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Identification of Thymocyte Subset by Multicolor Flow Cytometry ED LSR II FACSDriva – FlowJo Software Analysis

Harapan[1*], Wienands[2], Ichsan[3], Ana Indrayati[4]

1.School of Medicine - Syiah Kuala University, Banda Aceh, Indonesia.
2.Institute of Cellular and Immunology - Universitatsmedizin Gottingen Georg-August-University, Gottingen, Germany.
3.Institute of Microbiology - Universitatsmedizin Gottingen Georg-August-University, Gottingen, Germany.
4.School of Pharmacology - Bandung Technology Institute, Bandung, Indonesia.

Abstract

In their development, thymocytes express different cell surface molecules that important for identification of thymocyte subset. It's not easy to detect this cell surface molecules to determine the thymocyte subpopulation for research. Here we used multicolor flow cytometry ED LSR II FACSDriva - FlowJo software to identify of thymocyte subset from thymocyte sample solution using several antibodies such as mouse anti rat CD2-FITC, mouse anti rat CD45RC-PE, mouse anti rat CD4-APC, mouse anti rat CD8á-PerCP, mouse anti rat CD3-Biotin + PE-Cy7 or APC-Cy7. We determined double negative and single positive thymocyt subset (CD4 or CD8), found that the double negative thymocyte subset express CD2 and CD45RC. It was useful to determine the thymocyte subset using multicolor flow sitometry ED LSR II FACSDriva - FlowJo software

Keywords: thymocyte subset, multicolor flowcitometry, ED LSR II FACSDriva, FlowJo Software.

Introduction

The acquired immune response relies on the ability of thymus-derived (T) lymphocytes to recognize and discriminate among a wide range of different foreign antigens. As is the case with B lymphocytes, the enormous diversity of the T-cell repertoire stems from the ability of developing T cells to rearrange and modify the genes that encode their antigen receptors. An appreciation of the structure and function of the T-cell antigen receptor (TCR) is essential for understanding T-cell development and the complexities of the responses of mature T cells to antigen.

***Correspondent Author :**
Harapan , School of Medicine, Syiah Kuala University, Banda Aceh, NAD 23111 Phone : +6285260850805, Email: harapan-tumangger@yahoo.com.

Early in development, thymocytes express several cell surface molecules, such as CD2, that are characteristic of the T-cell lineage, but they lack many others, including CD4 and CD8, and thus are known as double-negative thymocytes (Crompton *et al*, 1994). Rearrangement of the TCR genes begins in the double-negative stage. Cells that are destined to become αα T cells rearrange the α gene first. If rearrangement at the TCR α locus is successful, the resulting β-chain polypeptide pairs with an invariant polypeptide called pTα. The pTα-TCRα dimer then associates with CD3 chains and is expressed on the cell surface as a pre-TCR (John and William, 2001). Expression of the pre-TCR serves to terminate further rearrangements at the TCR â gene locus and is required for efficient transition to the next major stage in development, when thy-

mocytes express both CD4 and CD8 and are called double-positive cells. Rearrangement of the TCR á gene occurs during the double-positive stage and, if successful, leads to low-level expression of an áâ TCR (John and William, 2001). As thymocytes mature into T cells, the level of TCR expression increases and the cells lose expression of either CD4 or CD8, becoming single-positive. At this stage, thymocytes have acquired the phenotype of mature peripheral T cells and soon exit the thymus.

The aim of this research is to determine double-negative thymocytes, double-positive, single-positive T cell and the other T cell subset by using multicolor flow cytometry ED LSR II – FACSDriva with FlowJo software.

Materials and Methods

We used several antibodies : mouse anti rat CD2-FITC, mouse anti rat CD45RC-PE, mouse anti rat CD4-APC, mouse anti rat CD8á-PerCP, mouse anti rat CD3-Biotin + PE-Cy7 and APC-Cy7.

Rat thymocytes (100µl of suspension) diluted with 100µl PBS and incubated with 20µl antibody for 15 min on ice (one tube with each antibody). Materials that were prepared were :

a.The mix contain all antibody mentioned above.

b.Single antibodies are needed for compensation control CD2, CD45RC and CD3 controls are prepared with lymph node cell.

c.One tube sample is left untreated as negative control.

After centrifuged in 1.200 rpm, 4 min, 23°C, pellet was washed with 4ml PBS, repeated 2 times. Sample was resuspended in 300µ PBS and stored on ice. The mix anti CD3 samples alternatively incubated with Streptavidine-PE-Cy7 or Streptavidine APC-Cy7 for 5 min on ice. Six color analysis at the flow cytometry ED LSR II were done and analyzed using Flow-Jo Software.

Results and Discussion

After flow cytometry ED LSR II standardized with control, maximum voltage adjusted. The maximum voltages that were used were: FSC 370, SSC 359, FITC 520, PE 520, PerCP 700, PE-Cy7 703, APC-Cy7 703 and APC 698. After sample was measured using flow cytometry ED LSR II, result is shown in Figure 1.

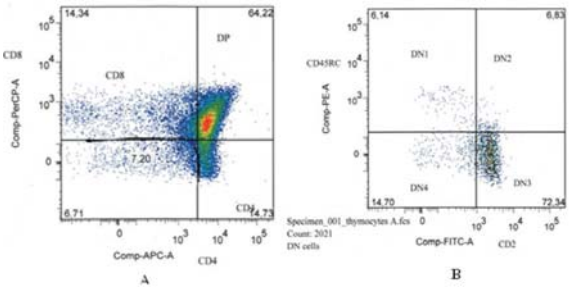

Figure 1. The result of sample with flow cytometry ED LSR II. A). The result of flow cytometry ED LSR II to determine the thymocytes cell which expressed CD4 (APC) and CD8 (PercCP). B). The result of flow cytometry ED LSR II to determine the thymocytes cell which expressed CD2 (FITC) and CD45RC (PE) from double negative sample. Double positive (DP), double negative (DN).

Figure 1A showed the different subpopulation of thymocytes; double-positive subset thymocyte which expressed CD4 and CD8, single positive subset thymocyte wich expressed only CD4 or CD8, and double negative subset thymocyte. In double negative subset thymocyte, the cell did not expressed CD4 or CD8 but expressed CD2 or/and CD45RC or none (Figure 1 part B).

We also used flow cytometry ED LSR II to determine the number of thymocytes subset base on their peak intensity (Figure 2), but real number for each thymocytes subset is not shown.

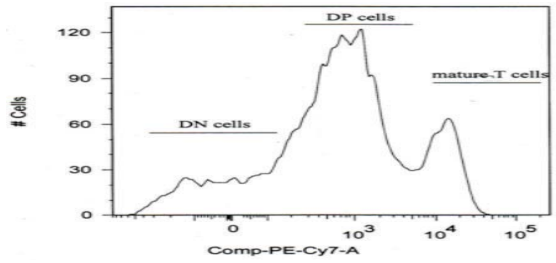

Figure 2 The number and differences peak each thymocyte subset which determined using flow cytometry ED LSR II. Double positive (DP), double negative (DN).

T cells develop from bone-marrow-derived progenitor cells that undergo maturation in the thymus (Figure 3).

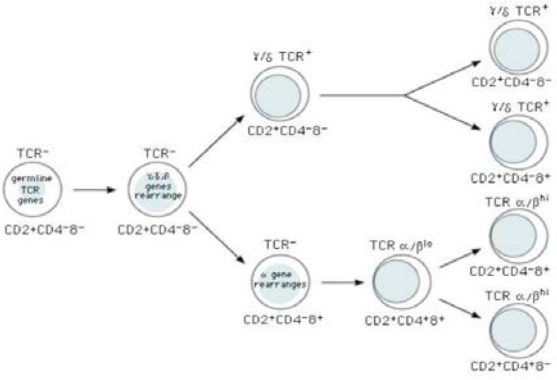

Figure 3. Stages in thymocyte development.

Progenitor cells migrate from the bone marrow to the thymus. At the earliest stages of development, thymocytes express several T-cell surface molecules, such as CD2, but still have germline configurations of their TCR genes. Thymocytes destined to become áâ T cells pass through a critical $CD4^+CD8^+$ phase during which positive and negative selection occur.

As the conlusion of this research, it was useful for using multicolor flow cytometry ED LSR II to determine differences and the number of thymocyte subset from thymus sample. In their development, thymocyt cell expressed CD2 or/and CD45RC or none (all this subset we called double negative), after that they expressed CD4 and CD8, this subset we called double-positive. As thymocytes were mature into T cells, the level of TCR expression increased and the cells lose expression of either CD4 or CD8, became single-positive.

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