

3rd International Congress on

Bacteriology and Infectious Diseases

August 04-06, 2015 Valencia, Spain

The role of *Candida albicans* in the activation of TLR-mediated mechanisms of innate immunity

Svitich O A¹, Blinkova L P¹, Gankovskaya L V², Grigorieva O Yu², Labdginov P A¹, Filina A B¹ and Zverev V V¹

¹L.Mechnikov Research Institute of Vaccines and Sera, Russia

²Pirogov Russian National Research Medical University, Russia

Introduction: Candidiasis of infants and immunocompromised people is the one of the main problems of medicine. *Candida albicans* and products of its metabolism play an important role in the pathogenesis of candidiasis. Previously it was found that Toll-like receptor (TLRs) system of innate immunity is directly involved in the process of recognition of fungal agents, such as *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans* and some others. Information about mechanisms of interaction of fungi (*C. albicans*) and system of innate immunity is extremely important because it allowed developing new methods of therapy.

Aim: The aim of this work was to investigate the levels of TLRs and cytokines gene expression *in vitro* after exposure to *C. albicans* antigens and in the epithelial cells of the mucosa of cervical canal of pregnant women with candidiasis.

Materials & Methods: The experiments were carried out on the Vero cells and mononuclear cells (MNCs). *Candida* cells were grown on minimal medium with 1% glucose. Antigens were presented after biomass was inactivated by heating. MNCs from peripheral blood of healthy donors were isolated by density gradient of ficoll pack. Selected mononuclear cells (at a concentration of 1×10^6 cells/ml) or 48-hour monolayer of Vero cells were cultured with lysates of *C. albicans* in 96-well plates (Costar, USA) at 37°C in the atmosphere of 5% CO₂. The study of gene expression of TNF, TLR1, TLR2, TLR3, TLR9 and TLR6 was performed using real-time PCR using the intercalating dye SYBR Green I and fluorescent probes (Synthol, RF). The number of copies of cDNA genes was standardized by expression of b-actin gene. Vero cells were incubated with *C. albicans* antigen for 3 and 24 hours, MNCs culture for 12 and 24 hours. In this study we also examined expression levels in the epithelial cells of the cervical canal of 20 pregnant women (27-30 weeks): 10 healthy pregnant women and 10 pregnant patients with candidiasis.

Results & Discussion: During experiments it was established that in Vero cells after 3 hours of antigenic exposure levels of TLR1, TLR2, TNF genes expression significantly increased and the level of TLR6 expression was unchanged. After 24 hours, levels of expression of innate immunity genes were not statistically different from control values. In the culture of MNCs levels of TLRs genes increased after 12 hours of antigenic exposure, the level of TNF gene expression after 24 hours. So, the level of TNF gene expression in the experiment corresponded to 3.38 ± 0.34 lg (number of copies of cDNA), relatively 2.56 ± 0.28 lg (number of copies of cDNA in the sample). It can be assumed that in response to *C. albicans* infection in cultures of Vero cells and mononuclear cells the expression of TLR2 and TNF α increase. Studies have shown that the level of expression of TLR1 gene in epithelial cells of the cervical canal of pregnant women with urogenital infection compared with healthy pregnant women increases 6 times or more and TLR2-more, then 10 times. The level of expression of TLR6 in cells of patients with candidiasis has not changed or has been lower than in the cells of healthy pregnant women. Significant correlation was shown between levels of expression of TLR2 and TLR1 ($r=0.8921$), which is an indirect evidence for the dimerization of these receptors. Thus, the antifungal immune responses, apparently, specified through TLR2/TLR2 and TLR1/TLR2. Further research of TLR-dependent mechanisms of the detection of antifungal immunity will allow creating the new approaches in treatment of fungal infections, based on TLRs ligands.

Biography

Svitich O A is currently working at Mechnikov Research Institute of Vaccines and Sera RAMS Moscow, Russian Federation.

svitichoa@yandex.ru

Notes: