Vaccination with Recombinant N-Terminal Domain of Als1p Improves Survival during Murine Disseminated Candidiasis by Enhancing Cell-Mediated, Not Humoral, Immunity

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***Candida* spp. are opportunistic fungal pathogens that are among the most common causes of nosocomial bloodstream infections. The mortality attributable to disseminated candidiasis is 40 to 50% despite antifungal therapy. Clearly, new strategies are needed to prevent this life-threatening infection. Because risk factors for disseminated candidiasis are well defined and frequently of limited duration, vaccination is an appealing prophylactic strategy. We have identified a cell surface protein, Als1p, that mediates adherence of *Candida albicans* to a variety of human substrates and plastic. Here we report that immunizing BALB/c mice with the recombinant N-terminal domain of Als1p (rAls1p-N) improved survival during a subsequent challenge with a lethal inoculum of *C. albicans*. The protective 20- μ g dose of rAls1p-N significantly increased *Candida* stimulation of Th1 splenocytes and increased in vivo delayed-type hypersensitivity. In contrast, antibody titers did not correlate with protection. Finally, the vaccine was not protective in T-cell-deficient mice but was protective in B-cell-deficient mice. These data indicate that the mechanism of action of the rAls1p-N vaccine is stimulation of cell-mediated, rather than humoral, immunity against *C. albicans*. The majority of efforts to date have focused on the development of passive immunization strategies to prevent or treat disseminated candidiasis. In contrast, our results provide proof of principle for vaccination with an adhesin of *C. albicans* and emphasize the potential for cell-mediated immune modulation as a prophylactic or therapeutic strategy against disseminated candidiasis.**

Candida spp. are the fourth most common nosocomial bloodstream isolates (53). The mortality attributable to disseminated candidiasis is 40 to 50%, even with modern antifungal therapy (16, 34, 40, 65). Furthermore, development of resistance to conventional antifungal therapies has created concern regarding the future ability to treat infections caused by *Candida* spp. (9, 23, 38, 63). Clearly, new strategies to prevent *Candida* infections are needed.

The major clinical risk factors for developing disseminated candidiasis have been well described (58). These key risk factors include colonization with the organism, gastrointestinal or cardiac surgery, a prolonged stay in an intensive care unit, burns, and use of central venous catheters, broad-spectrum antibiotics, and parenteral nutrition. Patients with these risk factors are extremely common. For example, they constitute the large population of patients recovering from surgery or those hospitalized in medical intensive care units for treatment of cardiac and respiratory failure. These patients are not profoundly immunosuppressed by cancer chemotherapies or drugs to prevent organ transplantation; they are likely to respond favorably to a *Candida albicans* vaccine. A smaller population of patients at risk for disseminated candidiasis includes those who are immunocompromised by cancer, neutropenia, corti-

costeroid use, or human immunodeficiency virus. While these patients may be less likely to respond to vaccination, there is extensive precedence for the efficacy of a variety of vaccines in these patient populations (1, 8, 10–12, 17, 24–27, 29, 30, 33, 39, 46, 52, 55, 56, 61, 62). Hence, these patients may also be considered candidates for vaccination.

Because the risk factors for disseminated candidiasis are often identifiable in patients prior to the development of infection, vaccination of these selectable, high-risk patients to prevent the onset of disseminated candidiasis is an appealing strategy. Furthermore, many of these risk factors are of relatively short duration, generally 4 to 6 weeks. Therefore, an immunization approach would need to protect patients for the short period of time during their increased susceptibility, in contrast to periods of years for vaccines such as those against tetanus, *Streptococcus pneumoniae*, and *Haemophilus influenzae*.

While searching for a dominant adhesin in *Candida*, by using surrogate genetics, we found that the protein product of the agglutination-like sequence 1 (*ALSI*) gene is an adhesin that mediates *C. albicans* binding to human cells (13, 14). *ALSI* is a member of a gene family composed of eight identified members (67). We and others have determined that Als proteins function as adhesins to biologically relevant substrates (13, 15, 32, 54). Consistent with their structural homology to the immunoglobulin superfamily, we have found that the substrate specificity of distinct Als proteins is determined by their N-terminal regions (32, 54). The recombinant N-terminal domain

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of Als1p (rAls1p-N) is an appealing candidate for use as a *C. albicans* vaccine because it is expressed at the cell surface (32), because antibody directed against it eliminates *C. albicans* adherence to endothelial cells (32), and because of its potential immunological cross-reactivity with other members of the Als family of proteins (54).

In these studies, we verified the potential for rAls1p-N to serve as an anti-*Candida* vaccine. The efficacy of the rAls1p-N immunogen was evaluated in a murine model of hematogenously disseminated candidiasis. Also, the mechanism of vaccine-mediated protection was identified.

MATERIALS AND METHODS

***C. albicans* and mice strains.** *C. albicans* SC5314, a well-characterized clinical isolate that is highly virulent in animal models (59), was supplied by W. Fonzi (Georgetown University). The organism was serially passaged three times in yeast peptone dextrose broth (Difco) prior to infection.

Female BALB/c mice were obtained from the National Cancer Institute (Bethesda, Md.). To explore the impact of age on vaccine efficacy, both juvenile mice (8 to 10 weeks old) and retired breeders (≥ 6 months old) were used. Female B-cell-deficient mice bearing a homozygous deletion of the *igh* loci (C.129B6-IgH-Jhd^{tm1Dhu}), T-cell-deficient nude mice (C.Cg/AnBomTac-Foxn1^{nu}N20), and congenic wild-type BALB/c littermates were obtained from Taconic Farms (Germantown, N.Y.). Mice were housed in filtered cages with irradiated food and autoclaved water supplied ad libitum. For survival experiments, mice were immunized with various doses of antigen (see below) and subsequently infected via the tail vein with the appropriate inoculum of *C. albicans* SC5314 blastospores or a phosphate-buffered saline (PBS; Irvine Scientific, Irvine, Calif.) control. Results of replicate survival studies were combined if the individual data sets demonstrated no statistical heterogeneity (see below). All procedures involving mice were approved by the institutional animal use and care committee, in accordance with the National Institutes of Health guidelines for animal housing and care.

rAls1p-N immunization protocol. rAls1p-N (amino acids 17 to 432 of Als1p) was produced in *Saccharomyces cerevisiae* and purified by gel filtration and Ni-nitrilotriacetic acid matrix affinity purification (13). The amount of protein was quantified by modified Lowry assay. A high degree of purity ($\sim 95\%$) was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, as well as circular dichroism and Fourier transform infrared spectroscopy, as previously described (54). Mice were immunized by intraperitoneal injection of rAls1p-N mixed with complete Freund's adjuvant (CFA; Sigma-Aldrich) at day 0, boosted with another dose of the antigen with incomplete Freund's adjuvant (IFA; Sigma-Aldrich) at day 21, and infected 2 weeks following the boost.

rAls1p-N ELISA. Antibody titers were determined by enzyme-linked immunosorbent assay (ELISA) in 96-well plates. Wells were coated at 100 μ l per well with rAls1p-N at 5 μ g/ml in PBS. Mouse sera were incubated for 1 h at room temperature following a blocking step with Tris-buffered saline (TBS; 0.01 M Tris HCl [pH 7.4], 0.15 M NaCl) containing 3% bovine serum albumin. The wells were washed three times with TBS containing 0.05% Tween 20, followed by another three washes with TBS. Goat anti-mouse secondary antibody conjugated with horseradish peroxidase (Sigma) was added at a final dilution of 1:5,000, and the plate was further incubated for 1 h at room temperature. Wells were washed with TBS and incubated with substrate containing 0.1 M citrate buffer (pH 5.0), 50 mg of *o*-phenylenediamine (Sigma), and 10 μ l of 30% H₂O₂. The color was allowed to develop for 30 min, after which the reaction was terminated by addition of 10% H₂SO₄ and the optical density (OD) at 490 nm was determined in a microtiter plate reader. Negative control wells received only diluent, and background absorbance was subtracted from the test wells to obtain final OD readings. The ELISA titer was taken as the reciprocal of the last serum dilution that gave a positive OD reading (i.e., more than the mean OD of negative control samples plus 2 standard deviations).

***C. albicans*-induced cytokine profiles.** To determine the effect of the rAls1p-N vaccine on cell-mediated immunity and in vivo cytokine profiles, mice were immunized as described above. Two weeks after the final boost, splenocytes were harvested and cultured in complete medium at a density of 4×10^6 cells/ml as previously described (59). To stimulate cytokine production, splenocytes were cocultured with heat-killed *C. albicans* SC5314 germ tubes. We used heat-killed *C. albicans* in lieu of rAls1p-N to stimulate the splenocytes to mimic the in vivo situation during infection. The *C. albicans* cells were pregerminated in RPMI 1640 medium with glutamine (Gibco BRL) for 90 min to induce expression of

Als1p (13). The resulting *C. albicans* germ tubes were heat killed by incubation for 90 min at 60°C (22). The heat-killed fungal cells were added to the splenocyte cultures at a density of 2×10^7 pseudohyphae/ml (a ratio of five fungal cells to one leukocyte). After 48 h, splenocytes were profiled for Th1 (CD4⁺ IFN- γ ⁺ [gamma interferon positive] IL-4⁻ [interleukin-4 negative]), Th2 (CD4⁺ IFN- γ ⁻ IL-4⁺), or CD4⁺ IL-10⁺ frequencies by intracellular cytokine detection and flow cytometry as previously described (59). Three-color flow cytometry was performed on a Becton Dickinson FACScan instrument calibrated with CaliBRITE beads (Becton Dickinson, San Jose, Calif.) with FACSCComp software in accordance with the manufacturer's recommendations. During data acquisition, CD4⁺ lymphocytes were gated by concatenation of forward and side scatter and fluorescein isothiocyanate-conjugated anti-CD4 antibody fluorescence properties. Data for each sample were acquired until 10,000 CD4⁺ lymphocytes were analyzed. Results are presented as the median $\pm$ the 25th and 75th quartiles of the percentage of all gated lymphocytes that were Th1 or Th2 cells.

Footpad swelling. Footpad swelling was determined by the method of Oomura et al. (41). Briefly, mice were immunized with the appropriate dose of rAls1p-N or CFA alone as described above. Two weeks following the boost, baseline footpad sizes of immunized mice were measured with an electronic digital caliper. Fifty micrograms of rAls1p-N in 25 μ l of PBS was injected into the right footpads and PBS alone was injected into the left footpads of the immunized mice. Twenty-four hours later, the footpads were again measured. Antigen-specific footpad swelling was calculated as follows: (right footpad thickness at 24 h – right footpad thickness at baseline) – (left footpad thickness at 24 h – left footpad thickness at baseline).

Statistical analysis. The nonparametric log rank test was used to determine differences in the survival times of the mice. Titers of antibody, frequency of Th1 or Th2 lymphocytes, and footpad swelling were compared by the Steel test for nonparametric multiple comparisons (47) or the Mann-Whitney U test for unpaired comparisons, as appropriate. Correlations were calculated with the Spearman rank sum test. To determine if heterogeneity existed in replicate survival studies, the Kolmogorov-Smirnov test was used. *P* values of <0.05 were considered significant.

RESULTS

An intermediate dose of the rAls1p-N vaccine induces protection against murine disseminated candidiasis. To determine the most effective dose of the rAls1p-N immunogen, a 10^7 -fold dose range was evaluated (20 pg to 200 μ g per mouse). Female retired breeder BALB/c mice were immunized with rAls1p-N plus adjuvant (CFA or incomplete Freund's adjuvant) or with adjuvant alone. Immunized mice were bled 2 weeks after boosting to determine anti-rAls1p-N antibody titers (see below). The mice were subsequently infected with a lethal inoculum of *C. albicans* (2×10^5 blastospores). The survival data from repeat experiments were combined since the individual experiments demonstrated no statistical heterogeneity ($P > 0.05$ by the Kolmogorov-Smirnov test). The 20- μ g dose of rAls1p-N resulted in long-term survival of 25% of the infected mice and a significant increase in overall survival compared to that obtained with adjuvant alone ($P = 0.044$ by log rank test, Fig. 1). Neither 10-fold higher (Fig. 1) nor lower (data not shown) doses significantly increased survival compared to that obtained with adjuvant alone.

The above findings established a protective dose for the rAls1p-N vaccine. Next we evaluated the efficacy of the vaccine in a more rapidly lethal model of mice infected with 10^6 blastospores (median survival, 3 versus 11 days for 10^6 - versus 2×10^7 -blastospore inocula in unvaccinated mice, respectively). Again the data from repeat studies were combined as the results of the individual experiments demonstrated no statistical heterogeneity ($P > 0.05$ by the Kolmogorov-Smirnov test). When administered as a 20- μ g dose plus CFA to BALB/c mice infected with 10^6 *C. albicans* blastospores, the rAls1p-N vaccine more than doubled the median survival and resulted in a

FIG. 1. Immunization of mice (retired breeders) with rAls1p-N improves survival during subsequent disseminated candidiasis. Survival of mice immunized with Als1p plus adjuvant was measured. Sixteen mice per group were used in duplicate experiments on different days. Adj. = adjuvant. *, $P < 0.05$ versus adjuvant.

significant increase in overall survival versus that of unvaccinated controls ($P = 0.001$ by log rank test, Fig. 2A). To determine if the age of the mice influenced their response to the rAls1p-N vaccine, we tested it in juvenile mice. A similar survival benefit was found when juvenile mice were vaccinated and infected with the same high inoculum ($P = 0.02$ by log rank test, Fig. 2B).

Protection induced by the rAls1p-N vaccine did not correlate with antibody titers. Although the 200- μ g dose of rAls1p-N resulted in inferior protection compared to the 20- μ g dose, only the 200- μ g dose of antigen induced a significant increase in serum anti-Als1p antibody titers ($P \leq 0.005$ for the 200- μ g dose versus all other groups, Fig. 3). No significant increases in anti-Als1p antibody titers were detected at the intermediate, protective antigen dose ($P = 0.1$ for the 20- μ g dose versus adjuvant). When the serum anti-Als1p antibody titers of individual mice were plotted against the survival time of each mouse, no correlation between antibody titer and survival was found ($R^2 = 0.03$, $P > 0.05$ by the Spearman rank sum test). Indeed, mice immunized with the highest dose of antigen (200 μ g) had anti-rAls1p-N antibody titers in excess of

Als1p Dose

FIG. 3. Anti-rAls1p-N titers do not correlate with survival. Titers of anti-rAls1p-N polyclonal antibodies (Ab) raised in BALB/c mice immunized with various doses of rAls1p-N with or without adjuvant were measured. Adj. = adjuvant. *, $P \leq 0.005$ for the 200- μ g dose versus all others.

1:100,000 but had survival durations no different from those of mice immunized with lower doses of antigen, whose titers were at the lower limit of detection ($\sim 1:100$).

Only the protective dose of the rAls1p-N antigen induced significant Th1 polarization and a delayed-type hypersensitivity reaction. Since humoral immunity did not correlate with rAls1p-N-induced protection, we examined the cell-mediated immune response induced by protective and nonprotective doses of rAls1p-N. Mice were immunized with 0.2, 20, or 200 μ g of rAls1p-N or with adjuvant alone as described above. Two weeks after the boost, splenocytes were harvested and cultured in the presence of heat-killed, pregerminated *C. albicans*, which is known to express Als1p (13). Following 48 h of culture, splenocytes were harvested for intracellular cytokine detection by flow cytometry. Only the lymphocytes from mice immunized with the protective 20- μ g dose of antigen developed a significantly increased frequency of Th1 cells compared to mice given adjuvant alone ($P = 0.03$, Fig. 4). No significant differences in Th2 frequency (Fig. 4) or in the frequency of

FIG. 2. Immunization with rAls1p-N improves the survival of both retired breeder and juvenile mice. Survival of retired breeder (A) and juvenile (B) mice infected with a rapidly fatal inoculum of 10^6 blastospores of *C. albicans* was measured. Sixteen mice per group were used in duplicate experiments on different days. Adj. = adjuvant. *, $P < 0.05$ versus adjuvant control.

FIG. 4. Only the protective dose of rAls1p-N induces an increase in *C. albicans*-stimulated Th1 splenocytes. Induction of Th1 ($CD4^+$ $IFN-\gamma^+$ $IL-4^-$) and Th2 ($CD4^+$ $IFN-\gamma^-$ $IL-4^+$) splenocytes by different doses of the rAls1p-N vaccine was measured. Splenocytes from immunized mice (nine per group) were stimulated for 48 h with heat-killed pregerminated *C. albicans* and then analyzed by three-color flow cytometry. *, $P = 0.03$ versus adjuvant.

$IL-10^+$ T lymphocytes (data not shown) were detected between mice immunized with adjuvant and those that received any of the doses of antigen.

To confirm that type 1 immunity was stimulated by r-Als1p-N in vivo, delayed-type hypersensitivity was tested by measuring footpad swelling. Only mice vaccinated with the protective 20- μ g dose of rAls1p-N developed a significantly increased delayed-type hypersensitivity reaction compared to the control, and this response was also significantly greater than that induced by the nonprotective 0.2- and 200- μ g doses (Fig. 5, $P < 0.05$ for all comparisons versus the 20- μ g dose by the nonparametric Steel test).

The Als1p vaccine is effective in B-cell-deficient mice but not in T-cell deficient nude mice. To define the role of antibody and T cells in vaccine-mediated protection, B-cell-deficient, T-cell-deficient nude, or congenic BALB/c wild-type control mice were immunized with 20 μ g of rAls1p-N plus adjuvant or given adjuvant alone and infected with a lethal inoculum (8×10^5 blastospores) of *C. albicans*. B-cell-deficient mice tended to be more resistant to infection, whereas T-cell-deficient mice were more susceptible, than were wild-type control mice given adjuvant alone ($P = 0.065$ and 0.01 for B-cell-deficient and T-cell-deficient mice versus adjuvant-treated wild-type mice, respectively, Fig. 6). Finally, the rAls1p-N vaccine maintained its efficacy in B-cell-deficient mice ($P = 0.04$ for rAls1p-N vaccination versus adjuvant alone, Fig. 6) but was ineffective in T-cell-deficient mice ($P = 0.4$ for rAls1p-N vaccination versus adjuvant alone, Fig. 6).

FIG. 5. Only the protective dose of rAls1p-N induces an increase in rAls1p-N-stimulated delayed-type hypersensitivity. Delayed-type hypersensitivity was assessed by measuring footpad swelling in mice (9 to 12 per group) vaccinated with rAls1p-N and CFA or with CFA alone. Mice were immunized with the indicated amount of rAls1p-N and then injected in the footpad with 50 μ g of rAls1p-N. Footpad swelling was assessed 24 h later. *, $P < 0.05$ versus adjuvant, 0.2 μ g, and 200 μ g.

DISCUSSION

By using surrogate genetics, we identified a cell surface adhesin, Als1p, that mediates binding of *C. albicans* to host constituents including human endothelial and oral epithelial cells (13, 14, 32, 54). Herein we report proof of principle that immunization with the N terminus of this protein improved the survival of both juvenile and mature BALB/c mice during subsequent hematogenously disseminated candidiasis. Interestingly, an intermediate dose of rAls1p-N (20 μ g) provided superior protection compared to both lower doses and a higher dose (200 μ g). Nevertheless, the nonprotective 200- μ g dose of rAls1p-N was immunogenic, as it induced 100-fold higher titers of antibody than did the protective 20- μ g dose.

The inverted-U-shaped dose-response efficacy curve, with lower protection at the highest dose of rAls1p-N, is reminiscent of the classical studies of Parish and of Parish and Liew, who first described the inverse relationship between the induction of humoral and cell-mediated immunity by a given dose of antigen (42–44). In the context of Parish's seminal data, an inverted-U-shaped dose-response efficacy curve could be explained if (i) vaccine efficacy depended on cell-mediated immunity and (ii) intermediate doses of rAls1p-N stimulated superior cell-mediated immunity compared to the high, antibody-stimulating dose. We therefore hypothesized that the inverted-U-shaped dose-response efficacy curve seen with the rAls1p-N vaccine was due to superior induction of cell-mediated immunity by the protective, intermediate doses of antigen.

To test this hypothesis, the abilities of high, intermediate, and low doses of antigen to stimulate Th1 cells and delayed-type hypersensitivity were determined. To stimulate cytokine production from splenocytes, we specifically activated the cells by exposure to heat-killed *C. albicans*, instead of rAls1p-N, to mimic the in vivo situation during infection. Only the protective 20- μ g dose significantly increased the frequency of *C. albicans*-stimulated splenic Th1 lymphocytes. The frequency of Th1 cells seen in ex vivo *C. albicans*-stimulated splenocytes was

FIG. 6. The rAls1p-N vaccine requires T cells, but not B cells, to induce protective immunity. Survival of B-cell-deficient, T-cell-deficient (nude), and congenic wild-type BALB/c control mice (seven or eight per group) was simultaneously assessed after vaccination with rAls1p-N plus adjuvant or with adjuvant alone. *, $P < 0.04$ versus adjuvant alone; †, $P = 0.003$ versus adjuvant-treated wild type.

similar to that detected in vivo during disseminated candidiasis in mice (59), underscoring the relevance of this approach.

To determine if the detected ex vivo Th1 cells were of functional significance in vivo, we compared the delayed-type hypersensitivity responses induced by different doses of rAls1p-N immunization. Concordant with the frequency of Th1 cells, only the protective 20- μ g dose of rAls1p-N stimulated a significant in vivo delayed-type hypersensitivity reaction. These results are consistent with the hypothesis that vaccine-induced protection was due to induction of type 1 cell-mediated immunity. Surprisingly, despite induction of markedly elevated antibody titers by the 200- μ g dose of rAls1p-N, we did not find an increase in splenic Th2 lym-

phocytes in mice vaccinated with this dose. One possible explanation is that Th2 cells were activated in peripheral lymph nodes rather than the spleen. Alternatively, other T-cell populations (e.g., NKT cells) may have been responsible for inducing the high antibody titers seen in response to the 200- μ g dose of rAls1p-N.

The lack of correlation between antibody titer and protection did not completely exclude a role for antibodies in mediating vaccine-induced protection. For example, ELISA titers are the result of enumeration of antibodies with a variety of specificities and affinities. Therefore, the possibility that small subsets of antibodies were generated that did participate in vaccine-mediated protection could not be excluded by measuring antibody titers. To confirm the role of cell-mediated and not humoral immunity in rAls1p-N vaccine-mediated protection, we tested the efficacy of the vaccine in B-cell- and T-cell-deficient mice. B-cell-deficient mice tended to be more resistant to disseminated candidiasis than did wild-type controls, and the efficacy of the vaccine was not eliminated in B-cell-deficient mice. In contrast, T-cell-deficient mice were more susceptible to disseminated candidiasis than were wild-type controls, and the efficacy of the vaccine was lost in T-cell-deficient mice. Our findings therefore confirm that the efficacy of the rAls1p-N vaccine is dependent on induction of T-cell-mediated, and not humoral, immunity. Also, because B-cell-deficient mice were not more susceptible to disseminated candidiasis than their congenic wild type littermates were, antibody is not a dominant effector against disseminated candidiasis in this model. These results are consistent with previous reports, including the seminal studies by Romani et al. and Cenci et al., indicating that cell-mediated (type 1), rather than humoral (type 2), immunity is the key to host protection against murine disseminated candidiasis (6, 49, 57, 59).

Although we and others (28, 64) have found that B-cell-deficient mice are not more susceptible to candidemia than wild-type controls are, numerous studies have reported that passive immunization against *C. albicans* improves survival during murine disseminated candidiasis (18, 20, 45, 60). Indeed, a monoclonal antibody directed against a *C. albicans* heat shock protein (35) is currently in clinical trials. The paradox that exogenous antibody can mediate protection against infection even when normal host protection is due to type 1 cell-mediated immunity has been noted previously (57). One likely explanation of this paradox is that administration of exogenous antibody is not equivalent to induction of an endogenous type 2 immune response. Rather, passive immunization with exogenous antibody garners the benefits of a humoral response without the added deficit of IL-4-, IL-10-, and IL-13-mediated suppression of phagocytic activity (57). Such benefits mediated by the exogenous antibody could include serving as opsonins to assist activated phagocytes, as well as interfering with the ability of a pathogen to adhere to host tissues. Another potential explanation of the paradox that passive immunization is effective despite the lack of protection afforded by endogenous antibodies is the passive transfer of "protective" antibodies versus endogenous production of "nonprotective" antibodies, as described by Casadevall's group in murine models of *Cryptococcus* infection (5, 66). Similarly, Bromuro et al. have shown that mice vaccinated with heat-killed *C. albicans* produced antibodies that prevented protective immunity and

that when these antibodies were adsorbed out of serum, the remaining antibodies were protective during passive immunization (2). Indeed, the lack of protection afforded by the high antibody titers induced by the 200- μ g dose of rAls1p-N in our model may be due both to a lack of induction of protective cell-mediated immunity and to induction of antibodies that bind to nonprotective epitopes on *C. albicans*.

In sum, we report that the novel rAls1p-N vaccine mediates protection against experimental disseminated candidiasis by inducing cell-mediated rather than humoral immunity. Enhancement of the modest protective effect of the rAls1p-N vaccine may therefore be accomplished with additional priming of cell-mediated immunity with optimized adjuvants and/or cytokines or an alternate route of immunization. Indeed, in our ongoing studies we have already found a marked increase in efficacy by administering rAls1p-N subcutaneously rather than intraperitoneally.

Despite the extensive basic immunology literature indicating that cell-mediated immunity is the key to host defense against *C. albicans* infection (6, 7, 37, 48–51, 58, 59), to date applied immunotherapies against *Candida* infection have largely focused on the humoral immune system (3, 18–21, 31, 35). The few recent studies of active vaccination against disseminated candidiasis have reported dramatic protection (2, 4, 36). However, these studies focused on the use of whole-cell vaccines, which are less likely to be used clinically than protein vaccines. A mannan-conjugated active vaccine strategy was also described by Han et al. (21). In contrast, to our knowledge, this is the first demonstration of in vivo protection by immunization with an adhesin from *C. albicans*. Our data support the potential for protein-based vaccination to modulate cell-mediated immunity as a prophylactic or therapeutic strategy against disseminated candidiasis and provide a basis for the pursuit of additional strategies to selectively enhance the cell-mediated immune response.

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