The Anti-Candida albicans Vaccine Composed of the Recombinant N Terminus of Als1p Reduces Fungal Burden and Improves Survival in Both Immunocompetent and Immunocompromised Mice

Brad J. Spellberg, Ashraf S. Ibrahim, Valentina Avenissian, Scott G. Filler, Carter L. Myers, Yue Fu, and John E. Edwards, Jr.

Division of Infectious Diseases, Los Angeles Biomedical Institute at Harbor-UCLA Medical Center, Torrance, California, and The David Geffen School of Medicine at UCLA, Los Angeles, California

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We have previously shown that intraperitoneal vaccination with the recombinant N terminus of Als1p (rAls1p-N) modestly improves survival during murine disseminated candidiasis. We now report marked efficacy with subcutaneous rAls1p-N vaccination. Efficacy is retained in neutropenic and corticosteroid-treated mice. The rAls1p-N vaccine is a promising candidate for the prevention of invasive candidiasis.

Candida spp. are the fourth most common nosocomial bloodstream isolates (14, 20), causing an attributable mortality of 40 to 50% during hematogenously disseminated disease (5, 10, 11, 19). The major clinical risk factors for disseminated candidiasis have been well described (17), allowing facile identification of at-risk patients. Furthermore, disseminated candidiasis typically occurs after multiple weeks of hospitalization (20), enabling the opportunity for prophylactic vaccination prior to disease onset.

We have developed a vaccine targeting Candida albicans, the most common species causing disseminated candidiasis (~50% of cases) (1, 2, 9, 12, 13), based on the agglutination-like sequence 1 (ALS1) gene product, which is an adhesin that mediates C. albicans binding to human cells (3, 4). The recombinant N-terminal domain of Als1p (rAls1p-N) demonstrated modest efficacy as a vaccine when administered intraperitoneally to immunocompetent BALB/c mice (6). The mechanism of protection was induction of type 1 immunity, and protection correlated with induction of delayed-type hypersensitivity (DTH) to the antigen. The present studies were performed to evaluate the efficacy of the rAls1p-N vaccine when administered by a different route (subcutaneously [s.c.]) in both immunocompetent and immunocompromised mice.

Efficacy of the rAls1p-N vaccine in immunocompetent mice. rAls1p-N, encompassing amino acids 19 to 433 of the full-length protein, was produced in Saccharomyces cerevisiae and purified as described previously (6). A control preparation was similarly purified from S. cerevisiae transformed with an empty plasmid. BALB/c retired breeder mice (25 to 30 g) were immunized by s.c. injection of rAls1p-N (20 µg) or control preparation mixed with complete Freund’s adjuvant at day 0, followed by a booster dose in incomplete Freund’s adjuvant at day 21. Two weeks following the boost, the immunogenicity of the vaccine was confirmed by evaluating the intensity of the footpad swelling reaction as a marker of DTH, as we have described previously (6). Vaccinated mice had marked increases in rAls1p-N-specific DTH (Fig. 1).

The efficacy of the rAls1p-N vaccine was evaluated by determining the impact of rAls1p-N vaccination on survival in infected BALB/c mice (Fig. 2A). Vaccinated or control mice were infected via the tail vein with rapidly lethal inocula (2.5 × 10⁵ to 5 × 10⁶ blastospores) of C. albicans. We have previously shown that mice infected with such inocula die of overwhelming septic shock (16). Vaccination markedly prolonged time to death (P < 0.05 for both inocula by log rank test) and improved 30-day survival (50 to 57% versus 0%, P < 0.05 for both inocula by Fisher’s exact test).

The impact of vaccination on tissue fungal burden during hematogenously disseminated candidiasis was then determined. Fourteen days following the boost, vaccinated and control BALB/c mice were infected via the tail vein with 5 × 10⁵ blastospores of C. albicans SC5314. Six days following infection, prior to onset of the first deaths in the control arm,

FIG. 1. Subcutaneous vaccination with rAls1p-N induces an in vivo DTH response in immunocompetent mice. Footpad swelling was assessed 24 h after injection of 50 µg of rAls1p-N into the footpad in BALB/c mice (n = 10 per group). Median values are displayed as horizontal black bars. *, P = 0.002 versus control by Wilcoxon rank sum test.
FIG. 2. The rAls1p-N vaccine improves survival of immunocompetent mice with hematogenously disseminated candidiasis and reduces tissue fungal burden. (A) Survival of vaccinated or control BALB/c mice (n = 7 or 10 per group for an inoculum of 2.5 x 10⁵ or 5 x 10⁵, respectively) subsequently infected via the tail vein with C. albicans. Each experiment was terminated at 30 days postinfection, with all remaining mice appearing well. *, P < 0.05 versus control by log rank test. (B) Kidney fungal burden in BALB/c mice (n = 7 per group) infected via the tail vein with 5 x 10⁵ blastospores of C. albicans. The y axis reflects the lower limit of detection of the assay. Median values are displayed as horizontal black bars. *, P = 0.01 versus control by Wilcoxon rank sum test.

Kidneys were harvested, homogenized, and quantitatively cultured in Sabouraud dextrose agar (Difco) (18). Subcutaneous vaccination with rAls1p-N resulted in a median 1.5-log CFU/g decrease in kidney fungal burden compared to that of the control (P = 0.01 by Wilcoxon rank sum test, Fig. 2B).

Efficacy of the rAls1p-N vaccine in immunocompromised mice. Having demonstrated efficacy in immunocompetent mice, the potential for the rAls1p-N vaccine to induce immunity in and protect neutropenic mice from disseminated candidiasis was evaluated. Vaccinated BALB/c mice were made neutropenic by administration of cyclophosphamide (200 mg/kg intraperitoneal on day −2 and 100 mg/kg intraperitoneal on day +9 relative to infection, resulting in approximately 12 days of neutropenia, as described previously [15]). The footpad swelling reaction was performed 2 days after the first dose of cyclophosphamide. Vaccinated neutropenic mice developed DTH reactions of similar magnitude to those in immunocompetent mice (Fig. 3A versus Fig. 1, experiments performed in parallel). In neutropenic mice infected via the tail vein with 2.5 x 10⁴ blastospores of C. albicans, vaccination also resulted in significant improvements in time to death (P = 0.007 by log rank test versus control), median survival time (>21 versus 12 days, P = 0.008 by Wilcoxon rank sum test), and overall survival (88% versus 38%, P = 0.005 by Fisher’s exact test) (Fig. 3B).

To determine the efficacy of rAls1p-N vaccination in mucosal infection, the vaccine was tested in our murine oropharyngeal candidiasis model (7, 8). Vaccinated or control mice were treated with cortisone acetate (225 mg/kg s.c. on days −1, 1, and 3 relative to infection) and infected sublingually as described previously (7, 8). In brief, mice were anesthetized by intraperitoneal injection with 8 mg xylazine and 110 mg ketamine per kg. Calcium alginate urethral swabs were saturated with C. albicans by placing them in a suspension of 10⁶ organisms/ml in Hanks balanced salt solution at 30°C. The saturated swabs were placed sublingually in the oral cavity of the mice for 75 min. Tongues were excised on day 5 postinfection, fixed in zinc-buffered formalin, mounted in paraffin, and sectioned and stained with periodic acid Schiff.

Because CFU of homogenized tongues cannot distinguish between invasive infection and surface-adherent colonization, we evaluated extent of invasion by histopathology. A blinded observer (B.J.S.) scored each section by scanning along the entire length of the tongues and quantifying the severity of fungal lesions per x40 high-powered field (0 = no lesion, 1⁺ = mild mucosal inflammation, 2⁺ = significant inflammation restricted to the epithelium, 3⁺ = inflammation extending through the entire epithelial layer, and 4⁺ = inflammation extending into the subepithelium). To avoid sampling bias, two sections of each tongue, separated by at least five intervening tissue sections, were scored. All control mice developed marked fungal invasion of their tongues in numerous locations, while only two vaccinated mice developed any tongue lesions. In total, the median number (75th, 25th quartile) of lesions per tongue in control mice was 6.5 (8, 5.75) as compared to 1 (2.5, 0) for vaccinated mice (P = 0.03 by Wilcoxon rank sum test).

Semiquantitative evaluation of the severity of infection dem-
proven a significant reduction in vaccinated mice compared to that in controls (Fig. 4, *P* = 0.03 by Wilcoxon rank sum test). These data provide proof of principle for the potential of the rAls1p-N vaccine to provide mucosal protection as well as systemic protection.

In light of the increasing incidence of candidemia and its continuing high mortality rate, development of a vaccine against *Candida* spp. is of great importance. Here we show that s.c. vaccination with rAls1p-N resulted in marked improvement in survival and significant reductions in fungal burden during otherwise rapidly fatal hematogenously disseminated candidiasis in both immunocompetent and immunocompromised mice. Of interest are the kidney fungal burden results from individual vaccinated mice, demonstrating that approximately half the mice had kidney fungal burdens under 5 log CFU/g. We have previously found that the threshold of kidney fungal burden indicative of a fatal infection is 5 log CFU/g; mice with kidney fungal burdens above this level typically die from infection, whereas mice with kidney fungal burdens below this burden survive the infection (16, 18). Therefore, breakthrough deaths in the vaccinated group likely reflect high fungal burden in spite of vaccination. The mouse-to-mouse variations in tissue fungal burden may reflect the complexities of host-pathogen interactions and/or variable vaccine responsiveness.

In summary, the rAls1p-N vaccine is a promising candidate for prevention of increasingly common and highly lethal disseminated candidiasis. The vaccine is efficacious in immunocompetent mice, and efficacy is retained even in immunopotent and corticosteroid-treated hosts. The successful development of an anticausal vaccine would greatly impact care of at-risk patients.

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