EVALUATION OF MYCOBACTERIAL ANTIGENS AS ACTIVATORS OF IL-2 CYTOKINE PRODUCTION BY T-CELLS FOR IMMUNODIAGNOSTICS OF TUBERCULOSIS

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Cytokines play an important role in cell mediated immune responses to various infections. The interferon gamma (IFN-γ) production by activated T cells has been widely considered to play a crucial role in protection against *M. tuberculosis* infection that makes this cytokine useful for immunodiagnostics of tuberculosis. The interleukin-2 (IL-2) cytokine is a new possible diagnostic biomarker, based on the fact that IL-2 is significantly differentially produced by individuals with latent and active tuberculosis patients.

The aim of this study was to assess the use of *M. tuberculosis* antigens for evaluation of T cell specific immune response as tuberculosis diagnostics.

The subjects enrolled in this study were classified as active tuberculosis patients, latent tuberculosis patients or healthy people. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples of each enrolled patient, within 8 hours after venipuncture to ensure cell activity, by Ficoll density gradient (1.077 g/mL) centrifugation. Cells were transferred into Roswell Park Memorial Institute complete medium (RPMI 1640) to obtain a concentration of $2.5x10^6$ PBMCs/mL.

Three recombinant *M. tuberculosis* antigens, 6 kDa early secretory antigenic target (ESAT-6), 10 kDa culture filtrate antigen (CFP-10) and L-alanine dehydrogenase (Ala-DH), were tested for stimulation of T cells to produce cytokines by using Elispot test platform.

The LIOSpot® TB anti-human IL-2 ELISpot kit was used. Phytohaemagglutinin mitogen was used as a positive control. RPMI 1640 complete medium was used as negative control. Test wells contained three different antigens ESAT-6, CFP-10 and Ala-DH. Then PBMCs from each patient were seeded in order to have $2.5x10^5$ cells per well and tested according to the instruction. The number of spot forming cells was counted. Results were expressed as number of spot forming cells per million of PBMCs.

The T cell specific response to each antigen was evaluated in terms of IL-2 production. Comparing the results of infected and non-infected patients, there were significant differences for all the antigens ($p<0,001$). In the group of healthy people, 0-18 spot forming cells per million of PBMCs were detected to all antigens. In the group of infected patients, results varied depending on antigen: Ala-DH – 3-1120, ESAT-6 – 30-1300, and CFP-10 – 78-1590 spot forming cells per million of PBMCs. In addition, it was found that Ala-DH antigen was able to stimulate IL-2 production in adults with active tuberculosis but not with latent infection.

Conclusion. *M. tuberculosis* antigens, ESAT-6, CFP-10 and Ala-DH, are potential candidates for immunodiagnostics of tuberculosis based on evaluation of T cell specific immune response. Using Ala-DH in Elispot test allows discriminating between active tuberculosis diseases and latent tuberculosis infection.