

The Use of *Blastomyces dermatitidis* Yeast Lysate Antigens to Stimulate Primary and Secondary Antibody Responses in Immunized Rabbits.

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Abstract

Blastomycosis, a systemic fungal disease caused by the dimorphic fungus *Blastomyces dermatitidis*, is a potentially fatal disease for which no vaccines are currently available. This study was performed to determine the potential of four *B. dermatitidis* yeast phase lysate reagents in the induction of primary and secondary antibody immune responses in immunized rabbits. Rabbits were immunized (day 0; primary response and day 42; secondary response) with lysate antigens prepared from four *B. dermatitidis* isolates (592, human; 394, soil, T-58, dog and 48938, bat). Serum specimens collected from these rabbits over a 56-day period were evaluated using the enzyme-linked immunosorbent assay (ELISA; indirect peroxidase method) to determine the efficacy of each lysate antigen with respect to antibody induction at the various time points. The 592 and 48938 reagents induced both primary and secondary antibody responses with mean absorbance values ranging from 1.249 to 2.699 respectively. In contrast the T-58 and 394 antigens exhibited only minimal reactivity with absorbance values of 0.792 and 0.393. This study indicates the potential of the two optimal lysate antigens as immunizing agents for stimulating an antibody response to *B. dermatitidis*.

Introduction

Blastomyces dermatitidis is a thermally dimorphic fungus that may infect an individual by inhalation of conidia resulting in blastomycosis. This can take place after contaminated soil is disturbed by activities such as excavation, construction, or wood

clearing. At 37C *B. dermatitidis* converts to an invasive yeast form that can produce chronic, progressive pneumonia. Blastomycosis is only acquired from the fungus that is in soil or spores in the air. It is not spread from person-to-person or from animal-to-person. This fungus may disseminate through the blood and cause osteomyelitis and central nervous system, skin, and genital disorders (3). Untreated blastomycosis is slowly progressive and usually fatal; however, spontaneous remissions occasionally occur. If not treated the disease may become fatal in an immunocompromised host. Blastomycosis is endemic to the Mississippi and Ohio River valleys and northern Wisconsin (4,5).

Our laboratory has been involved in research activities centered around the development of antigenic reagents from the yeast phase of *B. dermatitidis* for the immunodiagnosis of blastomycosis in humans and animals. Other investigators have used different antigens as potential vaccine candidates for blastomycosis (6). The objective of this present study was to utilize yeast phase lysate antigens prepared from various isolates of *B. dermatitidis* to investigate their potential as immunizing agents using the rabbit model for antibody production.

Materials and Methods

Antigens/serum specimens: Three rabbits were injected with yeast phase lysates of each *B. dermatitidis* isolate (592-human, 394-soil, T-58-dog, 48938-bat) subcutaneously and intramuscularly using a 23 gauge needle. We injected 0.5 ml of lysate suspended in TiterMax ® Gold Adjuvant (Sigma) into each site (24 ug/ml, protein concentration). Once this was done an 18 gauge needle was used to bleed the rabbits from the marginal ear vein. Wintergreen oil was utilized to aid in dilating the ear vein and approximately 5-10 ml of blood was collected each time.

Rabbits were bled on day 7, 21, 28, 35, 42, and then reinoculated on day 42 and bled on day 7 PI (post-inoculation) and day 14 PI. Serum was collected by centrifuging once the blood had clotted overnight at 4°C.

ELISA: An indirect enzyme linked immunosorbent assay (ELISA; 1,2) was utilized to determine the amount of antibody present in the sera. Yeast lysate antigen (as above) was added to each plate and incubated overnight at 4°C. The plate was rinsed 3 times with PBS-T (phosphate buffered saline + 0.15% Tween 20). Rabbit serum diluted 1:5000 was added in triplicate and the plate was incubated at 37°C for 30 minutes. Plates were rinsed three times to remove any unbound antibody. A secondary antibody conjugated to horseradish peroxidase was added and the plate was incubated at 37°C for 30 minutes followed by rinsing to remove any unbound secondary antibody. Finally, the substrate was added and allowed to incubate at room temperature for approximately 3 minutes to detect antibody. The reaction was stopped with 2 N H₂SO₄ and the absorbance was read at 450 nm using the BIO-RAD EIA reader.

Results

The amount of antibody in the immunized rabbit serum specimens detected with the ELISA indicated that the 592 and 48938 reagents induced both primary and secondary antibody responses with mean absorbance values ranging from 1.249 to 2.699 respectively (Figures 2,3). However, the T-58 and 394 antigens induced only minimal primary/secondary antibody responses with mean absorbance values of 0.792 and 0.393 respectively (Figures 1,4).

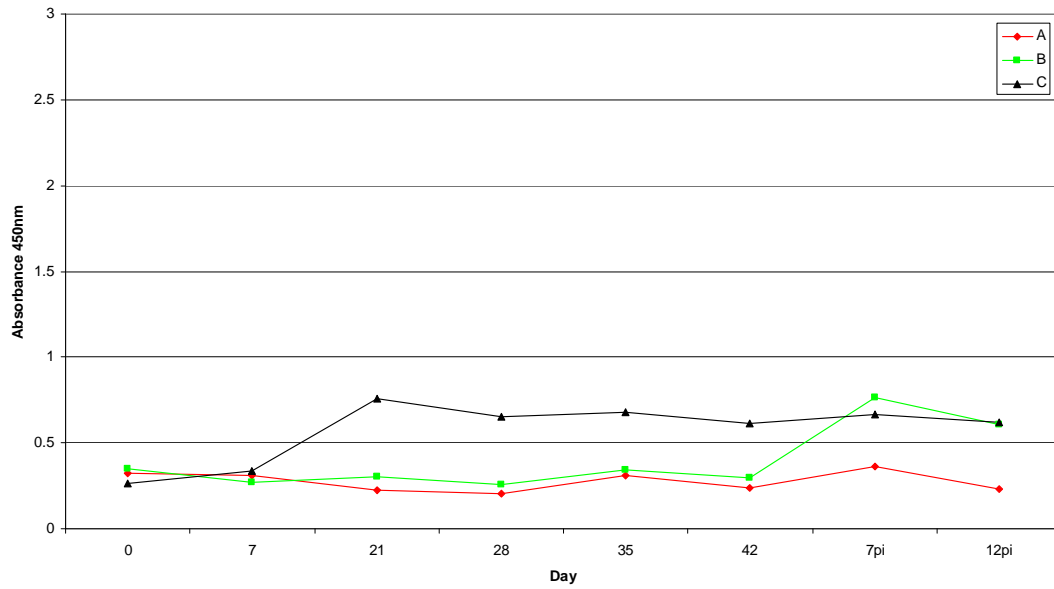


Figure 1: The ELISA detection of anti-*B. dermatitidis* antibodies with T-58 lysate antigen in serum specimens from rabbits immunized with T-58 lysate antigen. The three lines (A,B,C) represent antibody responses at the different time intervals of three individual rabbits.

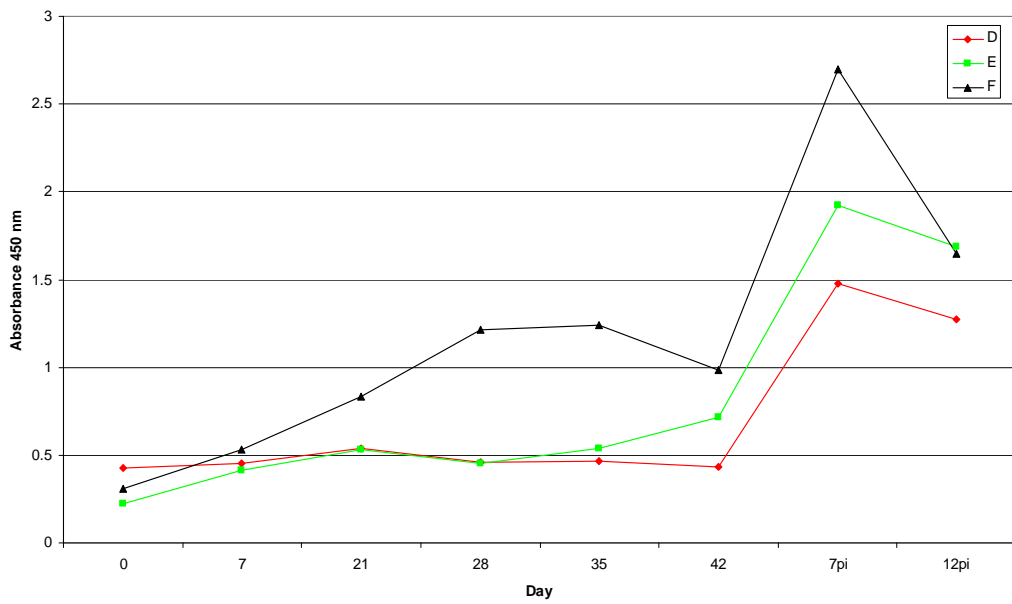


Figure 2: The ELISA detection of anti-*B. dermatitidis* antibodies with 48938 lysate antigen in serum specimens from rabbits immunized with 48938 lysate antigen. The three lines (D,E,F) represent antibody responses at the different time intervals of three individual rabbits.

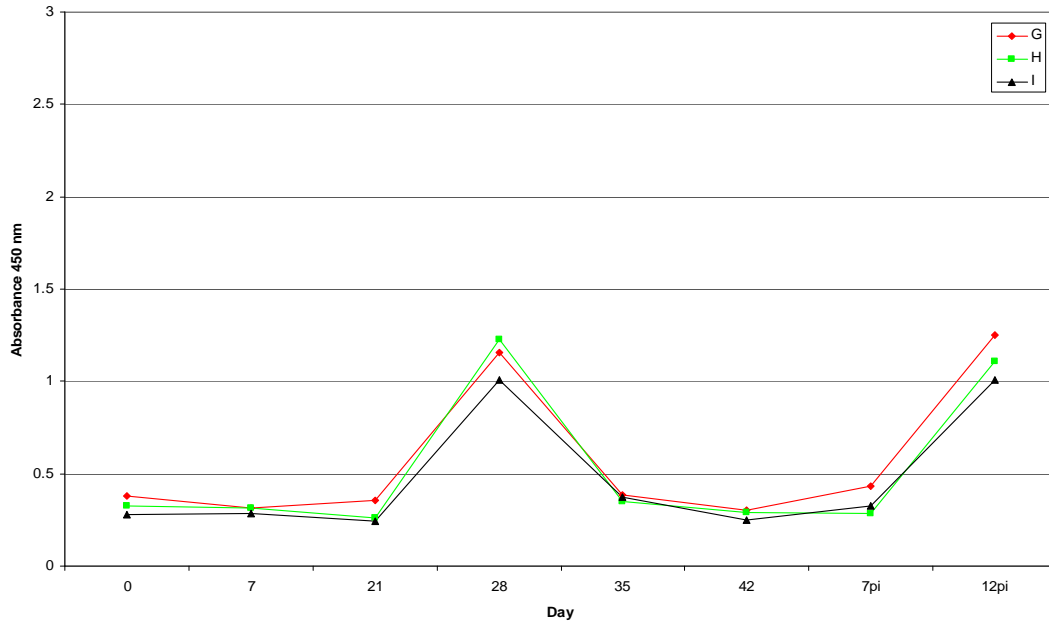


Figure 3: The ELISA detection of anti-*B. dermatitidis* antibodies with 592 lysate antigen in serum specimens from rabbits immunized with 592 lysate antigen. The three lines (G,H,I) represent antibody responses at the different time intervals of three individual rabbits.

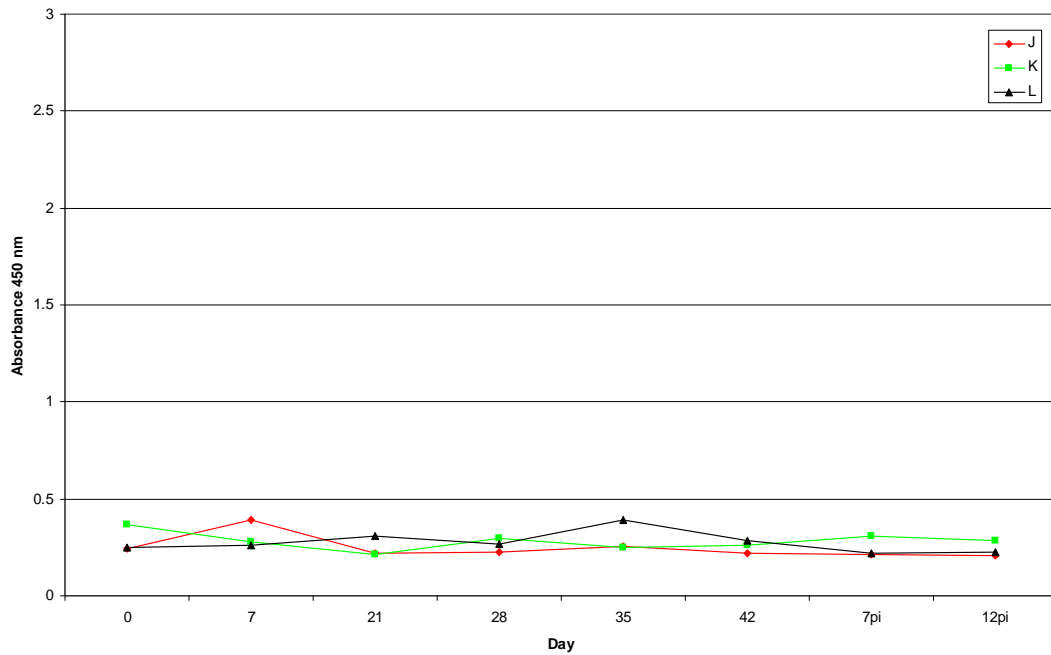


Figure 4: The ELISA detection of anti-*B. dermatitidis* antibodies with 394 lysate antigen in serum specimens from rabbits immunized with 394 lysate antigen. The three lines (J,K,L) represent antibody responses at the different time intervals of three individual rabbits.

Discussion and Conclusion

The importance of such a project must be emphasized since there are no vaccines available for the prevention of blastomycosis. The availability of a vaccine for this

disease, as well as other systemic fungal diseases, is at present becoming increasingly important due to the recognition of these infections in a variety of individuals (4).

Therefore our laboratory has become involved with respect to evaluating yeast phase lysate antigens prepared from *B. dermatitidis* as potential immunogenic agents for stimulating immune responses to *B. dermatitidis*.

The activity and existence of memory cells that resulted from the primary immune response is apparent in the rabbits inoculated with the 592 and 48938 reagents.

Conversely, the secondary antibody response in the rabbits immunized with the 394 and T-58 lysates showed minimal absorbance values. The poor immune response could be due to the immune system not properly recognizing and processing the antigen and subsequent production of antibody. The 48938 and 592 lysates generated good primary and secondary immune responses and perhaps may be vaccine candidates for this potentially deadly fungal disease of humans and various animals. Studies are continuing in our laboratory in attempts to optimize various antigenic reagents with regard to the development of agents that may stimulate antibody-mediated or cell-mediated immune responses to blastomycosis in humans and animals.

References

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