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Research Report

CHARACTERIZATION of CD4+ T LYMPHOCYTE FROM BONE MAROW STEM CELL USING INDIRECT IMMUNOFLUORESENCE FOR HIV & AIDS TREATMENT

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ABSTRACT

Acquired immune deficiency syndrome (AIDS) is caused by Human Immunodeficiency Virus (HIV). At the beginning of infection, gp120 virus interacts with CD4 receptor at the surface of the target cell. The interaction between gp120 and CD4 leads to the occurrence of the binding of specific chemokine receptor CXCR4 and CCR5, which are also present on the membrane of the target cell. Therefore, CCR5 and CXCR4 also determine the fate of the target cell. It is the performance of CCR5 and CXCR4, guided by controlling gene that determines susceptibility or resistance to HIV infection. Coding gene CCR5 may mutate to become protective or resistant against HIV infection. In homozygote individuals, it tends to be resistant against infection, while in heterozygote individuals it tends to be susceptible to HIV infection. Objective: To characterize TCD4 lymphocyte in the next that is resistant against HIV infection by using gene therapy deletion 32 CCR5 to use for HIV & AIDS treatment. Method: Sample collection, mononucleated cell collection, lymphocyte culture, CD4 identification, CCR5 variance analysis, co-cultivation with PBMC HIV and comparison to control. Result: This study was performed in several steps, such as mononucleated cell isolation, followed with cell culture, lymphocyte purification, lymphocyte and CD4 expression identification. Conclusion: Lymphocyte T CD4 had been mature after seven passages, once passage is about 5 days so for maturity lymphocyte T CD4 need 35 days and that cell as be candidate to resistant against HIV infection by using gene therapy deletion 32 CCR5 to use for HIV & AIDS treatment.

Key words: *characterization lymphocyte T CD4, HIV & AIDS*

INTRODUCTION

Acquired immune deficiency syndrome (AIDS), caused by Human Immunodeficiency Virus (HIV), is a life-threatening infectious disease. Human Immunodeficiency Virus (HIV) is an RNA virus from the family Retrovirus and subfamily Lentiviridae. There are two HIV serotypes, the HIV-1 and HIV-2. Morphologically, HIV-1 has a round shape and consists of the core and the envelope.[8]

At the beginning of infection, gp120 virus interacts with CD4 receptor at the surface of the target cell. The interaction between gp120 and CD4 leads to the occurrence of the binding of specific chemokine receptor CXCR4 and CCR5, which are also present on the membrane of the target cell. Therefore, CCR5 and CXCR4 also determine the fate of the target cell. It is the performance of CCR5 and CXCR4, guided by controlling gene that determines susceptibility or resistance to HIV infection. Coding gene CCR5 may mutate to become protective or resistant against HIV infection. In homozygote individuals, it tends to be resistant against infection, while in heterozygote individuals it tends to be susceptible to HIV infection.[1,9]

The objective of this study was to characterize TCD4 lymphocyte in the next that is resistant against HIV infection by using gene therapy deletion 32 CCR5 to use for HIV & AIDS treatment.

MATERIALS AND METHODS

This was an experimental study on in vitro allogenic stem cell transplantation using TCD4 bone marrow lymphocyte from donor for deletion in CCR5, which could be applied to HIV & AIDS patients. Samples

were taken from bone marrow of healthy donors. The inclusion criteria were aged 18–30 years, healthy, having no systemic or degenerative disease, no infectious disease, and signing the informed consent. The exclusion criteria were the presence of other infectious diseases, the presence of severe underlying diseases (DM, CKD, heart failure, hepatic failure, hepatic cirrhosis, etc), aged more than 30 years, and illiterate. The sites of the study were at Dr Soetomo Hospital, Surabaya, and Institute of Tropical Disease, Airlangga University, Surabaya.

Sample collection

Sample was taken from donors who had kinship relations (sibling). After 5 cc of blood was taken from the donor's BMA, we performed mononucleated cell isolation using Ficol Histopaque gradient 1.077, followed with culture.[15]

Mononucleated isolation

BMA was centrifugated at 1600 rpm for 15 minutes, and gently entered into 15 cc tube containing Ficol from the side of the tube. After being centrifugated, the buffy coat was isolated by putting in the pipette, sucked, contained within the eppendorf tube, washed with PBS, cultured, and kept within 5% CO2 incubator using alpha MEM medium containing 25% FSB serum.[15]

Lymphocyte culture

After the third day, it was cultured and hematopoietic stem-cell supernatant was separated and centrifugated at 1600 rpm for 5 minutes in 10 degree C. After the supernatant was removed, the pellet was resuspended with growing medium containing 20% FBS and penstrep antibiotics. After resuspension, it was entered into Petri dish and kept within 5% CO2 incubator.[15]

CD4 identification

An indicator was required to identify the maturation of the cell. In this case, the indicator was CD4. The identification was performed as follows: The lymphocyte culture was harvested and put into 15 cc tube, and fixed by means of methanol. After 15 minutes, anti T-CD4 cell reagent was added, labeled with Fit C. Subsequently, it was washed with PBS, and dripped onto object glass and analyzed under fluorescence microscope.[15]

RESULT

In the first year of this study, several stages have been performed, such as mononucleated cell isolation, followed with cell culture, lymphocyte purification, lymphocyte and CD4 expression identification

Isolation and culture of mononucleated cell

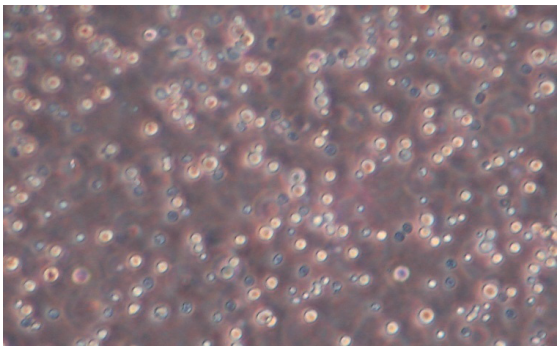

Figure 1. Cell was isolated using Ficoll histopaque 1.077 and cultured, observed under inverted microscope in 10× magnification.

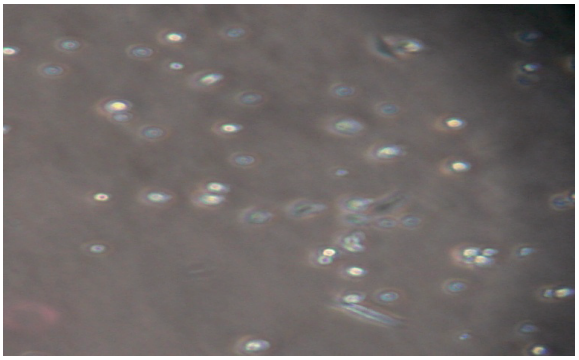

Figure 2. The cell, 24 hours after washing and observed under inverted microscope in 10 x magnification. Mononucleated cells attached in Petri dish

LYMPHOCYTE PURIFICATION

Three days after culture, supernatant that containing hematopoietic stem cell was separated and centrifugated in 1600 rpm for 5 minutes in 10 degree C. After the supernatant was removed, the pellet was resuspended with growing medium containing 20% FBS serum and penstrep antibiotics. After resuspension, it was put onto petri dish and kept within 5% CO_2 incubator.

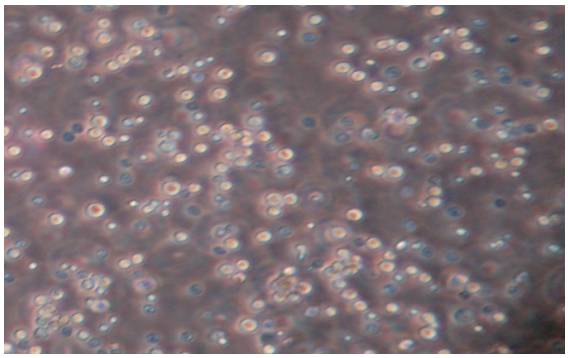

Figure 3. Mononucleated cell culture observed under inverted microscope in 10 x magnification.

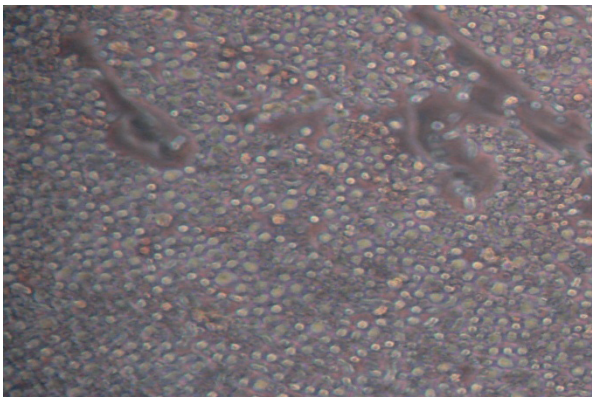

Figure 4. Day 5, after hematopoietic cells were cultured, mesenchymal cells also grew between hematopoietic cells, observed under inverted microscope in 10 x magnification. Then, the hematopoietic cells were separated from mesenchymal cells.

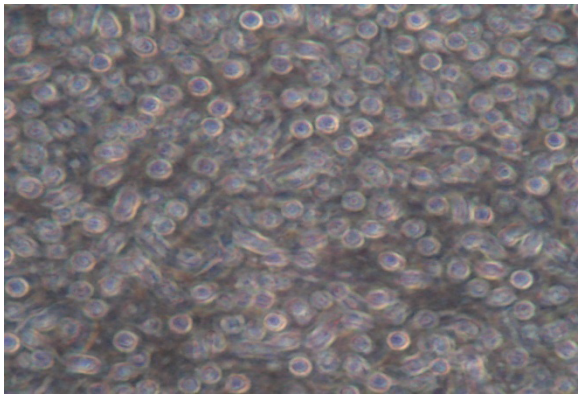

Figure 5. Lymphocytes are cultured until ready to be co-cultivated, observed under inverted microscope in 10 x magnification

Lymphocyte maturation identification with CD4

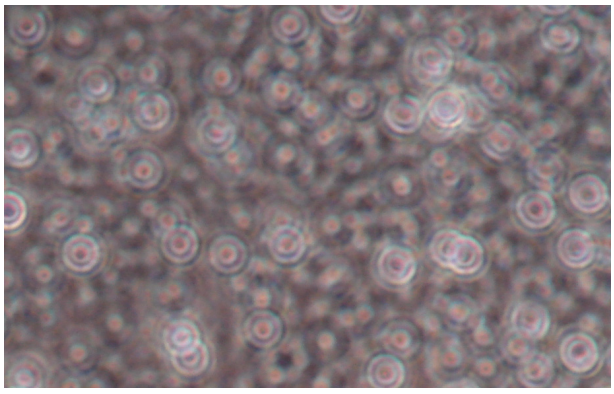

Figure 6. Mature lymphocytes are identified with CD4, observed under inverted microscope in 10 x magnification

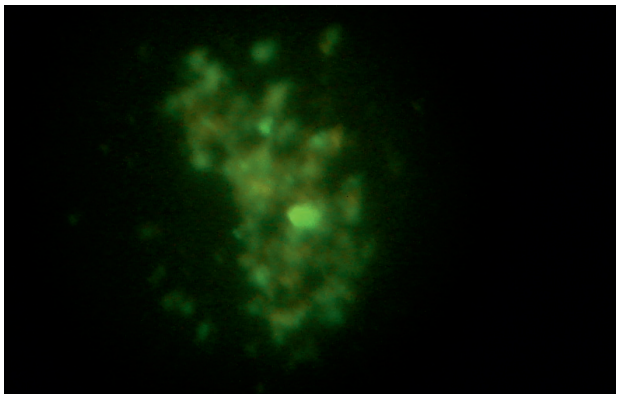

Figure 7. After being harvested, the lymphocytes were put into 15 cc tube and fixed with methanol. After 15 minutes, it is added with anti T-CD4 cell reagent labeled with Fit C. It was washed with PBS, and dropped onto glass object and analyzed under fluorescence microscope.

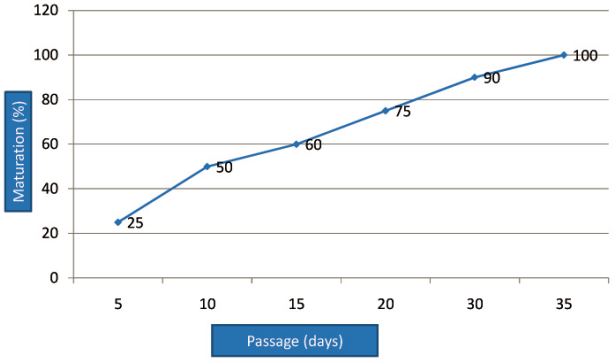

Figure 8. Lymphocyte maturation 100% achieved on 35 days (seven passages)

DISCUSSION

After performing studies in several stages, including isolation, identification, characterization, significant results were found, such as:

In this stage, after isolation we obtained cells as many as 2 × 10(7) per 3 ml BMA. However, in this stage, after isolation and cultivation, many cells could not grow. This was likely because the process between sample collecting and isolation needed faster time and more appropriate temperature as required by the cells, and medium pH should have been more suitable to the characteristics of the cell. This was proved during the culture with Petri dish, in which many cells could not develop due to less optimum adherence between cells and petri dish's matrix. Another point that should have been noticed in isolating process was sample collection. Sample collecting should use proper medium transport, so that cell death can be suppressed. This can also be seen in the result of mononucleated cell

isolation with cell grown on petri dish whose growth could reach more than 80%.[15,16]

Buffy coat containing mononucleated cell was separated or washed with PBS, deeper attention should be given to the pH of the PBS or washing medium, because if it is too acid or too alkali, it would cause membrane damage, resulting in difficult adherence of the cells to petri dish. This less capability of adherence results in cell death. Another point that should be noted is that when centrifugation is undertaken above 3600 rpm, adherence between cell often occurs, so that when resuspension is undertaken, cell membrane would be damaged, preventing the cell to adapt and adhere to the base of the petri dish.[2,15]

In hematopoietic cell culture, the isolation should be carried out carefully to prevent mesenchymal cell contamination in order to obtain optimum cells development, because those cells would become innate cells (innate immunity, such as macrophage, monocyte, PMN, eosinophil, and basophil). Therefore, this cell has better differentiation power. In this study, we did not carry out characterization in products that have important role, such as TGF, TNF, IL, IFN, and etc.[14] that serve as signaling transducer as well as immune cell triggering that enhances cell differentiation. This would be undertaken in the next step. Nevertheless, after culture was made on the result of the separation between mesenchymal and hematopoietic cells, contaminants are still found. Therefore, in this stage further purification was required, and the result can be seen in Figure 7.

In this stage, after lymphocyte purification was done and developed with growth medium, significant development was found as proved from CD4 receptor examination, which was found in almost all lymphocytes. This indicates that the cell underwent almost perfect differentiation, characterized by high density of CD4 expression. The result can be seen in Figure 10, which is using immunofluorescence approach. From the result it could be indicated that there was significant T-lymphocyte differentiation, even though in this study the cell had been differentiated and could be used as a basis for immunodeficiency therapy, such as in HIV & AIDS.[10] Another point to note is the CTL product has an important role in the process of apoptosis. This indication can be used as the marker of activity of lymphocyte against the exposure to foreign gene.

CONCLUSION

Lymphocyte T CD4 had been mature after seven passages, once passage is about 5 days so for maturity lymphocyte T CD4 need 35 days and that cell as be candidate to resistant against HIV infection by using gene therapy deletion 32 CCR5 to use for HIV & AIDS treatment.

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