US Open Medical Laboratory & Diagnosis Journal
Vol. 1, No. 1, June 2014, pp. 1 - 6
Available online at http://www.arepub.com/Journals.php

Research article

Induction and Detection of Antibodies with Antigens Prepared from Human and Dog Isolates of *Blastomyces dermatitidis*

Ryan Cox, Jessika Deschine, John Gilmer, Jordan Hammon, Maryam Kalifa, Anthony Valentine and Gene M. Scalarone

Department of Biological Sciences, Idaho State University, Pocatello, Idaho 83209, USA

Phone: (208) 282-3374 Fax: (208) 282-4570

E-mail: coxryan2@isu.edu

This work is licensed under a Creative Commons Attribution 4.0 International License.

Abstract

The fungal infection, blastomycosis, is a systemic infection caused by the dimorphic organism Blastomyces dermatitidis. Immunodiagnosis has presented problems to clinicians for years. Our laboratory has been involved in studies on the preparation and use of novel B. dermatitidis antigens in developing antibody immunoassays for the laboratory diagnosis of blastomycosis in humans and animals. The focus of this study was to evaluate and compare antibody induction and detection, using 4 B. dermatitidis antigens (B5896 and B5931; human isolates and ERC-2 and T-58; dog isolates) in serum specimens from rabbits, with regard to antibody induction with lysate and killed whole cell antigens and antibody detection with day 1 and day 7 lysates. The indirect enzyme linked immunosorbent assay (ELISA) was utilized to determine antibody content. The study results for induction range for the lysate cell 0.725-1.147, and the range for whole cell 0.952-1.894. Antigen B5896 had the best induction for whole cell at a mean absorbance of 1.894 and antigen B5931 had the best induction for lysate cell at a mean absorbance of 1.741. Our detection range for day 1 is 0.646-1.510, and for day 7 0.866-1.703. Antigen B5896 had the best detection for day 1 lysate and day 7 lysate at a mean absorbance of 1.510 and 1.703 respectively. All of the antigens prepared from the four isolates were able to induce and detect antibodies in this comparative evaluation, but the antigen

© American Research Publications

B5896 had the best detection for day 1 lysate and day 7 lysate. Given the results, the human antigen B5896 yielded the best results for both induction and detection.

Keywords: Blastmyces dermatitidis, blastomycosis, Indirect ELISA, Antibody detection, Antibody Induction, Lysate antigens

Introduction

Blastomycosis, produced by the dimorphic fungal organism Blastomyces dermatitidis, is a systemic fungal infection of humans and animals that 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. Blastomycosis is cause for concern in individuals with AIDS or other deficiency diseases that compromise the immune system [1-4].

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 [5,6].

Due to the increase in systemic fungal diseases researchers have begun to devote more attention to developing ways of diagnosing, preventing and treating these mycoses. Blastomycosis has been a concern because problems have existed with regard to diagnosis of the disease or even the misdiagnosis as some other infectious disease. 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 research 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 [4,7-10].

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 how antigens prepared from these isolates might be useful as immunodiagnostic reagents. Our laboratory has developed novel methods for the preparation of yeast phase lysate antigens and utilized these in various comparative immunoassays for both antibody and antigen detection [11-21], 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 this current study was to evaluate and compare antibody detection, using 4 B. dermatitidis lysate antigens, in serum specimens from rabbits in which antibody production was induced with yeast lysate reagents and killed whole yeast cells prepared from the same isolates.

Materials and Method

Serum Specimens

Serum specimens were obtained from rabbits that were immunized with either yeast cell lysate antigens or whole killed yeast cells prepared from B. dermatitidis isolates (B5896 and B5931, human, Minnesota; ERC-2 and T-58,

© American Research Publications

dog, Wisconsin and Tennessee respectively) and available in our laboratory. Four serum specimens were obtained from each rabbit at different intervals following immunization and assayed for antibody content with yeast lysate antigens as described below. The animals were housed in accordance to the NIH Guide for Care and Use of Laboratory Animals with approval from the Idaho State University IACUC.

Yeast Lysate Antigens

Mycelial phase cultures of the above 4 isolates were converted to yeast cells by culturing at 37 C on brain heart infusion agar. Yeast phase lysate reagents were prepared by a method similar to one that was previously used for the production of antigen from Histoplasma capsulatum [22-24] and modified in our laboratory for B. dermatitidis lysate antigen production [21]. The yeast phase 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 either 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.

Enzyme-Linked Immunosorbent Assay (ELISA)

The ability of each of the 4 yeast lysate reagents (day 1 and day 7 lysates, as above) 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 SureBlue Reserve TMB Microwell Peroxidase Substrate (KPL) was added to each well and incubated for approximately 2 min at room temperature. The reaction will be stopped by the addition of TMB Stop Solution (KPL) and the absorbance read at 450 nm using a BIO-RAD 2550 EIA reader.

Results and Discussion

Antibody induction in rabbits immunized with either yeast cell lysate antigens or killed whole yeast cells of B. dermatitidis is shown in figure 1. For the yeast cell lysate, the minimum mean absorbance value was 0.725 with the T-58 (dog isolate) antigen, and the maximum lysate mean absorbance value was 1.147 with the B5931 (human isolate). In contrast, more antibody was induced with the whole cell antigens with mean absorbance values ranging from 0.952 (dog T-58 antigen) to 1.894 (human B5896 antigen). All of the antigens prepared from the four isolates were able to induce antibodies in this comparative evaluation, but the human preparations were optimal with the B5931 inducing the greatest response with the lysate antigen at 1.147 and the B5896 isolate inducing the greatest response with the whole cell antigen at 1.894. The results obtained from human and dog B. dermatitidis antigens indicated that the greater antibody response was with the whole cell antigens rather than the lysates in the immunized rabbits.

Antibody detection using lysate antigens that were prepared following lysis in distilled water for 1 and 7 days,in sera from immunized rabbits, indicated that day 1 lysates were slightly less reactive than day 7 lysates (Figure 2). Mean absorbance values from day 1 preparations ranged from 0.646 (T-58 dog antigen) to 1.510 (B5896 human antigen). The antibody detection mean absorbance range with day 7 preparations varied from 0.866 (T-58 dog antigen) to

© American Research Publications

1.703 (B5896 human antigen). Lysates from all four B. dermatitidis isolates detected antibodies in sera from the immunized rabbits. For both day 1 and day 7 lysates antigen B5896 yielded the highest mean absorbance of 1.510 (day1) and 1.703 (day7). Based on this result, the optimal reagent for detection was the B5896 day 7 lysate antigen.

As previously noted in our studies, [16-20] variations in the activity of lysate antigens prepared from different B. dermatitidis isolates was evidenced. We have continued to study and compare yeast phase antigens from human, animal and environmental isolates in an effort to develop an optimal immunodiagnostic preparation for the laboratory diagnosis of blastomycosis. This study was concerned with not only antibody induction with lysate and whole cell antigens prepared from the four isolates, but also, and even more important with regard to immunodiagnosis, how different lysate antigens could detect an antibody response. One isolate (B5986) seemed to produce a lysate reagent that exhibited the greatest ability to detect antibody in the serum specimens from immunized rabbits. Studies are continuing in our laboratory to further evaluate yeast lysates by performing purification studies in order to isolate the optimal immunoreactive components and to ultimately use these antigens for the definitive diagnosis of human and animal blastomycosis.

Acknowledgement

This research was supported by the Department of Biological Sciences, Idaho State University, Pocatello, ID 83209-8007, USA

References

[1] R. W. Bradsher, S.W. Chapman, and P. G. Pappas, “Blastomycosis,” Infectious Disease Clinics of North America, vol.17, pp. 21-40, 2003.

[2] P.G. Pappas, “Blastomycosis,” Infectious Disease Clinics of North America, vol.17, pp. 21-40, 2003.

[3] J. R. Bariola and K. S. Vyas, “Pulmonary Blastomycosis,” Seminars in Respiratory Critical Care Medicine, vol. 32, no. 6, pp. 745-753, 2011.

[4] J. A. McKinnell, and P. G. Pappas, “Blastomycosis: new insights into diagnosis, prevention, and treatment,” Clinics in Chest Medicine, vol. 30, pp. 227-239, 2009.

[5] A. F. DiSalvo, “Blastomycosis,” in Topley and Wilson’s Microbiology and Microbial Infections, L. Collier, Ed., pp. 337-3559, Arnold Publishers, London, UK, 9th Edition,1998.

[6] M. A. Pfaller and D.J. Diekema, “Epidemiology of invasive mycoses in North America,” Critical Reviews in Microbiology, vol. 36, no.1, pp. 1-53, 2010.

[7] M. Saccente and G. L. Woods, “Clinical and laboratory update on blastomycosis,” Clinical Microbiology Reviews, vol. 23, no. 2, pp. 367-381, 2010.

[8] K. S. Vyas, J. R. Bariola, and R. W. Bradsher, “Advances in the serodiagnosis of blastomycosis,” Current Fungal Infection Reports, vol. 2, pp. 227-231, 2008.

[9] B. S. Klein, R. A. Squires, J. K. Lloyd, D. R. Ruge, and A. M. Legendre, “Canine antibody response to Blastomyces dermatitidis WI-1 antigen,” American Review of Veterinary Research, vol. 61, no. 5, pp. 554-558, 2000.

© American Research Publications

[10] D. Spector, A. M. Legendre, J. Wheat, D. Bemis, B. Rohrbach, J. Taboada, and M. Durkin, "Antigen and antibody testing for the diagnosis of blastomycosis in dogs," Journal of Veterinary Internal Medicine, vol. 22, pp. 839-843, 2008.

[11] T. R. Allison, J. C. Wright, and G. M. Scalarone, "Blastomyces dermatitidis: stability studies on different yeast lysate antigens," Open Journal of Immunology, vol. 3, pp. 98-102, 2013.

[12] W. O. Hatch and G. M. Scalarone, "Comparison of colorimetric and chemiluminescent ELISAs for the detection of antibodies to Blastomyces dermatitidis," Journal of Medical and Biological Sciences, vol. 3, no. 1, pp. 1-6, 2009.

[13] C. M. Sestero and G. M. Scalarone, "Detection of IgG and IgM in sera from canines with blastomycosis using eight Blastomyces dermatitidis yeast phase lysate antigens," Mycopathologia, vol. 162, pp. 33-37, 2006.

[14] R. C. Axtell and G. M. Scalarone, "Serological differences in two Blastomyces dermatitidis isolates from different geographical regions of North America," Mycopathologia, vol. 15, pp. 141-144, 2002.

[15] J. C. Wright, T. E. Harrild, and G. M. Scalarone, "The use of isoelectric focusing fractions of Blastomyces dermatitidis for antibody detection in serum specimens from rabbits immunized with yeast lysate antigens,"Open Journal of Veterinary Medicine, vol. 2, pp. 237-241, 2012.

[16] J. L. VanDyke, A. Boyd, J. Sorensen, T. Hine, C. Rayner, A. Zamora, and G. M. Scalarone, "Detection of antibodies in serum specimens from dogs with blastomycosis with lysate antigens prepared from four Blastomyces dermatitidis dog isolates: Individual antigens and antigen combinations," Open Journal of Veterinary Medicine, vol. 3, pp. 235-239, 2013.

[17] A. R. Boyd, J. L. VanDyke, and G. M. Scalarone, "Blastomyces dermatitidis yeast lysate antigen combinations: Antibody detection in dogs with blastomycosis," Veterinary Medicine International, ID 940126, 4 pages, 2013.

[18] K. Mondada, J. Fullmer, E. Hungerford, K. Novack, K. Vickers and Gene Scalarone, "Blastomyces dermatitidis: Antibody detection in sera from dogs with blastomycosis with yeast lysate antigens produced from human and dog isolates," Veterinary Medic8ine International, ID 376725, 4 pages, 2014

[19] A.R. Boyd, J.B. Poole, J. Sorensen and Gene M. Scalarone, "Antigen detection in urine specimens from dogs with blastomycosis: Comparative ELISA determinations with serum from rabbits immunized with Blastomyces dermatitidis dog isolates," US Open Animal Health & Veterinary Medicine Journal, vol. 1, pp. 1-7, 2014.

[20] J.B. Poole, R.T. Cox, E.R. Cobb, A.R. Boyd and Gene M. Scalarone, "Comparison of antibody detection with lysate antigens prepared from human and dog isolates of Blastomyces dermatitidis," US Open Infectious Diseases & Immunity Journal, vol. 1, pp. 1-5, 2014.

[21] S. M. Johnson and G. M. Scalarone, "Preparation and ELISA evaluation of Blastomyces dermatitidis yeast phase lysate antigens," Diagnostic Microbiology and Infectious Diseases, vol. 11, pp. 81-86, 1989.

[22] H. B. Levine, G. M. Scalarone, and S. D. Chaparas, "Preparation of fungal antigens and vaccines: studies on Coccidioides immitis and Histoplasma capsulatum," Contributions to Microbiology and Immunology, vol. 3, pp. 106-125, 1977.

[23] H. B. Levine, G. M. Scalarone, G. D. Campbell, R. C. Graybill, and S. D. Chaparas, "Histoplasmin-CYL, a yeast phase reagent in skin test studies in humans," American Review of Respiratory Diseases, vol. 119, pp. 629-636, 1979.

© American Research Publications

[24] G. M. Scalarone, H. B. Levine, and S. D. Chaparas, "Delayed hypersensitivity responses of experimental animals to histoplasmin from the yeast and mycelial phases of Histoplasma capsulatum," Infection and Immunity, vol. 21, pp. 705-713, 1978.

© American Research Publications