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Evaluation the Anti Proliferative Activity of Structural Proteins and Fraction of Supernatant from Culture of *Candida albicans*

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Abstract: In this study, anti proliferative activity of structural proteins and fraction of supernatant from culture of *Candida albicans* on proliferation responses of lymph node cells in Balb/c mice have been evaluated. For this reason *Candida albicans* was cultured in RPMI medium supplemented with 10% FBS at 37 in%5 CO_2 until reaching a confluent state for 2 weeks. The culture supernatant was obtained by centrifugation and purified by gel filtration chromatography and structural proteins of *C. albicans* were obtained from breakage of cell wall by vortexing with glass beads in suspension of PBS and 1 mM PMSF and then cultured with lymph node cells and evaluated by MTT assay. The results in this study demonstrated that both of structural proteins and fraction of supernatant from culture of *Candida albicans* suppress immune responses compared with Control group. Our study provides evidence that proteins of *C. albicans* have anti proliferative activity.

Key words: *Candida albicans*, structural proteins, supernatant from culture of *C. albicans*, MTT assay, antiproliferative activity

INTRODUCTION

Candida albicans is a component of the normal micro flora of the alimentary tract and mucocutaneous membranes of the healthy host (Newman and Holly, 2001). The genus Candida contain a heterogenous of anamorphic yeasts (Known-Chung and Bennet, 1992). When immune defenses are compromised or the normal microflora balance is disrupted, Candida transforms itself into an opportunistic pathogenic killer. The opportunistic pathogen *Candida albicans* is a serious agent of infection in the immunocompromised host (Verduyn Lunel *et al*., 1999; Rantala and PostoPerative, 1993). Despite appropriate mortality from systemic Candida infections in immunocompromised individuals is nearly 30% (Wenzel and Pfaller, 1991).

Some reports indicated that *C. albicans* and component of it suppress immune responses. *C. albicans* affects the differentiation of human monocyte into suppressor dendritic cells that produce IL-10 (Torosantucci *et al*., 2004). Systemic *C. albicans* infection can augment the effects of immunosuppressive therapies by promoting functional changes in immunosuppressive cells (Newman and Holly, 2001).

C. albicans suppresses NO production by Macrophages (Chinen *et al*., 1999). Soluble factors secreted by *C. albicans* suppress superoxide anion production in Neutrophils (Smail *et al*., 1998). As documents show, *C. albicans* can suppresses humoral and cellular immune responses. This study was designed to determine antiproliferative activity of structural proteins and fraction of supernatant from culture of *Candida albicans* on the proliferation response of lymphocytes by MTT assay because immunosuppressive effect of *Candida albicans* can be one of the factors that increasing tumor mass and promoting progression malignancy in immunocompromised patients.

For this reason, we prepared both structural proteins and fractionated of supernatant from long term culture of *C. albicans* and then evaluated anti proliferative activity of above proteins on the proliferation response of lymphocytes by MTT assay. The results of MTT assay revealed that structural proteins and also fraction of supernatant from culture of *Candida albicans* compared with control group had antiproliferative activity.

MATERIALS AND METHODS

Animals: Eight-to-ten-weeks-old inbred Balb/c mice were purchased from Pasteur Institute, Tehran, Iran. They were given sterilized water and autoclaved standard mouse chow *ad libitim* throughout the study.

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Preparation of structural proteins of *Candida albicans*: *Candida albicans* (confirmed by molecular tests) was cultured on SDA (Sabourauds Dextrose Agar) for 48 h. Yeast cells were washed twice with PBS (Phosphate Buffer Saline) and collected by centrifugation (3000 g, 10 min), then suspended in a small volume of 1 mM PMSF and broken by vortexing with glass beads (5 mg beads per mg cells); complete cell breakage was obtained. Cell breakage was assessed by examining the preparation with a phase-contrast microscope as published previously (Elorza *et al*., 1985). By this method we can obtain structural proteins of *C. albicans*. The next step was dialysis of structural proteins in PBS for 48 h.

We did proteins assay by Bradford then isolated proteins were freeze-dried and stored at -70°C until further use.

Preparation the supernatant fraction from culture of *Candida albicans*: *C. albicans* cells were maintained in Sabourauds liquid medium with shaking at 27°C. 5×10 (7) cells mL^{-1} of *C. albicans* was counted and cultured in 50 mL of RPMI 1640+10%FBS and incubated at 37°C and 5% CO_2 for 2 weeks. The culture supernatant was collected by centrifugation (2200 rpm/15 min) and concentrated by freeze drier. Concentrated supernatant from culture of *C. albicans* was fractionated by gel filtration chromatography. (Sephadex G-100) we used Bradford method for protein assay and stored at -70°C (Tang *et al*., 2004).

LYMPHOCYTE TRANSFORMATION ASSAY

Preparation of lymphocytes from brachial lymph nodes of mouse: Balb/c mice, 8-10 weeks of age, were used for lymphocyte preparation. Brachial lymph nodes were removed after killing the mice by cervical dislocation and passed through a stainless steel mesh into a culture plate using a syringe plunger. The mesh was rinsed twice with Phosphate Buffer Saline (PBS) under sterile conditions. Cells were washed and resuspended in RPMI+10%FBS.

MTT assay: A 100 μL cell suspension was added to each well of the micro plate. Then various concentrations of structural proteins and fraction of supernatant from culture of *C. albicans* (0.001, 0.01, 0.1, 1, 10 and 100 μg mL^{-1}) were added to the wells. In the negative control well, only RPMI-1640 was added without proteins of *C. albicans*. All experiments were performed in triplicate. Micro plates were incubated at 37°C in a 5% CO_2 for 72 h.

After this time, cell proliferation was measured based on the MTT (3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide) assay. MTT was dissolved in Phosphate Buffer Saline (PBS) at 5 mg mL^{-1} and filtered for sterilization and a small amount of insoluble residue present in some batches of MTT was removed. The MTT solution (25 μL) was added to each well and plates were incubated at 37°C for 4 h. A total of 100 μL dimethyl sulfoxide (DMSO) was added to all wells and mixed thoroughly to dissolve the dark blue crystals. After a few minutes at room temperature to ensure that all crystals were dissolved, the plates were read on MultiScan MS ELISA reader, using a wave-length of 570 nm.

Statistical analysis: All experiments were carried out two to three times. We used Mann-Whitney and Duncan tests to determine which dosages had maximum anti-proliferative effect on proliferation of lymphocytes. In all cases p value below 0.05 were considered significant.

RESULTS

Preparation of the fraction of supernatant from culture of *C. albicans*: Supernatant of *C. albicans* was gel filtered with Sephadex G-100 and fractions of 5 mL were collected. We used Bradford method for protein assay (595 nm). Diagram of fraction of supernatant from culture of *C. albicans* showed one peak. We collected the fraction (under diagram) (5-23) and stored at -70°C for further use (Fig. 1).

Anti proliferative activity of structural and supernatant fractions of *C. albicans*: In order to assess anti proliferative activity of structural and supernatant proteins, the protocol in Fig. 2 was performed and the

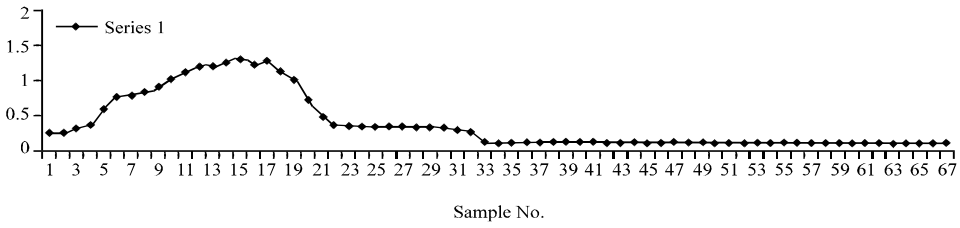

Fig. 1: Diagram of fraction of supernatant from culture of *C. albicans*

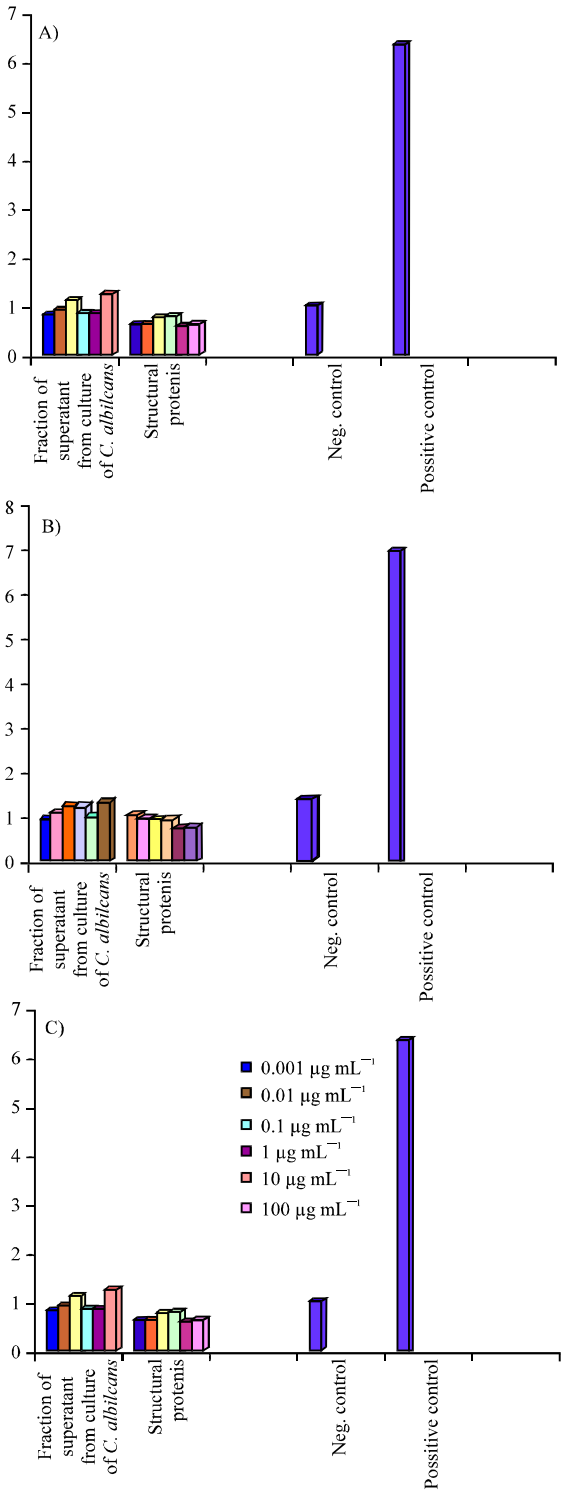

Fig. 2: Anti proliferative activity of structural proteins and fraction of supernatant from culture of *C. albicans* in MTT assay as different hours, (a) 24 h MTT (stimulation index) (b) 48 h MTT (Stimulation index) (c) 72 h MTT (Stimulation index)

results were evaluated by MTT assay. Results were obtained by various concentrations of structural proteins and fraction of supernatant of *C. albicans* (0.001, 0.01, 0.1, 1, 10, 100 µg mL^{-1}) then were analyzed by Mann-Withney test. Results indicated that all of concentrations of structural proteins after 24, 48, 72 h compared with control group had anti proliferative effect on proliferation of lymphocytes ($p<0.05$).

Also results showed that fraction of supernatant had anti proliferative effect at some concentration (1 µg mL^{-1}) but structural proteins are more anti proliferative effective than fraction of supernatant.

DISCUSSION

An intention of this study was to evaluation the antiproliferative activity of structural proteins and fraction of supernatant from culture of *Candida albicans* by MTT assay. Results of this test revealed that above proteins suppress proliferation responses of lymphocytes ($p<0.05$).

We used MTT Assay in order to evaluation the immunosuppressive effect of structural proteins and fraction of supernatant from culture of *C. albicans* on proliferation of lymphocytes. Most of the biological functions related to pathogenicity and virulence reside in the fungal cell wall. The cell wall of *Candida albicans* not only is the structure in which many biological functions essential for the fungal cells reside but also is a significant source of candidial antigens. The major cell wall components that have antiproliferative effect are proteins and glycoproteins, the latter being predominantly mannoproteins (Martinez *et al*., 1998). Numerous documents indicate that *Candida albicans* and its component have immunosuppressive effect on the microenviroment of the tissues. *C. albicans* affects the differentiation of human monocyte into suppressor dendritic cells that produce IL-10 (Torosantucci *et al*., 2004). killed form of *C. albicans* inhibits IFN-gamma production by NK cells (Murciano *et al*., 2006). Diamond *et al*., reported soluble factors secreted by *C. albicans* suppress superoxide anion production in Neutrophils.

Immunosuppression of cell mediated immunity by mannan (Podzorski *et al*., 1989). Above results show that *Candida albicans* and its component have immunosuppressive effect. For this reason we decided to examine antiproliferative activity of *Candida albicans* components. The results indicate that structural and fraction of supernatant from culture of *C. albicans* have immunosuppressive activity. MTT Assay showed that all concentrations (0.001, 0.01,0.1, 1, 10, 100 µg mL^{-1}) of structural-proteins of *C. albicans* compared with control

group had antiproliferative activity specially after 72 h (Fig. 2). But results of MTT assay about fraction of supernatant from culture of *C. albicans* (Secretion proteins) revealed that at concentrations of 0.001, 0.01, 10 μg mL^{-1}, had the maximum anti proliferative activity on lymphocyte proliferation responses ($p<0.05$) (Fig. 2). The results in this study demonstrated that both structural proteins and fraction of supernatant from culture of *C. albicans* (Secretion proteins) suppress immune responses compared to control group (Fig. 2). Present study provides evidence that proteins of *C. albicans* are immunosuppressive. Suppression mechanisms of *C. albicans* against immune responses can be one of the factors that increasing tumor mass and promoting progression malignancy in cancer patients who are affected by opportunistic infectious (candidiasis). Infections in general can promote malignancy progression by multiple mechanisms. Result of *C. albicans* immunosuppression is immunosurveillance reduction. In all forms of immunodeficiency, opportunistic infectious (candidiasis) is the relative risk of developing tumors (preliminary research).

Candidiasis is a life-threatening disseminated infection in immunocompromised patients and causes morbidity and mortality among this populations For this reason, scientists must have more attention to opportunistic infections and use new therapeutic strategies for the management of this type of infection. Early diagnosis, prompt treatment and prevention of opportunistic infections in immunocompromised patients may help in surveillance of patients and significant reduction on malignancy progression.

REFERENCES

Chinen, T., M.H. Qureshi, Y. Koguchi and K. Kawakami, 1999. *Candida albicans* suppresses nitric oxide (NO) production by interferon-gamma (IFN-γ) and lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages. Clin. Exp. Immunol., 115: 491-497.

Elorza, M.V., A. Murgui and R. Sentandreu, 1985. Dimorphism in *Candida albicans*: Contribution of mannoproteins to the architecture of yeast and mycelial cell wall. J. Gen. Microbiol., 131: 12209-12016.

Known-Chung, K.J. and J.E. Bennet, 1992. Medical Mycology. Leu and Febigez. New York, pp: 300-302.

Martinez, J.P., M.L. Gil, J.L. Lopez-Ribot and W.L. Chaffin, 1998. Serologic response to cell wall mannoproteins and proteins of *Candida albicans*. Clin. Microbiol. Rev., 1: 121-141.

Murciano, C., E. Villamon, J.E. Oconnor and M.L. Gil, 2006. Killed *Candida albicans* yeasts and hyphae inhibit gamma interferon release by murine natural killer cells. Infect. Immunity, 74: 1403-1422.

Newman, S.L. and A. Holly, 2001. *Candida albicans* is phagocytosed, killed and processed for antigen presentation by human dendritic cells. J. Infect. Immunity, 69: 6813-6822.

Podzorski, R.P., H.J. Herron, D.J. Fast and R.D. Nelson, 1989. Pathogenesis of candidiasis. Immunosuppression by cell wall mannan catabolites. Arch. Surg., 124: 1290-1294.

Rantala, A., 1993. Postoperative candidiasis. Ann. Chir. Gynaecol. Suppl., 205: 1-50.

Smail, E.H., D.A. Melnick, R. Ruggeri and R.D. Diamond, 1998. Anovel natural inhibitor from *Candida albicans* hyphae causing dissociation of the neutrophil respiratory burst response to chemotactic peptides from other post-activation events. J. Immunity, 140: 3893-3899.

Tang, N., L. Liu, K. Kang, P.K. Mukherjee, M. Takahara, G. Chen, T.S. McCormick, K.D. Cooper and M. Ghannoum, 2004. Inhibition of Monocytic Interleukin-12 Production by *Candida albicans* via Selective activation of Erk Mitogen-activated Protein Kinase. Infect. Immunity, 72: 2513-2520.

Torosantucci, A., G. Romagnoli, P. Chiani and R. Nisini, 2004. *Candida albicans* yeast and germ tube forms interfere differently with human monocyte differentiation into dendritic cells: A novel dimorphism-dependent mechanism to escape the host, simmune response. Infect. Immunity, 72: 833-843.

Verduyn Lunel, F.M., J.F. Mels and A. Voss, 1999. Nosocomial fungal infections: Candidemia. Diagn. Microbiol. Infect. Dis., 34: 203-220.

Wenzel, R.P. and M.A. Pfaller, 1991. Candida species: Emerging hospital bloodstream pathogens. Infect. Control. Hosp. Epidemiol., 12: 523-524.