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Enhancement of Resistance to Infectious Diseases by Oral Administration of Brewer's Yeast

YEHUDITH SINAI, A. KAPLUN, Y. HAI, AND BILHA HALPERIN
Israel Institute for Biological Research and Tel Aviv University Medical School, Ness-Ziona, Israel

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The effect of oral administration of brewer's yeast on resistance to infectious diseases was studied in laboratory animals. It was shown that there was a significantly increased enhancement of resistance to seasonal respiratory and enteric infections in rhesus monkeys. Similarly, enhanced resistance to experimental chronic infections was observed in mice after yeast administration. A 2-week lag occurred between the initiation of yeast treatment and the expression of enhanced resistance. Study of the mechanism of the yeast-induced enhancement of resistance to infection leads to the conclusion that it is based on in vivo stimulation of phagocytosis, as measured by the "phagocytic index." No effect of brewer's yeast on circulating antibody levels was detected.

Host resistance to invading microorganisms is based on two types of immune response: an acquired one, to which specificity is usually attributed; and an innate, or nonspecific, response, resulting from the participation of several components in the interaction between host and pathogen (24). Among the latter, inflammation (5), phagocytosis (13), natural antibodies (16), properdin (19), and complement (12) are considered to be the major participants. The nonspecific resistance of the host may be modulated by different agents of a nonspecific nature (8, 22). Recognition of the importance of enhancing agents in prophylaxis and therapy has spurred the search for these effectors. Among these, compounds like endotoxin (18), BCG (14), polynucleotides (4), and zymosan (11) have been widely studied.

In the present study, we describe some of our experiments in the enhancement of nonspecific resistance to infectious diseases in laboratory animals by oral administration of brewer's yeast. A mechanism for this stimulation is suggested, and the relationship of brewer's yeast to other resistance-enhancing agents is discussed.

MATERIALS AND METHODS

Monkeys. Monkeys of strain *Macaca mulata*, imported from India, were quarantined on arrival at our Institute for 6 weeks. Monkeys of both sexes, weighing 2 to 2.5 kg, were caged singly and fed a diet consisting of cereal-pelleted animal food (Ambar Ltd., Israel), seasonal vegetables, fruit, and a multivitamin preparation (Rafa-9, Rafa Laboratories Ltd., Jerusalem, Israel). Sick animals were given appropriate antibiotic and supportive treatment.

Mice. Female mice (25 to 35 g) of an inbred R III strain and a random-bred Swiss albino strain were used. They were fed pelleted chow (Ambar Ltd., Israel) and water ad libitum.

Brewer's yeast. *Saccharomyces cerevisiae*, dried, in tablets each containing 500 mg, was obtained from Pharmaceutical Products "Rekah" Ltd., Azor; or Taro Pharmaceutical Industries Ltd., Haifa. The daily doses employed were 500 mg per monkey or 35 mg per mouse, administered per os and mixed with food.

Microorganisms. *Staphylococcus aureus* strain Toro, obtained from Lepetit and Co., Italy, was grown for 18 h at 37 C on nutrient agar (Difco).

Yersinia pestis. An avirulent strain TRU unable to synthesize VW antigen (7) and vaccine strain EV 76, which develops resistance to phagocytosis upon incubation at 37 C (15), were grown at 28 C for 48 h on blood agar base (Difco) containing 2.5% hemolized rabbit blood.

Brucella melitensis. Rev. I. vaccine strain (9) was grown for 48 h at 37 C on Trypticase soy agar (BBL) supplemented with 3% glycerol and 5 μg of thiamine per ml.

All microorganisms were harvested and washed twice in saline shortly before inoculation into animals. The concentration of microorganisms was obtained from measurements of the optical density at 550 nm and verified by viable counts on appropriate media.

Assay of phagocytic activity. Phagocytosis was studied in vivo. Freshly harvested and washed bacteria (5×10^7 to 1×10^9 in 0.2 ml of saline) were injected intraperitoneally (i.p.) into each mouse of the series. At varying time intervals after inoculation, the mice were killed by ether, the peritoneal cavity was incised under aseptic conditions, and the inflammatory exudate was collected by washing the cavity with 2 ml of cold Tyrode solution. The cell suspensions were kept at 4 C. Eight mice were used in each determination. The following counts were made: (i) number of

exudate cells in the peritoneal cavity, counted in a hemocytometer; (ii) differential count of inflammatory cells in Giemsa-stained smears (200 cells were counted in each smear); (iii) number of noningested viable bacteria. The exudate was diluted 1:5 with cold Tyrode solution, and the cells were centrifuged in the cold at 1,200 rpm for 5 min in an International portable refrigerated centrifuge model PR-2. The number of bacteria in the supernatant fluid was estimated by viable plate counts; and (iv) number of ingested viable bacteria. The pelleted exudate cells obtained upon centrifugation [(iii), above] were resuspended in 0.2 ml of undiluted rabbit antiserum to mouse phagocytes. After incubation for 1 h at 37 C to rupture the cells, the number of bacteria was estimated by viable plate counts.

Phagocytosis was evaluated on the basis of clearance of the injected bacteria from the peritoneal cavity. In analogy to the clearance kinetics of carbon particles from blood (2), we assumed the rate of clearance of bacteria in the peritoneum to be exponentially expressed by the equation $C_1 = C_0 \times 10^{-K_1 t}$, where C_1 is the total concentration of viable bacteria in the peritoneal cavity, free or within phagocytic cells, at time t; C_0 is the concentration of bacteria at the time of injection; and the constant K_1 is the measure of the rate of phagocytosis, denoted as the "phagocytic index" (2), expressed as $K_1 = (\log C_0 - \log C_1)/t$.

Similarly, the rate of ingestion and the ingestion index were calculated from the equation $K_2 = (\log C_0 - \log C_2)/t$ where C_2 is the concentration of noningested bacteria free in the peritoneal exudate at time t.

Antiserum to mouse phagocytic cells. A preparation of antiserum to mouse phagocytic cells was prepared in rabbits. Mouse peritoneal phagocytic cells were mobilized by i.p. injection of 1 ml of saline. The exudate cells were harvested 6 and 24 h later in cold Tyrode solution to obtain polymorphonuclear cells and macrophages, respectively. The cell suspensions were diluted to 1.5×10^7 cells/ml. The rabbits were immunized by three intravenous injections of 1 ml of the freshly harvested cells, at intervals of 1 week, followed by two booster doses at monthly intervals. This antiserum completely lysed mouse polymorphonuclear and macrophage cells on incubation at 37 C for 1 h, and did not affect the microorganisms used in these experiments.

Assay of antibody formation. The assay was based on quantitation of the primary response after i.p. administration of a single dose of 3 mg of bovine gamma globulin (Mann Research Laboratories) into groups of mice. Antibody formation was measured in terms of concentration of circulating antibodies and length of induction period. Seven and 12 days after antigen injection, blood samples were collected and the sera were tested individually by the passive hemagglutination method of Boyden (3).

RESULTS

Effect of brewer's yeast on resistance to infection in newly arrived, quarantined monkeys. Fifty newly arrived monkeys were included in the experiment. On the day of arrival, they were randomly divided into two groups: control, comprising 35 animals, and the experimental group of 15 animals. The experimental animals were given 0.5 g of brewer's yeast per os daily. Yeast treatment began on the first day of quarantine and was continued throughout the experiment.

The effect of brewer's yeast was measured by comparison of morbidity and mortality rates as well as by duration of illness in experimental and control monkeys. Figure 1 illustrates the biphasic nature of the incidence of morbidity in the control group. The first peak occurred within the first 2 weeks of quarantine, followed by a short period of quiescence and then a second morbidity peak. Moreover, all deaths were clustered around the first peak of morbidity (Table 1). Comparison of yeast-treated and control monkeys (Table 1 and Fig. 1) indicates no significant difference either in mortality and morbidity rates or in the duration of infection during the first 2 weeks of treatment. However, in the period after the first peak of disease, the yeast-treated monkeys exhibited a high degree of resistance to infection. Out of the 13 survivors in the treated group, none were sick, whereas 40% in the control group became ill. This difference is statistically highly significant ($P < 0.001$). On the basis of these results, it was concluded that oral administration of brewer's yeast to monkeys significantly enhances their resistance to seasonal infections. This enhanced resistance is manifested only 2 weeks after initiation of treatment; thereafter, it persists at a constant high level for the duration of treatment.

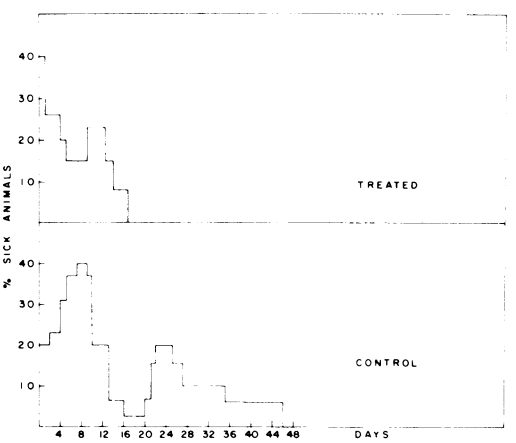

FIG. 1. *Incidence of disease in newly arrived, quarantined monkeys; resistance-enhancing effect of orally administered brewer's yeast.*

TABLE 1. *Effect of orally administered brewer's yeast on disease incidence in newly arrived, quarantined monkeys*

Determination	Control	Yeast treated
Mortality rate (%)	5/35 (14)	2/15 (13)
Mean survival time (days)	8	7
Morbidity rate in first 2 weeks (%)	21/35 (60)	7/15 (47)
Mean morbidity time (days)	5	4
Morbidity rate in subsequent, 5 weeks (%)	12/30 (40)	0/13
P		<0.001

Effect of brewer's yeast on experimental chronic infection of mice. After the demonstration that yeast treatment enhanced resistance to acute seasonal infections, it was of interest to investigate the effect of the treatment on chronic infections. Experimental brucellosis was chosen as a model. Forty mice of inbred strain R III were divided into two equal experimental and control groups. The former was given brewer's yeast per os daily, starting 2 weeks before inoculation and continuing for the duration of the experiment. Mice in both groups were inoculated i.p. with *B. melitensis* at two dose levels: 10^2 (approximately 2 mean infective doses), and 10^4. Four and 6 weeks after inoculation, five animals from each group were killed and examined for infection, as demonstrated by isolation of the microorganisms from the spleen and the presence of agglutinating antibodies in the serum (1). The effect of brewer's yeast on resistance to brucellosis was correlated with the rate of infection and its severity, as estimated by the number of microorganisms in the spleen and the duration of infection. These experiments demonstrate that both control and treated mice developed brucellosis at a similar rate (Table 2). Also, the severity of the disease, as measured by the number of microorganisms in the spleen, was similar in both groups during the first 4 weeks of infection. However, a significant difference was observed during the 6th week of infection. No viable microorganisms were detected in the spleens of any of the treated mice infected with 10^2 bacteria, whereas in all of the control mice at this time, up to 10^5 microorganisms per spleen were persistently present. From these data it was concluded that oral administration of brewer's yeast enhanced resistance to chronic infection in mice. This enhancement was one of degree; it was inadequate to prevent infection altogether but manifested itself in the accelerated elimination of the infecting microorganisms, thereby shortening the duration of the chronic disease. This enhancing effect was expressed at the lower challenging dose only. No differences were detected in agglutination titers in control and experimental groups.

Effect on phagocytosis. In an attempt to clarify the mechanism of enhancement of resistance to infection in the experimental animals, we tested the effect of orally administered brewer's yeast on in vivo phagocytosis of various microorganisms. In a typical experiment, *Y. pestis* strain TRU cells, freshly harvested and washed in saline, were injected i.p. into 60 strain R III mice at a concentration of 2.5×10^8 per animal. The experimental group comprised 30 mice treated orally with brewer's yeast for 2 weeks before inoculation. The 30 control mice did not receive yeast treatment. Similar experiments were performed using two other microorganisms, *Y. pestis* EV 76 (5×10^7 cells per mouse) and *S. aureus* (10^9 cells per mouse). Phagocytic activity at different time intervals after inoculation in the yeast-treated and control animals was measured as described in Materials and Methods by: (i) rate of clearance of bacteria from the peritoneal cavity; and (ii) rate of ingestion by phagocytic cells in the peritoneal cavity. Rates of clearance and ingestion were correlated with the corresponding cellular response in the peritoneal exudate, measured by the number of mononuclear and polymorphonuclear cells. The experiments were replicated twice; data for a representative experiment are given in Fig. 2 and Table 3.

These experiments indicate that orally administered brewer's yeast enhanced phagocytic activity in mice, as manifested by a statistically significant increase in the rate of clearance of different microorganisms from the peritoneal cavity. Phagocytic activity following i.p. injection of microorganisms can be divided into two phases: the initial phase of rapid clearing which may be attributed to the activity of peritoneal macrophages present at time of injection (Fig. 2); and a slower activity (second phase) which occurs between 2 and 3 h after inoculation, coinciding with the influx of polymorphonuclear cells into the peritoneum (Fig. 3). The enhancement of phagocytic activity in yeast-treated mice was more pronounced during the second phase (Fig. 2).

Also, the rate of ingestion was significantly higher in the treated animals. This was true for *S. aureus* but was particularly apparent in *Y. pestis* EV 76, known to develop resistance to phagocytosis at 37 C. Figure 2 depicts the emergence of resistance to phagocytosis in con-

TABLE 2. *Effect of orally administered brewer's yeast on resistance of mice to experimental brucellosis*

Weeks post-inoculation	Inoculum (no. of bacteria)	Infection					
		Rate		No. of microorganisms per spleen[a]		Agglutination titer	
		Control	Treated	Control	Treated	Control	Treated
4	10^2	5/5	5/5	8×10^4	10^5		
	10^4	5/5	5/5	3×10^5	10^5		
6	10^2	5/5	0/5	1.2×10^5		1:80	1:80
	10^4	5/5	5/5	2.8×10^4	1.1×10^4		

[a] Each value represents the mean of five determinations.

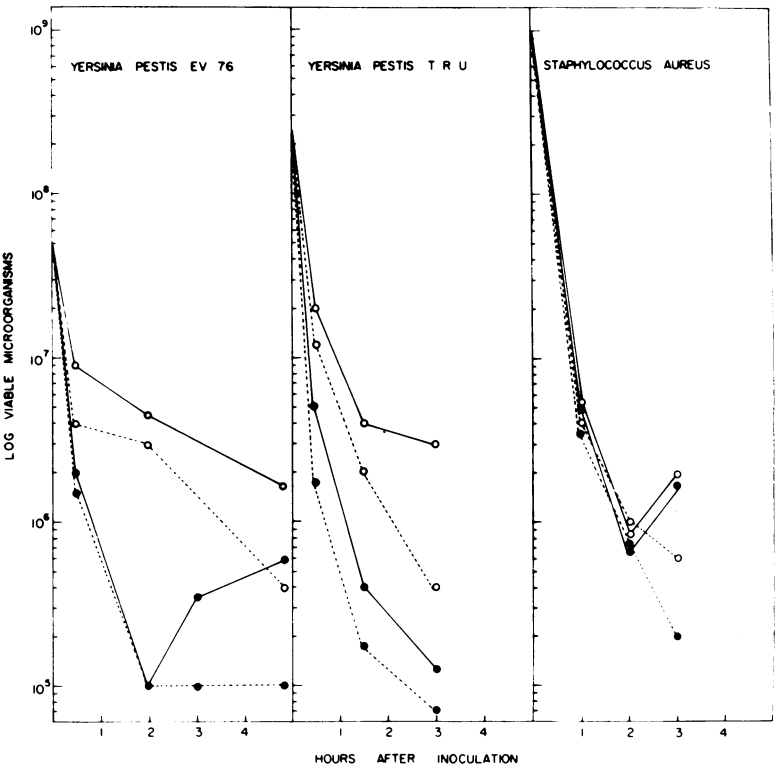

FIG. 2. *Enhancing effect of orally administered brewer's yeast on rate of clearance of microorganisms from the peritoneal cavity of mice. Yersinia pestis EV 76 (5×10^7), Y. pestis TRU (2.5×10^8), and Staphylococcus aureus (10^8) were injected i.p. into groups of mice. At different time intervals after inoculation, the rate of clearance was determined in control (——) and yeast-treated (-----) animals by total viabe counts (O) and viable counts of noningested microorganisms (●). Each point represents the mean estimate from eight animals.*

trol mice 2 h after inoculation, resulting in an increased number of extracellular microorganisms; no such increase was observed in yeast-treated mice. This may be taken as indicating that yeast-induced enhancement of phagocytic activity affects the resistance to phagocytosis of strain EV 76. However, an alternative explanation, i.e., a direct inhibitory effect on this microorganism caused by yeast treatment, cannot be excluded at present. The increased rate of clearance and ingestion in yeast-treated mice was probably related to heightened activity of the individual phagocytic cell. This assumption is based on measurements of the kinetics of the phagocytic cells in the peritoneal exudate (Fig. 3). The number of phagocytic cells in the peritoneal cavity at any given time after inoculation is the result of cell influx, migration to

TABLE 3. *Enhanced clearance of microorganisms from peritoneal cavity of mice treated with brewer's yeast*

Microorganisms injected	Yeast treatment	Clearance from peritoneal cavity[a]			
		Phagocytic index (K_1)[b]	P	Ingestion index (K_2)[b]	P
Yersinia pestis EV 76 (5×10^7)	Treated	0.447 ± 0.018	<0.01	0.517 ± 0.032	<0.02
	Control	0.297 ± 0.004		0.325 ± 0.010	
Yersinia pestis TRU (2.5×10^8)	Treated	0.930 ± 0.040	<0.05	1.120 ± 0.014	>0.05
	Control	0.650 ± 0.070		1.070 ± 0.056	
Staphylococcus aureus (10^9)	Treated	1.042 ± 0.016	>0.05	1.239 ± 0.055	<0.05
	Control	0.916 ± 0.113		0.914 ± 0.091	

[a] At t = 3 h.

[b] Calculated from equations given in Materials and Methods. The t test of significance was applied in the statistical analysis of phagocytosis data.

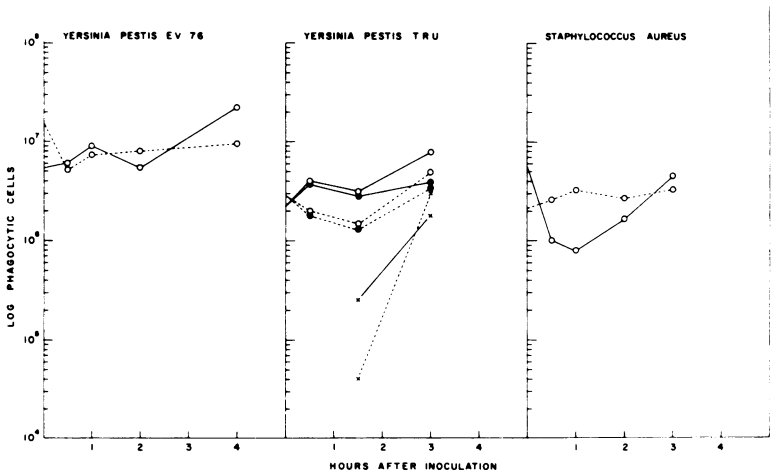

FIG. 3. *Kinetics of phagocytic cells in the peritoneal cavity of yeast-treated and control mice after i.p. inoculation of Yersinia pestis EV 76, Y. pestis TRU, or Staphylococcus aureus. At different time intervals the total number of phagocytic cells* (○) *and macrophages* (●) *and the number of polymorphonuclear cells* (×) *were determined in the peritoneal exudates of control* (——) *and yeast-treated* (-----) *mice.*

lymphoid organs, and cell destruction by the ingested microorganisms. The number of phagocytic cells in the peritoneal cavity of control animals fluctuated, reaching a peak representing a fourfold increase at 3 h; this increase was due mainly to the influx of polymorphonuclear cells. In yeast-treated animals there was only a twofold increase in the number of phagocytic cells. These fluctuations in the number of phagocytic cells could not account for the eightfold increase in clearance in yeast-treated mice. Therefore, our assumption that an "activated" phagocytic cell was induced by yeast treatment seemed plausible. In vitro experiments are needed to establish this point.

Effect on antibody synthesis. After the findings of enhancement of phagocytosis, the effect of yeast treatment on an additional parameter of immunity, antibody synthesis, was investigated. This was studied by measurements of the concentration of circulating hemagglutinins and the length of the induction period after a single i.p. injection of 3 mg of gamma globulin into control and yeast-treated mice. Yeast was orally administered throughout the experiment starting 2 weeks before antigen injection. Results (Table 4) show that brewer's yeast had no effect on the primary response in antibody synthesis, both with regard to antibody concentration and length of the induction period.

DISCUSSION

The experiments described demonstrate that brewer's yeast administered per os to experimental animals enhances nonspecific resistance to infection. This enhanced resistance was exhibited against a variety of agents, ranging from microorganisms causing seasonal infections of

TABLE 4. *Antibody synthesis in yeast-treated mice after a single injection of bovine gamma globulin (3 mg)*

Expt	Yeast treatment	Passive hemagglutination antibody titer[a] on day:	
		7	12
1	Treated	1:60	1:40
	Control	1:80	1:40
2	Treated	1:120	1:80
	Control	1:180	1:160
3	Treated	1:180	
	Control	1:160	

[a] Groups of 10 mice were used for each estimation. Each value represents the mean of 10 determinations.

the respiratory and intestinal tract in monkeys to microorganisms causing chronic infections such as experimental brucellosis in mice. In addition, we have found that prolonged feeding of brewer's yeast to an AKR inbred strain of mice developing autochthonous lymphoma resulted in a doubling of their life span as compared with control mice (unpublished data). These observations illustrate the general nature of the brewer's yeast-induced enhancement of resistance to disease.

Yeasts have been used by man for centuries in fermentation processes and as a food and vitamin supplement. Although yeast consumption has been recommended in folklore medicine as a remedy for various diseases, almost no basic studies, to the best of our knowledge, have been made to determine any therapeutic effect. Some crude preparations obtained by fractionation of yeast cells have been studied in connection with enhancement of resistance to disease. One, an extract of ribonucleotides, was used in humans to increase white blood counts (10), but no followup of this work was carried out. A crude preparation of baker's yeast cell walls termed "zymosan" has been extensively studied in connection with the properdin system in vitro and in laboratory animal experiments. The list of effects attributed to zymosan includes enhancement of resistance to disease, protection from radiation, and repression of tumor development (11).

The mode of action of zymosan remains a matter of controversy. It was suggested (20) that zymosan binds to properdin and complement, and that it acts through its ability to increase properdin titers in vivo. In this aspect, zymosan is not unique (21); a variety of endotoxin preparations and microbial cell walls possess this ability. In addition, the nature of properdin, as well as the correlation between its titer and the resistance to infection, has been challenged (6, 17). An additional effect of zymosan, related to enhancement, is its ability to stimulate phagocytosis upon intravenous injection in mice (B. Benacerraf and M. M. Sebestyen, Fed. Proc. **16**:860–872, 1957). Zymosan is a mixture of variable composition and difficult to standardize. This fact may be responsible for the conflicting reports on zymosan activity.

Our results suggest that the mechanism responsible for the enhancing effect of orally administered yeast on resistance to disease is based on an increase in the rate of phagocytosis. This is not unique to this system, but is found also for some other resistance-enhancing agents, e.g., the activation of the "angry macrophages" by BCG (14). The effect has also been attributed to zymosan, as mentioned earlier. We did not detect any enhancing effect upon formation of circulating antibody, as was demonstrated in the primary antibody response to injection of bovine gamma globulin in mice, as well as in the lack of increase in antibrucella agglutinin in mice recovering from experimental brucellosis. Although more experimental data will be needed to establish this contention, it is suggested that brewer's yeast exerts an enhancing effect on mechanisms related to cellular resistance rather than on those based on circulating antibody. The questions of whether other resistance-enhancing mechanisms are elicited by brewer's yeast or whether the action of brewer's yeast may be related to that of zymosan remain open for the time being.

An interesting feature of the yeast-induced effect is the 2-week period necessary for the expression of the enhanced resistance to disease. Our data are insufficient to explain the nature of this lag period.

These experiments indicate the feasibility of using brewer's yeast for enhancing nonspecific resistance to disease in humans. The merits of brewer's yeast as compared to other resistance-enhancing agents, such as BCG or poly(I:C), are that yeast has been consumed by man for centuries without any adverse effects being recorded and that it acts upon oral administration.

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