Coccidioides immitis Vaccine: Potential of an Alkali-Soluble, Water-Soluble Cell Wall Antigen

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C-ASWS-M, the alkali-soluble, water-soluble cell wall antigen of Coccidioides immitis mycelia, was evaluated for its vaccine potential in mice. Vaccination with 0.5-, 1.5-, or 3-mg doses of C-ASWS-M in complete Freund adjuvant provided a significant level of protection against intraperitoneal challenge with 1,500 arthroconidia \( (P < 0.0001) \) with each dose. Vaccination with 1 mg of C-ASWS-M protected mice against intranasal challenge with 50 \( (P < 0.05) \) and 500 \( (P < 0.01) \) arthroconidia, but not against intranasal challenge with 1,500 arthroconidia \( (P > 0.05) \).

Previous studies by Kong et al. (3–5), Levine et al. (6–8), and Pappagianis et al. (9, 10) have established that prior immunization of mice with killed mycelia or spherules of Coccidioides immitis affords protection against challenge with viable arthroconidia. Spherules were reported to be more efficacious than were mycelia or arthroconidia (3, 6, 7), and spherule cell walls were more efficacious than were intact spherules (4, 7). More recently, Pappagianis et al. (9) reported that a phosphate-buffered saline extract of spherule cell walls protected mice against intranasal challenge with 1,000 arthroconidia.

The present study was undertaken to determine whether C-ASWS-M, the alkali-soluble, water-soluble cell wall antigen of C. immitis mycelia (1, 11), might protect mice against experimental coccidioidomycosis. Male DBA/2J mice (Jackson Laboratories, Bar Harbor, Maine) weighing 18 to 22 g were immunized by a subcutaneous injection of C-ASWS-M in complete Freund adjuvant (CFA; Difco Laboratories, Detroit, Mich.). At 7 and 14 days after primary immunization mice were boosted by a subcutaneous injection of C-ASWS-M in complete Freund adjuvant. The doses of C-ASWS-M indicated within the text represent the total dose given throughout the immunization period. Control mice received three injections of a saline-adjuvant mixture.

At 30 to 40 days after immunization, mice were challenged by an intraperitoneal (0.5 ml) or intranasal (0.2 ml) inoculation of viable arthroconidia of C. immitis Silveira. Mice were observed for mortality at daily intervals for 30 to 40 days postinfection. Differences in the survival rates among groups were analyzed for statistical significance by the Mann-Whitney rank sums test (2). To assess the extent of disease involvement, we necropsied all mice which survived 30 to 40 days postinfection and those which had died before this period, and portions of their lungs, liver, and spleen were plated on Mycosel medium (Difco).

Vaccination with 0.5-, 1.5-, and 3-mg doses of C-ASWS-M in CFA afforded a significant degree of protection against intraperitoneal challenge with 1,500 viable arthroconidia (Fig. 1) \( (P < 0.0001) \) for all three groups compared with mice vaccinated with adjuvant alone. The highest level of protection was obtained with the 0.5-mg dose of C-ASWS-M; i.e., 20 (80%) of 25 mice vaccinated with this dose survived the 35-day period postinfection, compared with 12 (48%) of 25 mice vaccinated with 1.5 mg \( (P < 0.02) \) and 11 (44%) of 25 mice vaccinated with 3 mg \( (P < 0.01) \). Although vaccination conferred a significant degree of protection in terms of survival, C. immitis was recovered from cultures of the lungs, liver, or spleen of all vaccinated mice that survived for 35 days postinfection. Cultures were also positive in all mice which died before this time.

Studies were then conducted to determine whether C-ASWS-M would protect against intranasal challenge. Figure 2 shows the percent survivors in mice vaccinated with 0.2- and 1-mg doses of C-ASWS-M in CFA and with CFA alone before intranasal challenge with 50 viable arthroconidia, a dose which represents one 50% lethal dose for strain Silveira by this route (10). Of 25 mice vaccinated with 0.2 mg and 24 mice vaccinated with 1 mg of C-ASWS-M, 16 (68%) and 20 (83%), respectively, survived the 40-day period postinfection compared with 14 (56%) of 25 CFA-vaccinated mice. Statistical analyses...
established a significant level of protection in mice vaccinated with 1 mg of C-ASWS-M compared with control mice (*P* < 0.05). The survival rate of mice vaccinated with 0.2 mg of C-ASWS-M was not statistically significant compared with mice receiving adjuvant alone (*P* > 0.05). Despite the level of protection obtained in mice vaccinated with 1 mg of C-ASWS-M, all 20 survivors showed extrapulmonary spread of *C. immitis* to the liver, spleen, or both.

Two subsequent experiments were performed to determine whether the 1-mg dose of C-ASWS-M would protect against intranasal challenge with 500 (10 50% lethal doses) or 1,500 arthroconidia (30 50% lethal doses). Of 30 mice vaccinated with 1 mg of C-ASWS-M, 16 (53%) survived 30 days after challenge with 500 arthroconidia, compared with only 9 (30%) of 30 control mice (*P* < 0.01) (Fig. 3). Protection was not obtained, however, against pulmonary challenge with 1,500 arthroconidia (Fig. 4). Within 12 days postinfection, only 10 (42%) of 24 mice vaccinated with C-ASWS-M had survived, and by 30 days postinfection, only 5 (21%) mice remained alive, as compared with 2 (8%) of 25 control mice (*P* > 0.05).

The results presented in this study provide evidence that C-ASWS-M affords a significant level of protection against intraperitoneal challenge with 1,500 arthroconidia and against intranasal challenge with 50 or 500 arthroconidia. Previous vaccine studies for coccidioidomycosis were done with intact cells or cell walls of *C. immitis* mycelia and spherules (3-8, 10). Although these vaccines proved to be protective against intraperitoneal and intranasal infection with *C. immitis*, the insolubility of cells and cell
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LITERATURE CITED


walls and their heterogeneous composition diminishes their effectiveness as vaccines in humans. Additional studies are therefore needed to evaluate the vaccine potential of the soluble C-ASWS antigen from both mycelial and spherule cell walls.