DETECTION OF ANTIBODIES TO *CANDIDA ALBICANS* GERM TUBE BY IMMUNOFLUORESCENCE IN IMMUNOSUPPRESSED MICE WITH EXPERIMENTAL SYSTEMIC CANDIDIASIS

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Abstract- The increasing incidence of systemic candidiasis, which parallels the use of invasive and immunosuppressive medical procedures, necessitates development of rapid and cost effective tests for diagnosis of systemic candidiasis. Therefore in this study 85 mice were first immunosuppressed by cyclophosphamide and then infected by *Candida albicans* NCPF 3153. Other 85 mice were employed as control. The case and control mice were bled and then autopsied. Hearts and kidneys were checked by direct, histopathological and cultural examination for systemic candidiasis. The 85 sera from histological proven cases and 85 control mice were adsorbed with heat killed blastospores of same strain of *C. albicans*. Anti-Candida albicans germ tube antibodies were detected by indirect immunofluorescence assay for diagnosis of invasive candidiasis in case and control mice. In addition, sera from 35 mice with proven cryptococcosis were also tested. While 84 mice with proven systemic candidiasis (100%) had anti-germ tube antibodies, these antibodies were absent in all controls and mice with cryptococcosis. The specificity was 100%, indicating a high degree of discrimination was possible between systemic candidiasis and cryptococcosis in the mice studied. It must be concluded that anti-germ tube responses did not appear to be significantly reduced in immunocompromised mice.

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Key words: Antibody, Anti-germ tube, Candidiasis, Diagnosis

INTRODUCTION

Invasive Candida infections are a growing problem among patients put at risk by advances in medical technology, chemotherapy, cancer therapy, or organ transplantation. The management of serious and life-threatening candidiasis remains severally hampered by the lack of both specific clinical manifestations and the accuracy of conventional microbiological methods for diagnosis (1) because diagnostic methods must differentiate Candida colonization of mucous membranes or superficial infection from tissue invasion and candidemia requiring antifungal therapy (2). These problems have led to demands for the development of reliable serological tests for the diagnosis of invasive Candida infections.

Among the characteristics of such tests, relative simplicity, specificity and sensitivity may be the most important, since it will facilitate their adoption by clinical microbiology laboratories. Serological methods used in diagnosis of invasive candidiasis rely on the detection of antibodies against Candida,

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detection of Candida antigens and detection of non-antigenic Candida components, like D-arabinital, mannose, (1-3)β-D-glucan or nucleic acids. Nevertheless, each technique has limitations, and none of them have found widespread clinical use (3).

Detection of antibodies in patients with invasive candidiasis has two main limitations: 1) they may not be useful diagnostically, since antibodies titers can be high in patients who are only colonized (4), and 2) the antibody response may be delayed, reduced, or absent in immunocompromised patients (3). However, encouraging results in sensitivity and specificity have been obtained with the detection of antibodies to *C. albicans* germ tubes (CAGT), even in immunocompromised patients with invasive candidiasis (5).

Therefore, in the present study we have designed a test to detect antibodies against CAGT in diagnosis of invasive Candidiasis, that could be carried out in almost clinical microbiology laboratory all over the country after adaptation and evaluation for use in diagnosis of human systemic Candidiasis since indirect immunofluorescence technology is available in most clinical laboratories.

MATERIALS AND METHODS

Organism

C. albicans serotype A (NCPF 3153) was used throughout the study.

Experimental infection

Two groups of 85 Swiss white male mice with average weight of 20 g were employed, 85 for production of experimental infection (cases) and other 85 as controls.

As an initial step the case mice were given cyclophosphamide intraperitoneally (i.p.) at 200 mg Kg^{-}(1) four days before the challenge. The immunosuppressed mice were then intravenously (i.v.) inoculated with 0.1 ml of 10^4 or i.p. with 0.5 ml 10^8 yeast cell suspension. At the same time 85 normal mice were inoculated i.v. or i.p. with 0.1 or 0.5 ml sterile saline as controls. The animals were observed twice daily for 30 days after which inoculation and mortality were recorded. Procedures involving animals and their care were conducted in conformation with national policies.

The mice were bled after 28 or 30 days and sera were separated and stored at -70 °C until used. The animals were then autopsied and kidneys and heart were removed and examined with touch smears, histopathological and cultural examination. Touch smears were stained with Giemsa and Gram. Tissue sections were obtained and stained with Gomori methenamine sliver stain (GMS).

Duplicate culture of specimens from kidneys and heart were made on sabouraud dextrose agar (S) and incubated at 37 °C for 4 d.

Indirect immunofluorescent antibody assay

C. albicans was grown as germ tubes or blastospores in medium 199 (Gibco Laboratories, Grand Island. N.Y.), as described by Ponton *et al.* (6). Washed germ tubes and blastospores were used in the preparation of immunofluorescence slides (7). Briefly, to each well of Teflon coated 12 well immunofluorescence glass slides, 10^6 cells suspended in 10 µl of phosphate-buffered saline (PBS) were added, air dried, rinsed in PBS, and dried with a jet of air. The sera were adsorbed with heat-killed blastospores of the same strain to remove the antibodies against the blastospore cell wall surface antigen and allow the detection of antibodies to CAGT by method described by Quindos *et al.* (7).

Later, 10 µl of each sera was applied to each well of the immunofluorescence slides and incubated at 37°C for 30 min. Then the slides were washed and incubated with fluorescein-conjugated goat anti mouse immunoglobulin (Sigma USA) at 37 °C for another 30 min. The slides were washed again and examined with a Leitz Dialux microscope equipped to detect reflected fluorescence. The yellow-green fluorescence was considered as presence of antibody against germ tube antigen. This test also was done using sera from 35 mice with proven cryptococcosis which produced in our previous experiments.

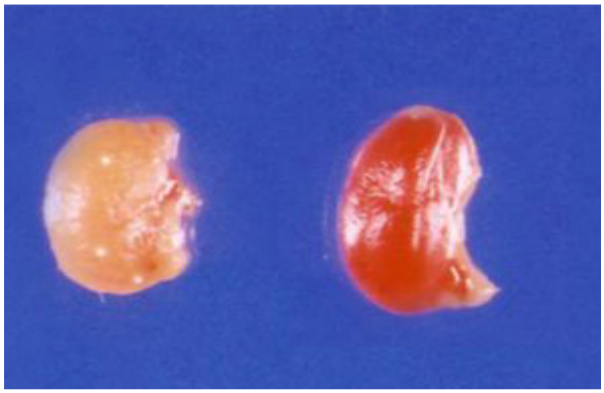

Fig. 1. Gross lesions on kidney of *C. albicans* infected mouse (left). Kidney of healthy mouse is shown on the right.

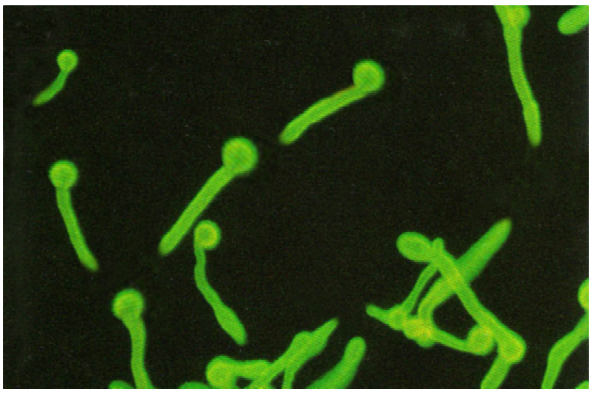

Fig. 2. Immunofluorescence of filamentous growth of *C. albicans* stained with blastospore-adsorbed (germ tube specific) antiserum. Note germ tubes are stained.

RESULTS

The results revealed that an infective inoculum of 10^8 yeast cells of *C. albicans* per immunosuppressed mouse was found to be an excellent infectious-dose through i.p. route than 10^4 yeast cells through i.v. route.

Twenty-eight to 30 days after fungal challenge, systemic candidiasis was diagnosed by direct, culture and histopathological examination. The main target organs of infection were the kidneys, and heart was the second involved organ by both infection routes. Gross lesions were frequently seen on these kidneys (Fig. 1) and occasionally on heart.

Anti-CAGTA were detected in 84 (100) histological proven case mice (Fig. 2) but all control mice were anti-CAGTA negative.

Touch smears of kidneys with two staining methods of Giemsa and Gram showed *C. albicans* in 73 (85%) and 40 (47%) of cases, respectively.

Histopathological examination of kidney samples showed tissue invasion in 84 (98.8%) of case mice (Fig. 3).

Touch smears from heart with two staining methods of Giemsa and Gram were positive in 63 (74%) and 34 (40%) of case mice, respectively. Tissue invasion was observed in heart of 30 (58%) cases (Fig. 4).

Cultures of kidneys and hearts of infected mice were yielded growth of *C. albicans* in 80 (95.2%) and 52 (62%) cases, respectively. The control mice, inoculated with sterile saline, did not develop any infection and direct culture and histopathological examination of kidneys and heart form these mice were negative. Anti-germ tube antibodies were also absent in all mice with cryptococcosis. Specificity, sensitivity, positive and negative predicative values and also efficacy, validity and reliability of five different methods are recorded in Table 1.

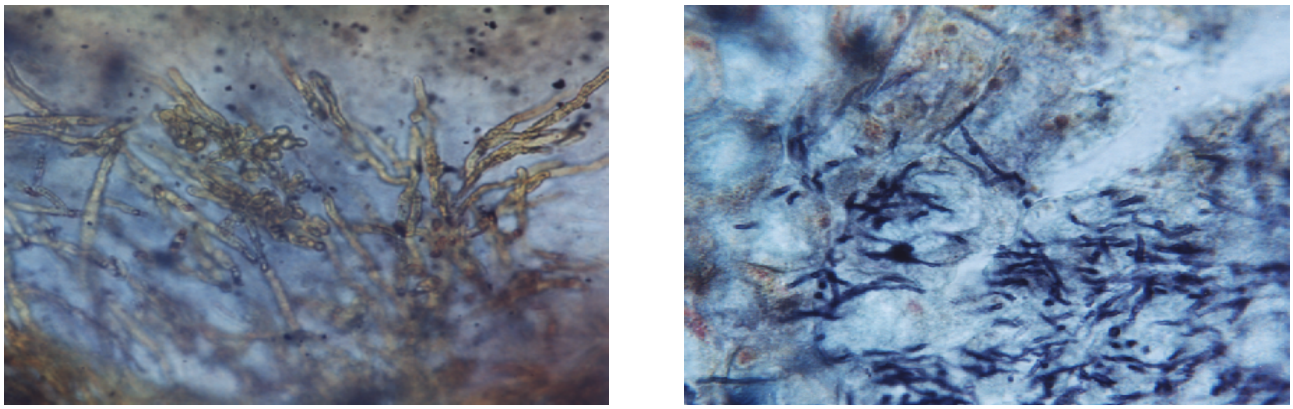

Fig. 3. Abscess in kidney of mouse 28d after infection with *C. albicans* showing multiple fungal filaments and yeasts (left GMS × 1000, right GMS × 400).

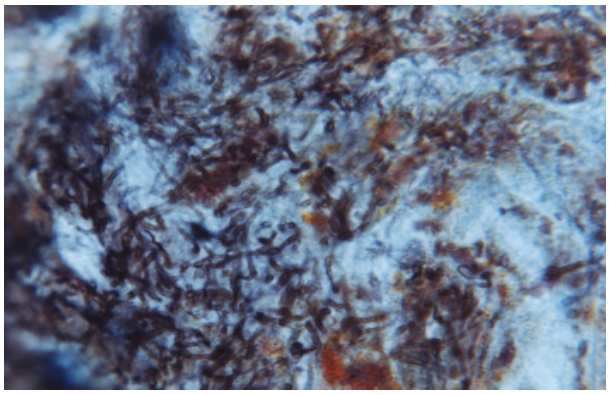

Fig. 4. The myocardium is invaded by both blastospores and hyphae (GMS × 400).

DISCUSSION

Although there is a general belief that antibody tests are both insensitive and non specific (4, 8-10), there is important recent evidence suggesting that detection of antibodies in highly immunocompromised patients is possible and useful for the diagnosis of invasive candidiasis (10- 13).

The ability of *C. albicans* to alter its cell morphology from blastospores to hyphae helps the fungus to adhere to the host epithelium and to penetrate the host tissue. During this morphological transition important changes in the antigenic composition of the fungus occur which may be useful for serodiagnosis of systemic candidiasis. A mannoprotein of 230- 250 KDa (>200 KDa) located on the germ tube cell wall surface (type I) is recognized by sera from patients with invasive candidiasis and is truly germ tube–specific (14-16). In contrast, mannan is an antigen type III which is expressed on both the blastospore and germ tube surface. Since the anti-Candida albicans blastospore antibodies detected are mainly anti mannan (8), the presence of this antibody among the general population (17) and cross reactivity among mannan present in the cell wall of different fungi (18, 19) may explain the lower specificity of this antigen in diagnosis of invasive candidiasis.

Since it was shown that detection of antibodies to CAGT appear to be a useful maker for diagnosis and therapeutic monitoring of immunocompromised patients with invasive candidiasis, we decided to design and carry out this indirect immunofluorescent antibody test for detection antibodies against CAGT antigen in animal model. The results presented in this study show that immunosuppressed mice produce detectable antibodies to CAGT.

In addition to the high values of sensitivity (100%) and specificity (100%) reached in the present study, detection of antibodies to CAGT allowed recognition of Candida infection, especially in mice with tissue-proven but negative by culture invasive candidiasis. The highest values for detection of anti-CAGT antibodies were obtained comparing mice with candidiasis and mice with cryptococcosis (specificity 100%).

Table 1. Comparison of different diagnostic methods in experimental renal and heart candidiasis

Diagnostic method	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Efficiency	Reliability	Rate of confidence
Renal							
Giemsa	86	100	100	88	93	93	0.8
Gram	47	100	100	65	73.5	73.5	0.7
Culture	95	100	100	20	95.5	97.5	0.2
GMS	100	100	100	100	100	100	1
IFA	100	100	100	100	99.4	100	1
Heart							
Giemsa	74	100	100	79	87	87	0.8
Gram	40	100	100	62.5	70	70	0.63
Culture	62	100	100	73	80.5	81	0.73
GMS	58	100	100	79.4	68	79	0.8
IFA	100	100	100	100	99.4	100	1

Abbreviations: GMS, Gomori methenamine silver; IFA, indirect immunofluorescence antibody

In conclusion, detection of anti-Candida albicans germ tube antibodies may be an important aid to diagnosis of systemic candidiasis since such antibodies are present in the all of case mice with invasive candidal infection at levels not significantly altered by immunosuppression. In addition our results showed that IFA test to detect antibodies to CAGT antigens can be developed for diagnosis of invasive Candida infection. Finally it is worth while to say that, there wasn't any commercially kit available for detection of antibodies against CAGT when we began this investigation at 2003, and at present we are trying to produce this kit after clinical trail for use in diagnostic laboratories.

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Conflict of interests

We have no conflict of interests.

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