Antibodies Targeting *Candida albicans* Als3 and Hyr1 Antigens Protect Neonatal Mice from Candidiasis

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ABSTRACT

Background. Pre-term infants in neonatal intensive care units are vulnerable to fungal sepsis. In these patients, *C. albicans* remains the predominant fungal pathogen causing high morbidity and mortality, despite antifungal therapy. Thus, new therapeutic strategies against neonatal candidiasis are needed. We have reported that vaccination with *C. albicans* cell wall N-termini recombinant proteins Als3 (rAls3p-N) and Hyr1 (rHyr1p-N) protected adult mice from disseminated candidiasis. NDV-3A vaccine (rAls3p-N+alum) protected women from recurrent vulvovaginal candidiasis, which was correlated with anti-Als3p IgG2 titers. Here, we evaluated the efficacy of Als3p and Hyr1p based vaccine in a neonatal candidiasis mouse model.

Methods. Inbred BALB/c mice strain was used for both active and passive vaccination studies. Female 4-6 weeks old mice were vaccinated with rAls3p-N or rHyr1p-N antigens mixed with complete Freund's adjuvant (CFA, priming)/incomplete Freund's adjuvant (IFA, boosting). Mice were mated after the boost and pups (3 days old) born to vaccinated mice were infected intraperitoneally (IP) with C. albicans. For passive vaccination, 3 days old naive pups were IP infected with *C. albicans* and treated with serum obtained from vaccinated adult mice. Fungal burdens were determined in the kidneys of infected neonate mice at 3 days post-infection. Antibody titers were determined by ELISA.

Results. CFA/IFA formulated Als3 and Hyr1 vaccines induced a robust antibody response with a ten-fold higher titer of IgG2, than attained by either antigen formulated with alum. Transplacental transfer of these antibodies significantly reduced the fungal burden in the kidneys of mice pups, and adoptive transfer of vaccinated mothers' sera into pups displayed similar levels of protection. Neutrophils were found important for this efficacy. Anti-Hyr1 antisera potentiated the activity of fluconazole in protecting from *C. albicans* infection.

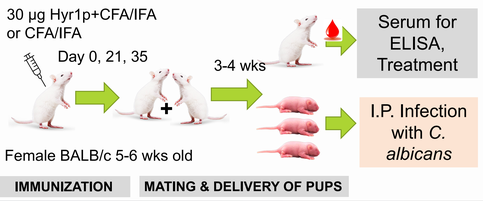
Conclusions. Our current studies are the first to emphasize the importance of anti-Als3 and anti-Hyr1 antibodies in preventing neonatal candidiasis. Considering that Candida infections in low-birth-weight infants is a lethal infection, active and passive vaccination strategies using these antigens could have profound clinical significance.

INTRODUCTION

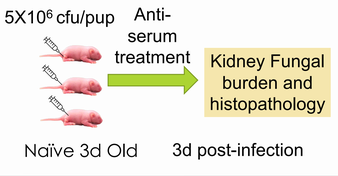
- About 11.4% are born preterm (28 - 36 weeks of gestation), 8% have low birth weight and 1.4% are of very low birth weight.
- It is recognized that 75% of infants admitted to the neonatal intensive care unit are colonized by Candida by the first month.
- Despite empirical antifungal therapy, mortality related to the disease remains considerably high (20-30%), with even higher rates (59-73%) of long-term neurodevelopmental impairment in survivors.
- Premise for vaccine / immunotherapeutic strategies to combat neonatal candidiasis:
  - *C. albicans* antigens Als3p and Hyr1p have been successful in previous pre-clinical studies in adult animal models and are available as targets for immunotherapeutic development.
  - Immunotherapeutic approaches have been successful in protection against other diseases in newborn infants.

METHODS

1. Active Vaccination



2. Passive Therapeutic Vaccination



RESULTS

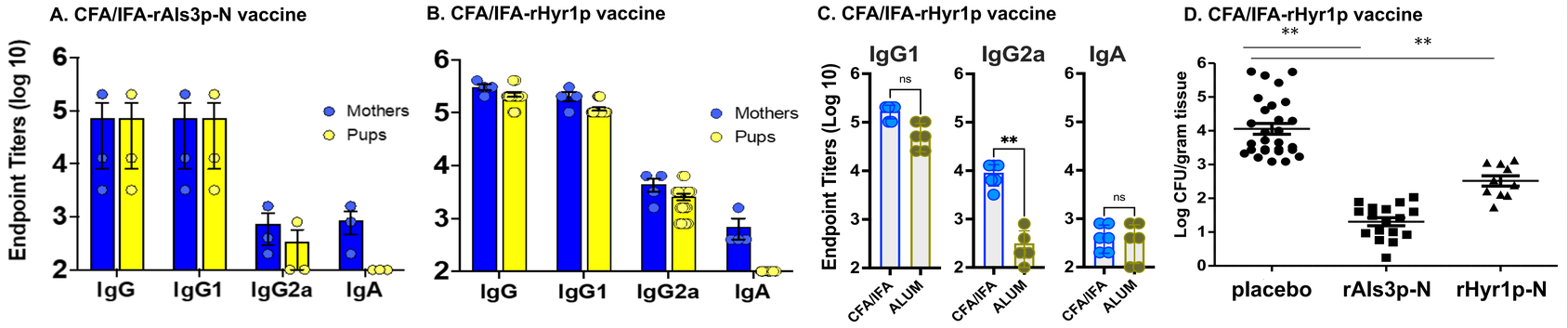


Figure 1. Active vaccination of Female BALB/c mice induce robust antibody titers that cross through placenta to induce protection in their newborn mice against C. albicans infection. Female BALB/c mice (6-weeks old) were vaccinated with Als3p-N or Hyr1p-N vaccine adjuvanted with CFA/IFA or adjuvant alone (placebo group) on day 0, 21 and 35. On day 35, mice were mated and kept in cages until the pups were delivered. Sera were obtained on day 6 post-partum both from mothers and their pups for antibody determination (A-C). Als3p-N vaccinated female BALB/c mice (A) and Hyr1p-N vaccinated mice (B) induce robust total IgG and IgG isotype and IgA antibodies, which efficiently transferred (equivalent titers) to their pups (except IgA). (C) CFA/IFA adjuvanted Hyr1p-N vaccine showed similar IgG1 and IgA isotype antibodies, but higher levels of IgG2a in CFA/IFA vaccinated mice. (D) Fungal burden in kidneys harvested from 3-day old pups delivered from mothers vaccinated with placebo, CFA/IFA or rAls3p-N or rHyr1p-N + CFA/IFA, three days after infecting with *C. albicans* (3 x 10^7 cells). $^{**}P$ <0.01 treatment vs. placebo sera vaccinated mice.

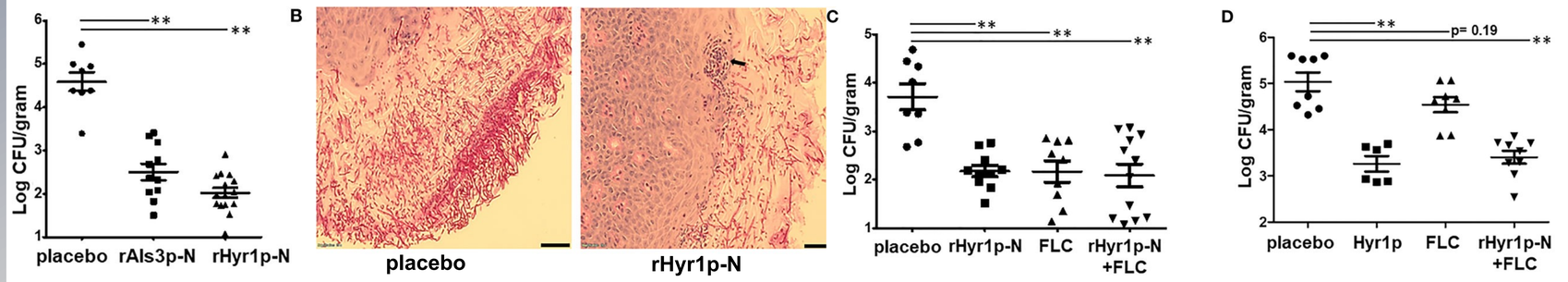


Figure 2. Passive vaccination by rAls3p-N or rHyr1p-N antisera protect pups from candidiasis. (A) Fungal burden in kidneys harvested from pups infected with *C. albicans* on day 3 of birth and treated with sera obtained from mice vaccinated with rAls3p-N, rHyr1p-N or adjuvant alone (placebo). Treatment was on day 0 (4 h post infection) and day 2 relative to infection. (B) Histopathology during systemic *C. albicans* infection in pups. Sections stained with PAS stain. Arrows indicate *C. albicans* hyphae invaded kidney tissues. Scale bar = 10 µm. (C) Kidney fungal burden in pups infected with a fluconazole (FLC) sensitive strain SC5314 and treated with control placebo sera (n= 8 pups), rHry1p-N anti-sera (n= 10 pups), or FLC alone (n= 9 pups), or a combination of rHyr1p-N+FLC (n= 12 pups). (D) Kidney fungal burden in pups infected with a FLC resistant strain CA6 and treated with control placebo sera (n= 8 pups), rHry1p-N antisera (n= 6 pups), FLC alone (n= 8 pups), or a combination of rHyr1p-N + FLC (n= 9 pups). $^{**}P$ <0.01 treatment vs. placebo sera vaccinated mice.

CONCLUSIONS

- rAls3p-N and/or rHyr1p-N based maternal vaccination strategies provide protection from from debilitating and lethal candidiasis in neonates.
- Anti-Als3p or Hyr1p antibody-based immunotherapies can protect neonatal mice from lethal systemic candidiasis.

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