PROTECTIVE EFFECT OF ACIDIC MANNAN FRACTION OF BAKERS' YEAST AGAINST *STAPHYLOCOCCUS AUREUS* INFECTION IN MICE

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Protective activity of an acidic fraction of bakers' yeast mannan containing protein and phosphorus, designated as WAM025, against infection of *Staphylococcus aureus* β H 248 strain in mice was investigated. WAM025 elicited a marked increase in the survival ratio of mice challenged with viable cells of the *S. aureus* strain, 5×10^8 cells per mouse, when the fraction was administered to mice 150 mg/kg/d, 5 times, intraperitoneally. This effect was stronger than that of WNM, a neutral fraction of mannan obtained from the same bakers' yeast. The difference seemed to correlate with the strength of activating effects of WAM025 and WNM on the reticuloendothelial system of the host animal. WAM025 induced higher activities of serum lysozyme and carbon clearance in mice than WNM. Also, mice treated with WAM025 showed a greater increase in number and activity of oxygen-generating blood polymorphonuclear leucocytes than mice treated with WNM.

Keywords — acidic mannan; *Staphylococcus aureus*; infection; serum lysozyme; carbon clearance; polymorphonuclear leucocyte; chemiluminescence

INTRODUCTION

Many immunopotentiators have been reported to stimulate the resistance of animals against microbial infections. Di Luzio and Williams,¹⁾ and Kokoshis *et al.*²⁾ have shown that prior treatment of mice with a glucan of *Saccharomyces cerevisiae* significantly increased the survival ratios of mice when the animals were systemically challenged with *Staphylococcus aureus*. We have previously reported that a neutral mannan of bakers' yeast (WNM) conferred protection against *S. aureus* infection in mice.³⁾ In the present paper results are presented that indicate that an acidic mannan fraction of bakers' yeast designated as WAM025 protected ddY strain mice from *S. aureus* β H 248 strain infection. Experimental findings relative to the protective mechanism of this acidic mannan are also presented.

MATERIALS AND METHODS

Mice — Male ddY mice 6- to 8-week old were used.

Mannan Fraction — Bulk mannan of bakers' yeast were fractionated on a column of DEAE-Sephadex A-50 according to the method of Suzuki *et al.*^{4,5)} Mannan subfractions, eluted from the DEAE-Sephadex column with water and 0.25 M NaCl, were designated as WNM and WAM025, respectively, and used in this study. An analysis of fraction WAM025 showed this fraction to contain sugar (93.6%), nitrogen (1.0%) and phosphorus (0.6%) and fraction WNM to lack inorganic phosphate.⁶⁾

Treatment of Mice — Mice were injected intraperitoneally with appropriate mannan in doses of 10, 50 or 150 mg/kg/d, for 5 d. Assays were performed at day 6 after of the first mannan administration.

Protective Assay against Infectious Challenge of

S. aureus — A protection assay was performed in accordance with the method described in a previous paper.³⁾ The cells of *S. aureus* β H 248 strain in saline solution (5×10^8) were injected intravenously into the tail vein of the mice one day after the last injection of WAM025 or WNM. Subsequently, the mice were observed for survival for 60 d.

Assay of Serum Lysozyme Activity — Blood was drawn from the carotid of mice treated with each mannan in a dose of 150 mg/kg/d, for 5 d, and the serum was separated by centrifugation. Lysozyme activity was assayed according to Parry *et al.*⁷⁾ as described previously.³⁾

Assay of Phagocytic Activity — WAM025 was administered intraperitoneally to mice for 5 d in a dose of 150 mg/kg/d. The phagocytic activity of reticuloendothelial system was examined by measuring the clearing rate of colloidal carbon in the mouse blood according to the method of Biozzi *et al.*⁸⁾ Phagocytic index (*K*) was calculated by the following formula:

$$K = \frac{\log C_1 - \log C_2}{T_2 - T_1}$$

where C_1 and C_2 are the carbon concentrations at time T_1 and T_2 .

Preparation of Polymorphonuclear Leucocytes in Blood — Preparations of polymorphonuclear leucocytes (PMN) were obtained by a procedure

described in a previous paper⁹⁾ that combined dextran sedimentation and lympholyte-M centrifugation methods.

Assay of Active Oxygen-Generating Activity — Chemiluminescence response of PMN was measured according to the method of an earlier paper⁶⁾ using Biolumat LB 9500 (Berthold Co., Wildbad, West Germany).

RESULTS

Protective Effect of the Acidic Mannan Fraction, WAM025, on S. aureus Infection

Mice treated with WAM025 received an infectious challenge of *S. aureus*, 5×10^8 cells, per mouse, at day 6 after the beginning of administration of mannan. The dose dependency of the protective activity of WAM025 was observed at doses of 10 to 150 mg/kg (Table I). It was found that the protection activity induced by WAM025, at all doses was greater than that induced by WNM, a neutral mannan fraction of the same bakers' yeast. When mice were treated with 150 mg/kg of WNM and WAM025, 74 and 89% of the animals, respectively, survived. Figure 1 shows the result of the survival experiment. The control mice which were not treated with WAM025 or WNM succumbed to *S. aureus* infection by day 4. On the other hand, 80 and 60% of mice treated with WAM025 and WNM, respectively, survived.

TABLE I. *Effect of WAM025 on the Survival of Mice Infected with S. aureus*

	Dose (mg/kg/d $\times$ 5)	Mortality (death/total mice)				Survival (%)
		Exp 1	Exp 2	Exp 3	Total	
Control		11/15	7/10	10/10	28/35	20
WAM025	10		5/10		5/10	50
WAM025	50		3/10		3/10	70
WAM025	150	1/15	1/10	2/10	4/35	89 ^{a,b)}
WNM	10		8/10		8/10	20
WNM	50		5/10		5/10	50
WNM	150	2/15	3/10	4/10	9/35	74 ^{a)}

a) Significant difference from the values of control at $p < 0.01$. b) Significant difference from the values of WNM group at $p < 0.05$. *p* values were obtained by the adjusted chi-square method.

Serum Lysozyme Activity in Mice Administered with Either WAM025 or WNM

Serum lysozyme activity of mice treated with either WAM025 or WNM was significantly higher than that of the mice of the untreated

FIG. 1. Survival Rates of WAM025 and WNM Treated Mice Infected with *S. aureus*

WAM025 or WNM was administered at 150 mg/kg daily for 5 d intraperitoneally. Mice were infected with 5×10^8 cells of *S. aureus* intravenously 1 after day the final administration of mannan.

●, control; ▲, WNM; ■, WAM025.

FIG. 2. Serum Lysozyme in Mice Administered with Either WAM025 or WNM

The mice were injected intraperitoneally with WAM025 or with WNM on days 0–4. Serum lysozyme activity was determined on day 5. Bar: mean $\pm$ S.D.

1, control; 2, WNM 150 mg/kg/d; 3, WAM025 150 mg/kg/d.

FIG. 3. Phagocytic Activity of Mice Administered with Either WAM025 or WNM

The mice were injected intraperitoneally with WAM025 or with WNM on days 0–4. Phagocytic activity was determined on day 5. Bar: mean $\pm$ S.D.

1, control; 2, WNM 150 mg/kg/d; 3, WAM025 150 mg/kg/d.

FIG. 4. Number of Blood Polymorphonuclear Leucocytes in Mice Administered with Either WAM025 or WNM

WAM025 or WNM was administered i.p. on days 0–4, and the number of PMN was determined on day 5.

Bar: mean $\pm$ S.D.

1, control; 2, WNM 150 mg/kg/d; 3, WAM025 150 mg/kg/d.

control (Fig. 2). The difference between the activities of WAM025- and WNM-administered groups was small.

Phagocytic Activity of Mice Administered with Either WAM025 or WNM

Figure 3 shows the carbon clearance activity of mice treated with either WAM025 or WNM at a dose of 150 mg/kg/d for 5 d. WAM025 and WNM stimulated carbon clearance by the reticuloendothelial system but WAM025 was the stronger of the two mannan preparations.

Number of Blood PMN in Mice Treated with Either WAM025 or WNM

The effects of WAM025 and WNM on blood PMN are shown in Fig. 4. The number of blood PMN in mice treated with WAM025 increased about 2 times the number in the control group, whereas the cell number of mice treated with WNM remained unchanged.

Chemiluminescence Response of Blood Polymorphonuclear Leucocytes in Mice Administered with WAM025

FIG. 5. Chemiluminescence Response of Blood Polymorphonuclear Leucocytes in Mice Administered with Either WAM025 or WNM

WNM or WAM025 was administered i.p. on days 0–4, and chemiluminescence response of PMN was determined on day 5.

Bar: mean ± S.D.

1, control; 2, WNM 150 mg/kg/d; 3, WAM025 150 mg/kg/d.

Figure 5 shows chemiluminescence response of blood PMN in mice treated with WAM025 or WNM. The activity for 10⁵ cells of blood PMN of mice treated with WNM showed a slight increase compared with the cells from the control. However, the activity per fixed number of cells from mice treated with WAM025 increased about 3 times the activity of the cells from the control mice. However, the total activity per mouse increased about 1.5 and 6 times with WNM and WAM025.

DISCUSSION

Bacillus Calmette-Guérin (BCG),¹⁰⁾ *Corynebacterium parvum*,^{10,11)} *Streptovorticillium cinnamomeum* cell wall,¹²⁾ yeast glucan^{1,2)} and neutral mannan³⁾ have been known as effective immunopotentiators for *S. aureus* infection in mice. In the present study, a comparison of the protective effect of two bakers' yeast mannan fractions, WNM and WAM025, on *S. aureus* infection was conducted. The most significant finding of this comparative protection study was that WAM025 was more active than WNM in protecting mice from *S. aureus* infection.

For the study on the mechanism of WAM025 protection of mice to *S. aureus* infection, experiments concerning the stimulative effect of this mannan on the function of mouse reticuloendothelial system were performed. It is well-known that macrophages are important effector elements in the defense mechanism of host, and therefore sera were assayed for indices of macrophage function.¹³⁾ Additionally, as suggested by Cappuccino *et al.*,¹⁴⁾ the augmentation of lysozyme activity may be the result of stimulation of the reticuloendothelial system of the host. In a previous study,⁶⁾ we reported that lysosomal enzyme activities of peritoneal phagocytes of mice treated with WAM025 were much higher than those of the cells from mice treated with WNM. Klockars and Roberts,¹⁵⁾ and Namba *et al.*¹⁶⁾ also suggested that increased lysozyme concentration might contribute to bactericidal effect by providing the host with a non-specific defense mechanism. The lysozyme activity of

sera of mice treated with WAM025 was much higher than the activity of sera from mice treated with WNM. We also assayed the carbon clearance activity of mice treated with both mannans. As expected, activity to clear carbon in mice treated with WAM025 was clearly greater than that in mice treated with WNM.

Polymorphonuclear leucocytes (PMN) are found to generate a variety of active oxygen radicals, *i.e.*, superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen (1O_2) and hydroxyl radical ($\cdot OH$), which might participate in bactericidal function of PMN against *S. aureus* as reported by several workers.¹⁷⁻²¹⁾ Therefore, an assay of the number of PMN in mice administered with the WAM025 and chemiluminescence response of the same PMN were conducted. Although both WAM025 and WNM increased protection of mice against *S. aureus* infection, serum lysozyme level, carbon clearance activity and active oxygen-generation of blood PMN, when compared with the results of the control group, it is obvious that the treatment of mice with WAM025 rather than with WNM resulted in a pronounced elevation of these activities. In a previous paper,⁶⁾ we reported that mice administered with WAM025 showed a potent increase in number of peritoneal exudate cells with elevation of lysosomal enzyme and active oxygen-generation activities. Thus, it is considered that activation of the increase of number of phagocytes and potentiation of these cells in the whole mouse serve as strong factors of protection against *S. aureus* infection.

In a previous report,⁶⁾ it was demonstrated that WAM025 was able to display a marked antitumor activity to allogeneic and syngeneic ascites tumors, while WNM did not display such antitumor activity. Because WAM025 contains both protein and phosphorus, constituting phosphomannan-protein complex(es), while WNM consisted of a simple polysaccharide with a very small amount of protein, it is suggested that one of the constituents in WAM025 may display some important role in evoking antitumor activity.⁶⁾ The results of the present study indicate

that WAM025 was able to exhibit a higher protective effect against *S. aureus* infection in mice than that by WNM. Even though WNM was found to be protective against *S. aureus* infection³⁾ and ineffective in growth-inhibition against ascites tumors in mice, it is reasonable to assume that either a peptide moiety or a phosphate group, or both, in addition to the polysaccharide moiety in WAM025 may be essential in inducing both antitumor and antimicrobial effects.

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