were suspended in saline solution. The cells (1.5×10^9) were injected intravenously into the tail vein of the mice one day after the last in-

jection of D-mannan. Subsequently, the mice were observed for survival for 60 days.

Assay of phagocytic activity. — This assay was conducted according to the method of Leijh *et al.*[8] as follows: D-mannan was administered to the mice intraperitoneally 6 times in a dose of 150 mg/kg/day. The peritoneal exudate cells were obtained one day after final administration of D-mannan. The cells (1×10^6/0.5 mL) in Hanks' BSS were plated in microplate of 1.5 cm diameter (A/S NUNC, Roskilde, Denmark). After the nonadherent cells were removed, the adherent cells were incubated with the preopsonized bacteria (1×10^4/0.3 mL) for 30 min at 37°. Preopsonized bacteria were obtained by incubation of the suspensions of *S. aureus* in Hanks' BSS with 10% (v/v) fresh, normal-mouse serum for 30 min at 20°. The macrophages were washed with Hanks' BSS containing heparin (0.5 unit per mL) to remove all free extracellular bacteria, and distilled water (1.0 mL) containing 0.01% (w/v) bovine serum albumin was added to the cell pellet. Lysis of the macrophages was obtained by alternately freezing the suspension in a dry ice-acetone bath and quickly thawing it in a water bath (37°), three times. The solution was diluted 10 times with saline solution, and an aliquot (0.1 mL) was plated in the Budokyukin (Staphylococci) medium. The plate was incubated for 24 h at 37°, and the number of colonies of viable bacteria counted with a colony counter.

Assay of enzyme activity. — D-Mannan was administered intraperitoneally to the mice 10 times in a dose of 150 mg/kg/day[1-3]. The cells of peritoneal exudate at day 2, 5, 8, 11, 17, 20, and 25 after D-mannan administration were obtained by washing the peritoneal cavity with Hanks' BSS containing 5 units of heparin per mL. The PEC in Hanks' BSS was plated in Petri dishes (A/S NUNC, Roskilde, Denmark) of 3-cm diameter, and incubated for 2 h at 37° in a carbon dioxide incubator. After the nonadherent cells had been removed, the adherent cells were suspended according to the method of Schorlemmer *et al.*[12] and counted with a hemocytometer. More than 95% of these adherent cells were macrophages determined by their nuclear shape after staining with the Giemsa stain, and by their phagocytic activity for latex particles. Treatment of macrophages in monolayers prepared *in vitro* were treated with D-mannan as follows: Peritoneal macrophages (2×10^5) from ddY normal mice were placed in each well of a microplate (167008 A/S NUNC, Roskilde, Denmark). After change of the medium, various doses (mg/mL) of D-mannan were added to each well, and the cells were cultured for 24 h. After the incubation period was complete, the medium was removed and the cells were lyzed with water (0.1 mL). Blood was drawn from the carotid of mice treated with D-mannan, and the serum was separated by centrifugation. The activities of acid phosphatase and β-D-glucuronidase were determined by measuring the *p*-nitrophenol liberated from *p*-nitrophenyl phosphate and *p*-nitrophenyl β-D-glucopyranosiduronic acid, respectively, according to the method of Komatsu *et al.*[13]. One milliunit (mU) was defined as the amount of enzyme releasing 1 nmol of *p*-nitrophenol within 1 min. Lysozyme activity was measured generally by its action on a suspension of *Micrococcus lysodeikticus* cells (Miles Laboratories Inc., Kankakee, U.S.A.) according to the method

of Parry *et al.*[14]. Lysozyme activity was also measured with the method of Woollen *et al.*[15]. The *t* test was used to compare the data of test groups and their controls.

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REFERENCES

1 S. SUZUKI, M. SUZUKI, H. HATSUKAIWA, H. SUNAYAMA, T. SUZUKI, M. UCHIYAMA, F. FUKUOKA, M. NAKANISHI, AND S. AKIYA, *Gann*, 60 (1969) 273–277.
2 T. MATSUMOTO, M. TAKANOHASHI, Y. OKUBO, M. SUZUKI, AND S. SUZUKI, *Carbohydr. Res.*, 83 (1980) 363–370.
3 M. SUZUKI, F. CHAKI, AND S. SUZUKI, *Gann*, 62 (1971) 553–556.
4 Z. A. COHN AND B. BENSON, *J. Exp. Med.*, 121 (1965) 153–170.
5 N. R. DILUZIO, *J. Reticuloendothel. Soc.*, 26 (1979) 67–81.
6 N. R. DILUZIO AND D. L. WILLIAMS, *Infect. Immun.*, 20 (1978) 804–810.
7 P. L. KOKOSHIS, D. L. WILLIAMS, J. A. COOK, AND N. R. DILUZIO, *Science*, 199 (1978) 1340–1342.
8 P. C. J. LEIJH, M. T. VAN DEN BARSELAAR, T. L. VAN ZWET, M. R. DAHA, AND R. VAN FURTH, *J. Clin. Invest.*, 63 (1979) 772–784.
9 M. KLOCKARS AND P. ROBERTS, *Acta Haematol.*, 55 (1976) 289–295.
10 Y. NAMBA, Y. HIDAKA, K. TAKI, AND T. MORIMOTO, *Infect. Immun.*, 31 (1981) 580–583.
11 C. D. WRIGHT, M. J. HERRON, G. R. GRAY, B. HOLMES, AND R. D. NELSON, *Infect. Immun.*, 32 (1981) 731–738.
12 H. U. SCHORLEMMER, J. H. EDWARDS, P. DAVIES, AND A. C. ALLISON, *Clin. Exp. Immunol.*, 27 (1977) 198–207.
13 N. KOMATSU, K. EGAWA, AND N. NAGUMO, *Jpn. J. Antibiot.*, 26 (1973) 61–67.
14 R. M. PARRY, R. C. CHANDAN, AND K. M. SHAHANI, *Proc. Soc. Exp. Biol. Med.*, 119 (1965) 384–386.
15 J. W. WOOLLEN, R. HEYWORTH, AND P. G. WALKER, *Biochem. J.*, 78 (1961) 111–116.