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Research article

Antibody Detection in Immunized Rabbits with *Blastomyces dermatitidis* Yeast and Mycelial Lysate Antigens

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Abstract

The laboratory diagnosis of blastomycosis has met with problems with regard to the development of immunodiagnostic assays for the detection of antibodies in a sensitive and specific manner. This present study was designed to compare the ability of *Blastomyces dermatitidis* yeast (YP) and mycelial phase (MP) lysate antigens, prepared following lysis for 1 or 7 days for the detection of antibodies in 25 serum specimens from rabbits immunized with either lysate preparations or killed whole yeast cells. The results from an indirect enzyme linked immunosorbent assay (ELISA) indicated that the mean absorbance values obtained with the day 1 reagents ranged from 0.995 (YP) to 0.935 (MP) and from 1.308 (YP) to 1.265 (MP) with the day 7 preparations when used to detect antibody in sera from rabbits immunized with the *B. dermatitidis* antigens. Specificity determinations when the above YP and MP day 1 reagents were used to detect antibodies in 7 sera from rabbits immunized with *Histoplasma capsulatum* lysate antigens ranged from 0.431 (YP) to 0.439 (MP) and from 0.474 (YP) to 0.489 (MP) with the day 7 reagents. Potential reactivity (mean absorbance values of the *B. dermatitidis* sera minus the *H. capsulatum* sera values) ranged from 0.564 (YP) to 0.496 (MP) with the day 1 preparations to 0.834 (YP) to 0.776 (MP) with the day 7 antigens respectively.

Keywords: *Blastomyces dermatitidis*, ELISA, blastomycosis, antibody detection, yeast and mycelial lysate antigens

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Introduction

Blastomycosis is a systemic fungal disease of humans and animals produced by the thermally dimorphic fungal pathogen *Blastomyces dermatitidis*. The geographic distribution of this disease has been associated with states that border the Ohio and Mississippi Rivers and upper Midwestern states including highly endemic regions of Wisconsin and Minnesota. The mycelial form of the fungus grows in slightly acidic soils and other organic matter and produces microscopic airborne spores. These spores are infectious particles that enter the respiratory system of humans and animals as a portal of entry. Once in the body at a temperature of approximately 37°C, the spores transform into large broad-based budding yeast cells [1, 2]

The growth of the yeast cells in the lungs leads to similar symptoms that mimic tuberculosis or other bacterial/viral diseases including fever, chills, chest pain, and productive cough. This pulmonary form may be followed by dissemination to other organs or to the production of cutaneous skin lesions. The fungus can produce extensive disease and possibly death in patients with AIDS or other diseases that compromise the immune system [3, 4].

In recent years investigators have been concentrating on trying to improve the laboratory diagnosis of blastomycosis. One concern is that the disease is either not diagnosed or misdiagnosed as some other microbial disease. The standard laboratory methods including culturing and histopathology have been utilized and are desirable, but these procedures may be time consuming or may fail to yield the desired results. Researchers have developed immunoassays that provide for a more rapid diagnosis, but many of the assays for antibody detection have been of limited value due to problems with sensitivity and specificity [3-5].

Our laboratory has been concentrating on the development and evaluation of various yeast-phase and mycelial-phase lysate reagents for antibody detection from a number of isolates of *B. dermatitidis* for the past several years [6-10]. These studies have provided data concerning possible approaches to improving the current immunodiagnostic methods for antibody detection, but they have also indicated the need for more evaluations on the use of various antigenic reagents in enzyme immunoassays for the sensitive and specific diagnosis of this disease. This current study was designed to compare *B. dermatitidis* yeast and mycelial antigenic preparations produced following lysis of the two growth phases for 1 or 7 days in distilled water for their ability to detect antibodies in sera from immunized rabbits to determine the sensitivity and specificity of the reagents.

Materials and Methods

Lysate Antigens

A mycelial phase culture of a *B. dermatitidis* isolate (T-58, Tennessee dog) was converted to yeast cells by culturing at 37 C on brain heart infusion agar. Yeast phase and mycelial phase lysate reagents were prepared by a method similar to one that was previously used for the production of antigen from *Histoplasma capsulatum* [11-13] and modified in our laboratory for *B. dermatitidis* lysate antigen production [6]. The yeast and mycelial cells were grown for 7 days at 37 C in a chemically defined medium in an incubator shaker, harvested by centrifugation (700 x g; 5 min), followed by washing with distilled water, re-suspended in distilled water and then allowed to lyse for 1 or 7 days at 37 C in water with shaking. The preparations were centrifuged, filter sterilized, merthiolate added (1:10,000) and stored at 4 C for further use. Protein determinations were performed on the lysates using the BCA Protein Assay Kit (Thermo-Fisher Pierce) and dilutions of the antigenic reagents used in the assays were based on protein concentration.

Serum specimens

Twenty five serum specimens from rabbits immunized with various yeast lysate antigens or killed whole yeast cells prepared from *B.dermatitidis* and 7 sera from rabbits immunized with *H. capsulatum* yeast lysates were assayed to

determine and compare the sensitivity and specificity of the *B. dermatitidis* day 1 and day 7 lysates as immunodiagnostic reagents.

Enzyme-linked immunosorbent assay (*ELISA*)

The ability of each of the 4 (YP) or (MP) lysate reagents to detect antibodies in the above serum specimens was determined using the indirect enzyme-linked immunosorbent assay (ELISA). Each lysate antigen was diluted (2000 ng/ml of protein) in a carbonate-bicarbonate coating buffer (pH 9.6) and then added to triplicate wells (100 ul) of a NUNC 96-well microplate (Thermo-Fisher). The plates were then incubated overnight at 4 C in a humid chamber followed by washing three times with phosphate buffered saline containing 0.15% Tween 20 (PBS-T). The serum specimens (1:2500 dilution; 100 ul) were added to the microplate wells and incubated for 30 min at 37 C in a humid chamber. Following this incubation the wells were washed as above and 100 ul of goat anti-rabbit IgG (H & L) peroxidase conjugate (Kirkegaard and Perry; KPL) was added to each well and incubated for 30 min at 37 C. The plates were again washed as above and 100 ul of Sure Blue Reserve TMB peroxidase substrate (KPL) was be added to each well and incubated for approximately 2 min at room temperature. The reaction will be stopped by the addition of Stop Solution (KPL) and the absorbance read at 450 nm using a BIO-RAD 2550 EIA reader.

Results/Discussion

The sensitivity and specificity of *B. dermatitidis* yeast phase lysate antigens (T-58) prepared following lysis for one day and seven days are shown in figure one. The day seven yeast and mycelial lysates detected antibody to a greater degree than the day one yeast and mycelial preparations. The yeast lysates were more reactive than the mycelial reagents at both intervals. Specificity determinations when the below YP and MP day 1 reagents were used to detect anti *H. capsulatum* antibodies ranged from 0.431 (YP) to 0.439 (MP) and from 0.474 (YP) to 0.489 (MP) with the day 7 reagents, Potential reactivity (mean absorbance values of the *B. dermatitidis* sera minus the *H. capsulatum* sera values) ranged from 0.564 (YP) to 0.496 (MP) with the day 1 preparations to 0.834 (YP) to 0.776 (MP) with the day 7 antigens respectively.

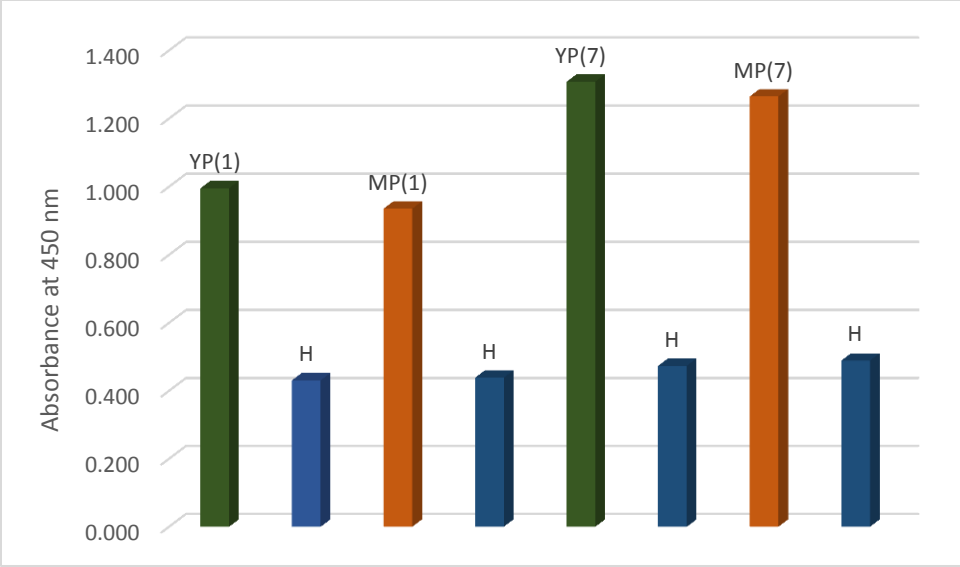

Figure 1: Comparison of antibody detection in rabbit serum specimens with *B. dermatitidis* (YP and MP, yeast and mycelial lysates, day 1 and day 7 reagents). Cross reactivity is also indicated with anti *H. capsulatum* (H) serum specimens.

Conclusion

This study indicates that the yeast phase lysate showed greater reactivity at both day one and day seven when compared to the mycelial reagents. The day seven lysates were more reactive than the day one lysates with both the yeast and mycelial preparations. The cross reactivity with *H. capsulatum* at day seven was approximately one third of the reactivity of the yeast and mycelial lysates. Studies are continuing to investigate the potential of *B.dermatitidis* lysate antigens as immunodiagnostic reagents for laboratory diagnosis of blastomycosis in humans and animals.

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