US Open Infectious Diseases & Immunity Journal
Vol. 1, No. 1, May 2014, pp. 1 - 5
Available online at http://www.arepub.com/Journals.php

Short Communication

Comparison of Antibody Detection with Lysate Antigens Prepared from Human and Dog Isolates of *Blastomyces dermatitidis*

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Abstract

Blastomycosis, caused by the systemic fungal organism *Blastomyces dermatitidis*, has presented a diagnostic challenge to medical personnel for many years. Our laboratory has been involved in the preparation and evaluation of yeast phase lysate antigens in attempts to develop immunodiagnostic assays that may provide a reliable diagnosis of the disease of humans and animals. The objective of this present study was to compare yeast lysate antigens prepared from four different isolates of the fungus (day 1 and day 7 periods of lysis) with respect to ELISA antibody detection in serum specimens from rabbits immunized with *B. dermatitidis* lysates or killed whole yeast cells. Mean absorbance values when the 8 lysate antigens were used to detect antibody in the sera from rabbits immunized with killed whole cells ranged from 1.904 (B5896-day 7) to 2.663 (ERC-2-day 7) and from 0.626 (B5896-day 7) to 1.205 (ERC-2-day 7). All antigens were able to detect antibody with absorbance values well above the 3X SD value of normal sera from non-immunized rabbits. The optimal yeast lysate antigen for antibody detection in the rabbits immunized with the lysate preparations and the whole yeast cells was produced from the dog isolate ERC-2 by allowing yeast cells to lyse for a period of 7 days in distilled water at 37 degree C.

Keywords: *Blastomyces dermatitidis*, Indirect ELISA, Antibody detection, Lysate Antigens

Introduction

Blastomycosis, produced by the dimorphic fungal organism *B. dermatitidis*, is a systemic fungal infection of humans and animals. Traditionally the geographic distribution of blastomycosis has been associated with southeastern and south-central states that border the Ohio and Mississippi Rivers and upper Midwestern states including areas in Wisconsin and Minnesota, which are highly endemic for the disease. Recent studies have indicated that blastomycosis may be present in other regions with sporadic cases being reported in Colorado, Texas, Kansas and Nebraska [1-3].

The infection is initiated by the inhalation of conidia (spores produced by the filamentous phase of the fungus). The organism exists in this stage in nature or in the laboratory at 25 C and has the ability to convert to the yeast phase at 37 C in the lungs of the infected host. The disease may be self-resolving or it may exist as an acute or chronic state in the pulmonary tissue, where it may be misdiagnosed as tuberculosis. If the disease is not diagnosed or untreated while in the lungs it may become invasive and disseminate to other organs and possibly to the central nervous system where fatal meningitis may develop [4-7]. Blastomycosis, as well as other systemic mycoses, are termed "emerging fungal threats" since they can not only infect persons with normal immune systems, but also they are a cause for concern in individuals with AIDS or other deficiency diseases that compromise the immune system [8,9].

Due to the increase in systemic fungal diseases researchers have begun to devote more attention to developing ways of diagnosing, preventing and treating blastomycosis. For the past several years the thrust of research in our laboratory has been associated with studies on various strains of *B. dermatitidis* from human, animal or environmental specimens from many geographical locations in an effort to better understand the immunobiology of the organism. Diagnosis of the disease has presented major problems. In some instances culturing or histopathological examination may be beneficial, but in some patients these methods may not yield the desired results. This has led to more and more work being done to improve immunological assays which tend to provide a more rapid diagnosis, but problems still exist with regard to the sensitivity and specificity of immunoassays [10-14]. Our laboratory has developed novel yeast phase lysate antigens and utilized these in various immunoassays for both antibody and antigen detection [15-18], but these studies have only opened up new avenues of approach with regard to how we might improve immunodiagnostic methods in the future.

The focus of our research has been to continue studies associated with the production and evaluation *B. dermatitidis* lysate antigens for the detection of antibodies in serum specimens. This current study was designed to compare *B.dermatitidis* lysate antigens produced by two different methods for the detection of antibodies in immunized rabbits.

Materials and Methods

Yeast phase lysate antigens were prepared from four different *B. dermatitidis* isolates (B5896, human, Minnesota; B5931, human, Minnesota; T-58, dog, Tennessee; ERC-2, dog, Wisconsin) by a method similar to one previously used for the production of lysate antigen from *Histoplasma capsulatum* [20-23] and modified in our laboratory for *B. dermatitidis* lysate antigen development [15]. Antigens were prepared following lysis in distilled water for 1 or 7 days. The lysate antigens were used to detect antibody in 8 sera from rabbits immunized with lysate or killed whole cell preparations (B5931; B5896; T-58; ERC-2).

An indirect ELISA (peroxidase system) used for detecting antibodies in the rabbit serum specimens with the above lysate antigens. The antigens were diluted (2000 ng of protein/ml) in a carbonate-bicarbonate coating buffer (pH 9.6) and added to triplicate wells (100 ul) of a Corning Easy Wash96-well modified flat bottom microplate. The plates were incubated overnight in a humid chamber at 4 °C, and then washed three times with 0.15% Tween 20 in phosphate-buffered saline (PBS-T; pH 7.4). The primary rabbit serum was diluted 1:2500 with PBS-T and added

(100 µl) to the wells. After 30 minutes incubating at 37 °C in the humid chamber, the wells were washed three times with PBS-T and 100µl of secondary goat anti-rabbit IgG + horseradish peroxidase enzyme (KPL, Gaithersburg, MD), diluted 1:2000 with PBS-T, was added to the wells and incubated again for 30 minutes at 37 °C. The plates were then washed with PBS-T as above and 100 µl of TMB peroxidase substrate was added and incubated for approximately 3 minutes at 25 °C. TMB Stop Solution was added and the absorbance was read at 450 nm using a BIO—RAD 2550 EIA reader.

Results and Discussion

The mean absorbance values obtained with the 8 *B. dermatitidis* yeast lysate antigenic reagents when used to detect antibody in the rabbit serum specimens is shown in Figure 1. All of the lysate antigens (both day 1 and day 7 preparations) detected antibody in the sera obtained by immunizing the rabbits with either lysate antigen or killed whole cell antigen with mean absorbance values considerably greater than a value obtained when 3X the SD value of the normal sera was added to the mean absorbance value of normal sera from non-immunized rabbits. The lysate reagent prepared from a *B. dermatitidis* dog isolate from Wisconsin (ERC-2; day 7 lysate) was optimal when used to detect antibody in the above serum specimens. The results from the study indicated only minimal differences in reactivity between the day 1 and day 7 preparations of the 4 isolates used in this study (Figure 2).

Based on the results of this present study, we intend to perform additional investigations on evaluating *B. dermatitidis* yeast lysate antigenic reagents prepared by allowing the yeast cells (from additional isolates of the fungus from human, animal and environmental sources) to lyse for one day in distilled water. In addition studies are in progress to purify and isolate the imunoreactive components from the optimal yeast lysate preparations with the ultimate aim of developing a reliable, sensitive and specific antigen for the laboratory diagnosis of human and animal blastomycosis.

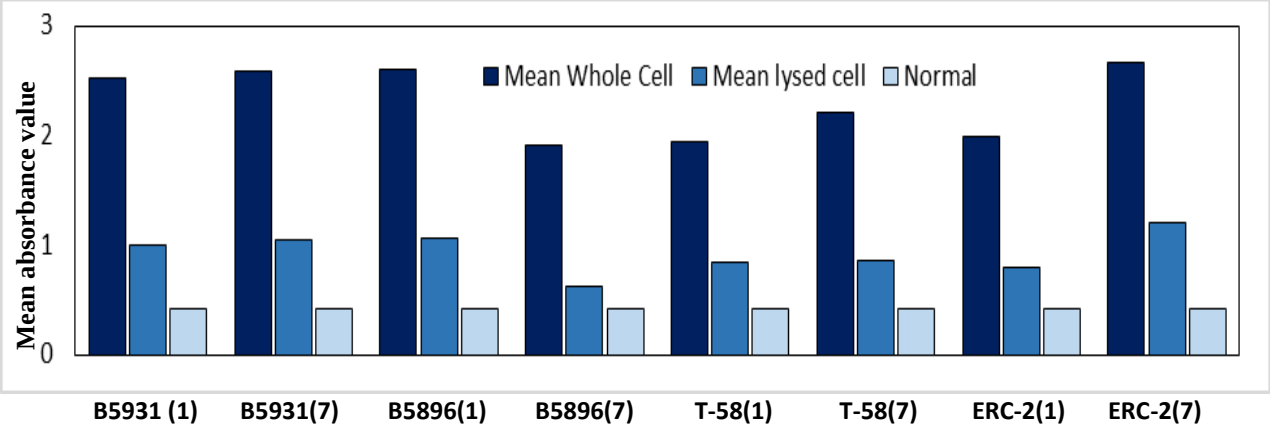

Figure 1: Antibody detection (mean absorbance values) with *B. dermatitidis* antigens

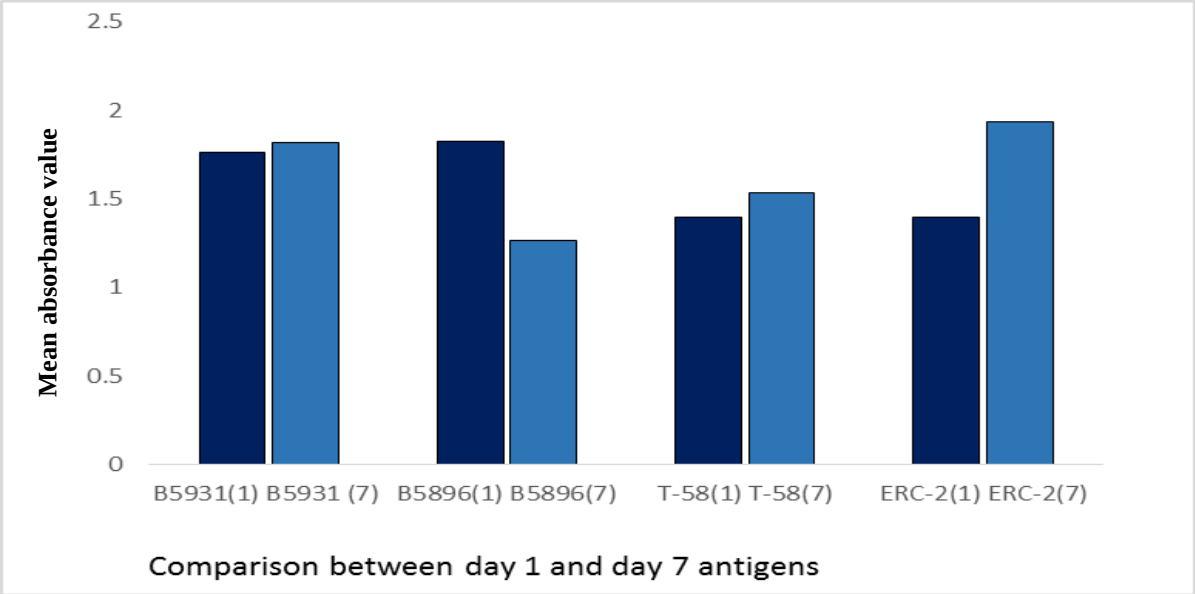

Figure 2: Comparison of antibody detection with *B. dermatitidis* antigens prepared following lysis for 1 or 7 days

Acknowledgement

This research was supported by the Department of Biological Sciences, Idaho State University, Pocatello, Idaho. USA

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