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Saccharomyces cerevisiae as a vaccine against coccidioidomycosis

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ABSTRACT

Disseminated coccidioidomycosis is a life-threatening infection. In these studies, we examined protection against systemic murine coccidioidomycosis by vaccination with heat-killed *Saccharomyces cerevisiae* (HKY). CD-1 mice received HKY subcutaneously or by oral gavage with or without adjuvants once weekly beginning 3 or 4 weeks prior to infection; oral live *Saccharomyces* was also studied. All HKY sc regimens were equivalent, prolonging survival ($P \leq 0.005$) and reducing fungal burden versus controls. Oral live *Saccharomyces*, but not HKY, prolonged survival ($P = 0.03$), but did not reduce fungal burden. Survival of mice given HKY was equivalent to vaccination with formalin-killed spherules, but inferior in reduction of fungal burden. HKY was superior to a successful recombinant vaccine, PRA plus adjuvant. This novel heterologous protection afforded by HKY vaccination offers a new approach to a vaccine against coccidioidomycosis.

1. Introduction

Coccidioidomycosis is a disease caused by the dimorphic fungi *Coccidioides immitis* and *C. posadasii* endemic in the Americas. The disease ranges from mild respiratory with spontaneous resolution to severe life-threatening mycosis. The average number of reported cases is 40,000 per year, but during epidemic years (dust-storm, rainfalls and earthquake-related events) the symptomatic population can be more than 10-fold increased [1]. The development of an effective vaccine would reduce the acquisition of coccidioidomycosis among populations from endemic areas, as well as transient visitors to the endemic areas, such as tourists or military personnel. This reduction would result in fewer severe infections, as well as reduce the overall costs of healthcare related to this organism.

Over the course of the last 50 years a variety of vaccine preparations have been examined. The most robust among these has been the formalin-killed spherule (FKS) vaccine, which provided significant protection against *Coccidioides* in animal studies [2–4]. Intramuscular administration of FKS of *C. immitis* in mice demonstrated full protection against lethal infection with the fungus [3]. Overall, the experimental work with killed coccidioidal vaccines demonstrated protection in four animal species, including primates [4]. However, when tested in phase 3 clinical trials, FKS vaccination failed to demonstrate significant reduction of incidence of disease or severity [5]; failure was thought to be due to inadequate dosing of the FKS because of associated side-effects [5]. The current focus of *Coccidioides* vaccine efforts is on more purified antigens, including those produced by recombinant DNA technology [6,7]. Various subcellular and recombinant protein vaccine preparations have been shown to be protective in murine models of infection [8–16,17a]. Recently, we demonstrated that heat-killed yeasts (HKY) of *S. cerevisiae* protected mice against lethal aspergillosis [17b]. We have expanded that line of research to include *Coccidioides* infection and tested whether HKY can protect against lethal systemic infection. In the present study we show that HKY also has a beneficial effect in protecting mice against systemic coccidioidomycosis.

2. Materials and methods

2.1.1. Vaccines

Two clinical strains (98–108 and 98–116) of *Saccharomyces cerevisiae* from our collection stored at −80 °C in 40% (vol/vol) glycerol were used. These strains were identified as *S. cerevisiae* by molecular methods used in previous studies [18]. The strains were grown in broth containing 1% yeast extract, 1% peptone, and 2% dextrose for 24 h at 37 °C in an orbital shaker, centrifuged (1000 r.p.m. for 5 min) and the cell pellet washed three times in phosphate-buffered saline (PBS, pH 7) by centrifugation. Yeasts were killed by heating in a waterbath at 70–75 °C for 3 h. Viability was determined by culturing on Sabouraud's dextrose agar. No growth was noted after

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Table 1
Vaccine regimens and groups in Experiment 1.

Group	Vaccine (per dose)	Route	Dose prior to infection (days)
HKY	HKY (2.5 mg)	s.c.	21, 14, 7
HKY + CpG	HKY (2.5 mg) + CpG (10 μg)	s.c.	21, 14, 7
HKY 5 mg	HKY (5 mg)	PO	21, 14, 7
FKS	FKS (1.5 mg)	i.m.	47, 40, 33, 26
Control	PBS	s.c.	21, 14, 7

5 days' incubation. Heat-killed yeasts (HKY) were adjusted to the desired number of cells by hemacytometer count; dry weight was determined after desiccation of a sample of HKY suspension.

Formalin-killed spherules (FKS) of *C. immitis* [19] were stored in sterile vials at 4 °C as a suspension in physiologic saline and thimerosal (1:10,000). FKS were centrifuged 7 min at 1500 r.p.m. to remove the diluent, suspended and washed two additional times by centrifugation in PBS.

Monophosphoryl lipid A (MPL; Sigma Chemical Co., St. Louis, MO) and recombinant proline rich antigen (PRA_{1-172}) preparations were provided by Dr. J. Galgiani, and the components prepared as described previously [20]. Prior to vaccination 150 μl of 80 ng/μl PRA–GPI plus 300 μl of MPL (1 μg/μl) were mixed and used as vaccine stock; each mouse was given 37.5 μl for vaccination subcutaneously in the inguinal region.

2.2. Animals

Five-week-old to seven-week-old male CD-1 mice were used in these experiments. Animals were housed in standard conditions in cages of 5 animals. Thirty-two groups of mice total (10 per group) were used in 4 independent experiments. After infection, animals were housed in micro-isolator cages under ABSL 2 conditions. All animal experiments were done with the approval of the Institutional Animal Care and Use Committee of the California Institute for Medical Research.

2.3. Vaccinations

Various routes of administration were tested to determine their effectiveness. These included administration orally by gavage (PO) of 1.2×10^8 (5 mg per dose) HKY or live *Saccharomyces*, and subcutaneous (s.c.) dosing of HKY using two dorsal injection sites (0.075 ml each, total of 6×10^7 HKY (2.5 mg per animal). FKS at 1.5 mg per dose was given intramuscularly (i.m.), a regimen shown to provide optimal protection against pulmonary infection in prior studies [3]. In some instances the vaccine regimen included an adjuvant. The adjuvants tested included: oligonucleotide CpG (CpG; Operon Biotechnologies Inc., Huntsville, AL), sesame oil (Sigma–Aldrich Inc., St. Louis, MO), live Bacillus Calmette-Guérin (BCG; Aventis Pasteur Inc., Swiftwater, PA), monophosphoryl lipid A (MPL; Sigma), muramyl dipeptide (MDP; Sigma), and Imject® Alum (Thermo Scientific, Rockford, IL). These were administered in combination with HKY using same route. Recombinant mouse IL-12 (IL-12, Sigma) was administered intraperitoneally (i.p.) for 5 doses per week for 3 weeks. Vaccination regimens and experimental groups are presented in Tables 1–4.

Table 3
Vaccine regimens and groups in Experiment 3.

Group	Vaccine (per dose)	Route	Dose prior to infection (days)
HKY	HKY (2.5 mg)	s.c.	21, 14, 7
HKY 2.5 mg d28	HKY (2.5 mg)	s.c.	28, 21, 14
HKY 2.5 mg d39	HKY (2.5 mg)	s.c.	39, 32, 25
HKY 5 mg	HKY (5 mg)	s.c.	28, 21, 14
HKY 96–116 2.5 mg	HKY 96–116 (2.5 mg)	s.c.	21, 14, 7
Live Y 5 mg d21	Live Y (5 mg)	PO	21, 14, 7
Live Y 5 mg d39	Live Y (5 mg)	PO	39, 32, 25
Live Y 5 mg ×9	Live Y, 9 doses (5 mg)	PO	21, 19, 17, 14, 12, 10, 7, 5, 3
Control	PBS	s.c.	21, 14, 7

2.4. Infection

Experimental systemic infection was established using *C. posadasii*, strain Silveira, as previously described [21,22]. In brief, the organism was grown at room temperature on glucose yeast-extract agar slants in a Class III Biosafety Cabinet. Arthroconidia were harvested as a suspension in 0.9% saline, quantified by hemacytometer and verified for viability by plating dilutions of the inoculum on Mycosel (BD, Sparks, MD) plates. Animals were infected intravenously (i.v.) with 180–280 viable arthroconidia per mouse, see individual experiments for inoculum used. Animals were examined daily. Previous studies have quantitatively demonstrated the close correlation of survival curves, in both naïve and vaccine-immunized mice, when i.v. and intranasal challenges of various inoculum sizes are compared, thus underscoring the validity of i.v. challenge as a vaccine screening tool [16].

2.5. Fungal burden

Twenty-eight days after infection, surviving animals were euthanized by CO_2 anoxia. The kidneys, spleen and lungs were removed and homogenized in 0.9% saline. Ten-fold dilutions of tissue homogenates were placed on Mycosel plates for CFU determination. An arbitrary value of $\log_{10} 7$ (a value higher than that from the organ of any mouse that survived) is assigned to data points missing due to the death of an animal due to infection [23,24]. This assures that death is considered as a worse outcome than is survival with any amount of organism burden and allows us to include these animals in the nonparametric analysis of CFU. This value represents, as determined in prior studies, the CFU present in the organ just prior to death.

Table 2
Vaccine regimens and groups in Experiment 2.

Group	Vaccine (per dose)	Route	Dose prior to infection (days)
HKY 2.5 mg (3 weeks)	HKY (2.5 mg)	s.c.	21, 14, 7
HKY 2.5 mg (4 weeks)	HKY (2.5 mg)	s.c.	28, 21, 14, 7
HKY 5 mg (3 weeks)	HKY (5 mg)	s.c.	21, 14, 7
HKY 5 mg PO (3 weeks)	HKY (5 mg)	PO	21, 14, 7
HKY + oil	HKY (5 mg) + oil	PO	21, 14, 7
HKY + CpG	HKY (5 mg) + CpG (50 μg)	PO	21, 14, 7
HKY + MDP	HKY (5 mg) + MDP (50 μg)	PO	21, 14, 7
HKY + MPL	HKY (5 mg) + MPL (12.5 μg)	PO	21, 14, 7
HKY + IL-12	HKY (5 mg) + IL-12 (100 ng)	PO + i.p., respectively	21, 14, 7 (IL-12 on 21, 20, 19, 18, 17, 14, 13, 12, 11, 10, 7, 6, 5, 4, 3)
HKY + BCG	HKY (5 mg) + BCG (1.2×10^8 microorganisms)	PO	21, 14, 7
Live Y	Live Y (5 mg)	PO	21, 14, 7
Control	PBS	s.c.	21, 14, 7