WHOLE BLOOD LYMPHOCYTE PROLIFERATION ASSAY IN HEALTHY THAIS: COMPARISON OF HEPARINIZED BLOOD AND ACID CITRATE DEXTROSE BLOOD

Chuenchitra T\textsuperscript{1}, Sukwit S\textsuperscript{1}, Chaitaveep P\textsuperscript{1}, Kuwanont S\textsuperscript{2}, Amlee P\textsuperscript{1}, Songprasom K\textsuperscript{1}, de Souza M\textsuperscript{1,3} and Nitayaphan S\textsuperscript{1}

\textsuperscript{1} Armed Forces Research Institute of Medical Sciences, Bangkok 10400, Thailand, \textsuperscript{2} Army institute of Pathology, Bangkok 10400, Thailand, \textsuperscript{3} Henry M. Jackson Foundation, Rockville, Maryland 20850, USA

Abstract

Background: Lymphocyte proliferation assay (LPA) is technique to determine T-lymphocyte functions in vitro. The standard LPA using peripheral blood mononuclear cells (PBMC) separated from heparinized blood requires a large blood sample, time consuming and expensive. Moreover, it is more useful if acid citrate dextrose (ACD) blood could be used not only in LPA but also multiassays.

Objectives: To determine whether whole blood could be substituted for standard LPA and to compare using heparinized blood and ACD blood.

Methods: Heparinized and ACD blood from 35 healthy Thais were collected. PBMC separated by density gradient centrifugation and 1:10 diluted heparinized and ACD blood were used to test for lymphoproliferative responses to phytohemagglutinin (PHA), pokeweed mitogen (PWM), and tetanus toxoid (TET). A stimulation index (SI) for each mitogen or antigen was calculated by dividing the count per minutes (cpm) in stimulated cultures by the cpm in control cultures. An SI > 3 was considered a positive response.

Results: All healthy Thais demonstrated positive proliferative responses to PHA and PWM by using PBMC and whole blood culture assays from both heparinized and ACD blood. However, the difference in the frequency of positive proliferative responses to TET by using PBMC and whole blood culture assays was significant [88.6\% (31/35) vs 45.7\% (16/35), p=0.0001 for heparinized blood and 82.9\% (29/35) vs 40.0\% (14/35), p=0.0001 for ACD blood, respectively]. Nevertheless, no significant difference in frequency of positive responses to TET between heparinized and ACD blood was observed.

Conclusions: These results suggested that no significant difference between using heparinized and ACD blood in standard LPA using PBMC. However, the whole blood LPA for measuring mitogen (PHA and PWM) induced lymphoproliferation could be substituted for standard LPA from heparinized and ACD blood. Thus, it would be applicable in a field laboratory and in research setting.