IL-12 PE, CD 69 PERCP, CD3 FITC, AND CD4 APC OPTIMIZATION WITH ACTIVATION OF ISOLATED AGENT HEAT-KILLED SONICATED *MYCOBACTERIUM TUBERCULOSIS* BEIJING STRAIN

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ABSTRACT

Infection caused by *Mycobaterium tuberculosis* exists in form of intracellular infection, which leads to lymphocyte activation. CD69 is the first lymphocyte activation marker expressed in Th1 lymphocyte, which follows by IL-12 release. Flow cytometry analysis can identify the subpopulations of lymphocytes and intracellular cytokines such as IL-12, yet precise preparation needs to be done. This research aims to conduct optimization with four color lyse/wash flow cytometry assay system FastImmune™ FACSCalibur examination, with monoclonal antibody IL-12, CD69, CD3, and CD4 in succession uses fluorochrome PE, PerCP, FITC, and APC.To activate the lymphocytes from heparinized whole blood, we used activation agent which derives from isolated heat-killed sonicated *Mycobacterium tuberculosis* Beijing strain. Optimal concentration from the according activation agents is 40 µL. To determine the compensation, BD™ CompBead and blank-cell unstainning are used, but the maximum result showed by blank-cell unstainning.Each monoclonal antibody dosage of IL-12PE, CD69 PerCP, and CD3 FITC is 40 µL, while CD4 APC 5 µL. Total event lymphocyte is determined minimally by 10,000 events. With 18,510 total events and Th gated events quantity are 4,692, the result obtained is IL12-PE has 7.4% gated (347 events); $CD69^+$ perCP/$CD3^+$ FITC 18.2% (850 events); and $CD69^+$ perCP/$CD4^+$ APC 3.9%.

Keywords: heat-killed sonicated *Mycobacterium tuberculosis* Beijing strain; IL-12; CD69; flow cytometry

Introduction

Infection caused by *M. tuberculosis* has broad variety of courses, characteristics, and effects in each individual because the victim's immune responses act differently for both responses from nonspecific immune and specific immune. In early stage of infection, nonspecific immune response, which involving macrophages, dendrite cells, NK cells, and lymphocyte T γδ, develops. *Mycobacterium* antigen will be presented via MHC-II to the cell surface and cytokine release follows. Subsequent activities depend on cytokine released, event of macrophage activation, inflammation process, or lymphocyte activation. (Rumende, 2000; vanCrevel R, 2002; Hingley-Wilson *et al*, 2003; Kayser *et al*, 2005; Abbas *et al*, 2007).

Mycobacterium tuberculosis, which lives intracellular inside the macrophages, will induced cellular immune response where T lymphocyte plays main role. T lymphocyte and its subset can be differerentiated based on its protein marker present on molecule surface, called cluster of differentiation (CD). CD3 found in every T lymphocyte and CD4 found in Thelper lymphocytes (Abbas *et al*, 2007; Takeshita, 2007; Paraskevas, 2012). T lymphocytes, with the presence of IL-12, will differentiate to Th1, which plays important roles in immune response for *M. tuberculosis* infection. T lymphocytes heterodimer receptors, *T-cell receptor* (TCR)-α/β and TCR-γ/δ, owns functions in introducing foreign peptide or antigen, which expressed jointly