The Effect of Macrophage Addition to Interleukin 10 (IL-10) in Tuberculosis Granuloma Model In Vitro

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Keywords: Mycobacterium tuberculosis, Granuloma, IL-10

Abstract: Tuberculosis (TB) caused by the bacterium Mycobacterium tuberculosis has caused more deaths than other infectious diseases over the past 200 years. Granulomas are characteristic of mycobacterial infection. Doubling of Mycobacterium tuberculosis bacilli in any place will cause specific inflammation to form characteristic granulomas. Granulomas contains of different types of cells, but the main cellular component of the structure is macrophages. Macrophages are cells for the formation of granulomas and major cell types in most granulomas. One function of macrophages in granulomas is the production of anti-inflammatory cytokines IL-10. IL-10 can reduce inflammation and maintain homeostasis. This study aims to determine the effect of the addition of macrophages to levels of interleukin-10 (IL-10) in granuloma model in vitro. This type of research is true experimental with the object of research in the form of PBMC which comes from 1 healthy volunteer. Analysis using twoway ANNOVA with P <0.05, and tukey comparing for comparison. The results showed that without macrophage group with $1x10^5$, $2x10^5$, and $3x10^5$ macrophage groups, the value of p = 0.3197 was greater than 0.05 (p> 0.05), so there was no significant difference. While the variation day in the treatment group obtained p = 0.2407 which showed no effect of macrophage addition to interleukin-10 (IL-10) level on granuloma model in vitro, with IL-10 concentration on the 1st day until the 5th day are low. There is no effect of adding macrophages to IL-10 levels in tuberculosis granuloma models in vitro.

1 INTRODUCTION

Tuberculosis (TB) has caused more deaths compared to other infectious diseases over the past 200 years (Heemskerk et al., 2015). This disease is caused by Mycobacterium tuberculosis mostly attacks people living in low-income and middle-income countries where it reaches 9.4 million people a year worldwide. Most individuals who are exposed do not develop the disease because it can produce an immune response to resist or eliminate bacteria (Fox and Menzies, 2013).

Tuberculosis granuloma is a hallmark of mycobacterial infection (Heemskerk et al., 2015). Characteristic granuloma formation originates from the presence of specific inflammation caused by multiplication of Mycobacterium tuberculosis bacilli in any place (Elorriaga et al., 2015). Granulomas have a dynamic process in which the structure of the size will increase as more cells move in (Orme and Basaraba, 2014).

Mycobacterium tuberculosis infection causes granuloma formation where bacteria enter in an inactive state and can live for decades before reactivation to develop active disease when the host's immune system is weakened (Kapoor et al., 2013). Bacteria will become latent when trapped in granulomas and are under hypoxic conditions, lack of nutrition, and pressure from adaptive immunity (Orme and Basaraba, 2014). A person infected with latent TB can store a small amount of inactive Mycobacterium tuberculosis bacilli contained in microgranuloma. This organism can continue to live but is not active (Kapoor et al., 2013).

IL-10 is called an anti-inflammatory cytokine, an inhibiting cytokine for a balance between inflammatory and immunopathological responses (Cyktor et al., 2013). IL-10 plays an important role

Puspodewi, D., W, A. and Nugraha, J.
The Effect of Macrophage Addition to Interleukin 10 (IL-10) in Tuberculosis Granuloma Model In Vitro.
DOI: 10.5220/0007543503860391
In *Proceedings of the 2nd International Conference Postgraduate School (ICPS 2018)*, pages 386-391
ISBN: 978-989-758-348-3

in Mtb infection, where cytokines have been shown to reduce immunity (Cavalcanti et al., 2012; Cyktor et al., 2013; Adrian et al., 2015).Immunosuppressive cytokines IL-10 have been associated with susceptibility to TB in both humans and animals (Cyktor et al., 2013). IL-10 plays an early inhibitory role in preventing the development of protective immunity associated with the containment of Mtb infection (Cyktor et al., 2013).

This study aims to detect whether there is an effect of the addition of macrophages on IL-10 levels in tuberculosis granuloma models in vitro.

2 MATERIALS AND METHODES

2.1 RPMI

The growth media used in this study was the Roswell Park Memorial Institute (RPMI) 1640 which was obtained already in the form of ready-to-use solution. RPMI 1640 media is a medium used for cell and tissue culture, usually used for the growth of human lymphoid cells. This medium contains a large amount of phosphate and is formulated for use in air with a 5% CO_2 atmosphere. RPMI 1640 uses the bicarbonate buffer system so that it enables the growth of several types of cells, especially T lymphocytes, hybridomas. There are several series of RPMI most often used is RPMI 1640.

2.2 PBMCs

Peripheral Blood Mononuclear Cells = PBMCs are cells made from human blood which are then processed for the PBMC cell capture. Sample criteria are adult blood, the blood used should be new blood taken, it can not be blood that has been stored for too long, blood comes from healthy people and does not suffer from tuberculosis infection, there is no specific provision for sex either male or female. Suggestions from the blood researcher used should come from one person only, because the immune response of each individual is different so if coming from more than one person in worry affects the outcome. The number of PBMC used in this study was 10^5 in each well.

2.3 Mycobacterium tuberculosis

This study used bacterial isolates Mycobacterium tuberculosis H37Rv obtained from the Laboratory of Microbiology, Institute of Tropical Disease, Airlangga University Surabaya with concentration 10^5 in each well.

2.4 Macrophage

Taken buffy-coat about 60 ml. Then prepare 50 ml conical tubes with histopags each 15 ml. Prepared one tube every 10 ml of buffy-coat. Histopags are used at room temperature. Plate at 10 cm culture dishes (10 ml / dish) incubated at 1-2 hours at 37ºC 5% CO_2. Observe macrophage cells under a microscope and then make doses 1,2 and $3x10^5$.

2.5 Procedure

There are four groups on this procedure. Group I was given PBMC and Mycobacterium tuberculosis bacteria on RPMI media as control. Group II was given PBMC, Mycobacterium tuberculosis and $1x10^5$ macrophages on RPMI media. Group III was given PBMC, Mycobacterium tuberculosis and $2x10^5$ macrophages on RPMI media. Group IV was given PBMC, Mycobacterium tuberculosis and $3x10^5$ macrophages on RPMI media. Prepared well plates that already contain RPMI media. Enter $1x10^5$ PBMC cells into all wells. Inoculated with $1x10^5$ M. tuberculosis strain H37Rv bacterial isolates into all wells. Added macrophages as much as $1x10^5$, $2x10^5$ and $3x10^5$ cells into each well of group II, III, and IV. Plate was incubated at 37°C with 5% CO_2 condition. Observed on days 1,2, 3, 4 and 5. Every day 100 uL of supernatant was taken to test IL-10 levels using the ELISA test at 450 nm wavelength.

3 RESULTS

3.1 Direct Granuloma Observation

Results from observations under an inverted microscope with 400x magnification of PBMC samples from 1 healthy volunteer were added with $1x10^5$, $2x10^5$, $3x10^5$ and Mtb macrophages showing the difference from day one to fifth day. On the first day there has not been a good aggregate on culture without macrophages with a culture plus macrophages (Figure 1). The second day has already begun to form aggregate on both treatments i.e., without the addition of macrophages and the added macrophages (Figure 2). On the third day (Figure 3) and the fourth day (Figure 4) is still aggregate, while on the fifth day the aggregate has begun to decrease (Figure 5).

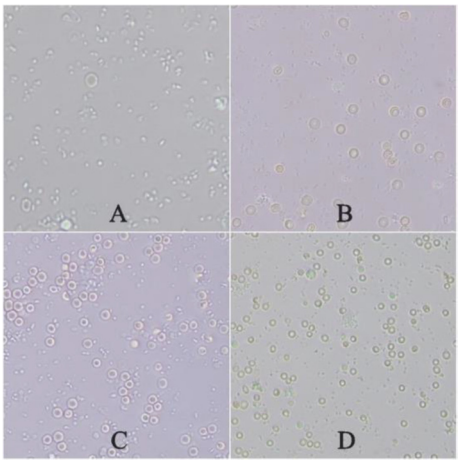

Figure 1. First Day Culture. A. Without Macrophages, B. Addition of macrophages $1x10^5$,

C. Addition of macrophages $2x10^5$, D. Addition of macrophages $3x10^5$.

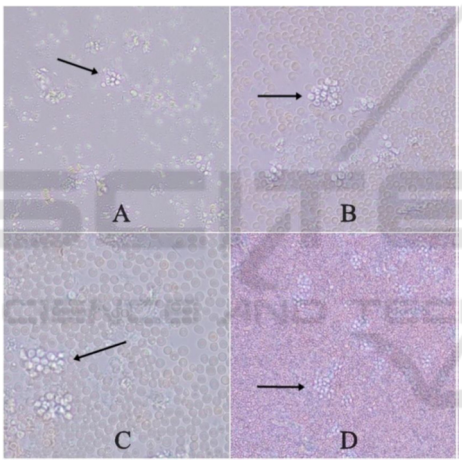

Figure 2. Culture Second day. A. Without Macrophages, B. Addition of macrophages $1x10^5$,

C. Addition of macrophages $2x10^5$, D. Addition of macrophages $3x10^5$.

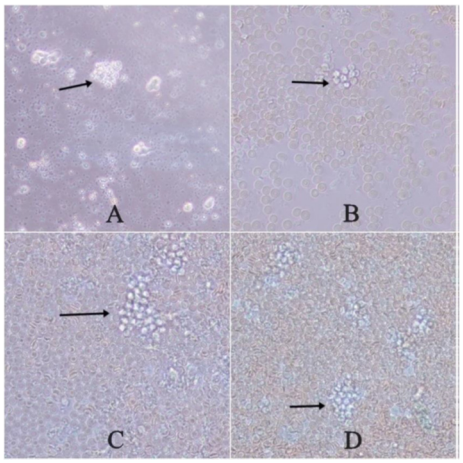

Figure 3. Third Day Culture. A. Without Macrophages, B. Addition of macrophages $1x10^5$,

C. Addition of macrophages $2x10^5$, D. Addition of macrophages $3x10^5$.

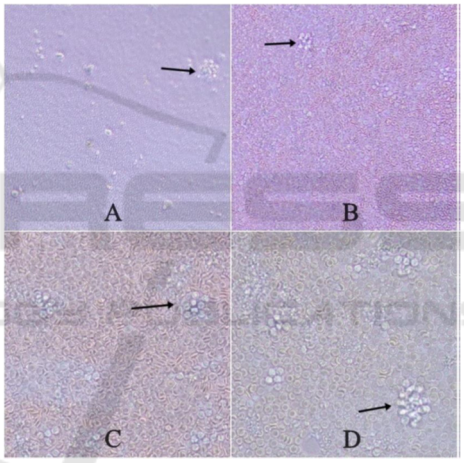

Figure 4. Fourth Day Culture. A. Without Macrophages, B. Addition of macrophages $1x10^5$,

C. Addition of macrophages $2x10^5$, D. Addition of macrophages $3x10^5$.

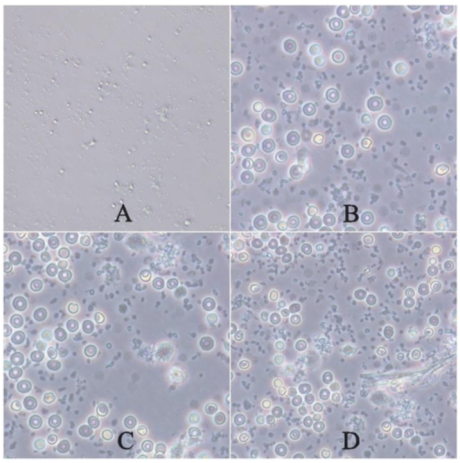

Figure 5. 5th Day Culture. A. Without Macrophages, B. Addition of macrophages 1x10^5,

C. Addition of macrophages 2x10^5, D. Addition of macrophages 3x10^5.

3.2 Examination the levels of IL-10

Result of examination of level of IL-10 with elisa method from 4 group that is without the addition of macrophages, with the addition of 1x10^5, 2x10^5, 3x10^5 macrophages were analyzed by day variation.

From the research, it was found that the levels of IL-10 without addition the highest value of macrophages occurred on the 2nd day with an average of 3,733 and the lowest average value occurred on the 3rd day of 1,595 shown on figure 6.

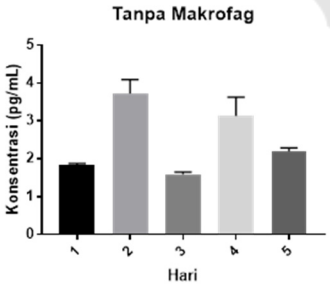

Figure 6. Levels of IL-10 without the addition of macrophages

The level of IL-10 with the addition of 1x10^5 macrophage the lowest value occurred on the 1st day with an average of 1.435 and the highest average value occurred on the 3rd day ie 3,506 shown in Figure 7.

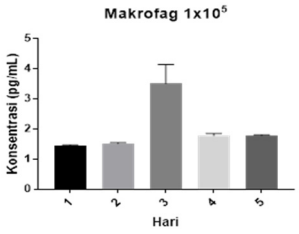

Figure 7. Levels of IL-10 with the addition of 1x10^5 macrophages

The level of IL-10 with the addition of 2x10^5 macrophage the highest value occurred on the 2nd day with an average of 3.135 and the lowest average value occurred on the 5th day that is 0.804 shown in figure 8.

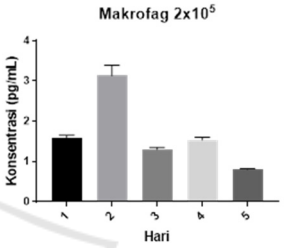

Figure 8. Levels of IL-10 with the addition of 2x10^5 macrophages

It was found that the level of IL-10 with the addition of 3x105 macrophage of the lowest value occurred on the 5th day with an average of 1,191 and the highest average value occurred on the second day that is 3,521 shown in Figure 9.

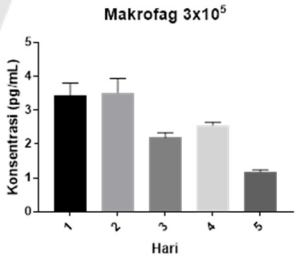

Figure 9. Levels of IL-10 with the addition of 3x10^5 macrophages

The results of IL-10 examination with elisa method of 4 groups, i.e., without the addition of macrophages with the addition of macrophages 1x10^5, 2x10^5, 3x10^5 showed low levels of IL-10.

All groups experienced increased concentrations on the second day. On the third day, the fourth and fifth groups of 1x105 macrophages were different from the other groups, i.e., the third and fifth day

increases and the decrease on the fourth day, while the group without macrophages, the 2x105 and 3x105 macrophages decreased the concentration on the third and fifth days and increased the fourth day (Figure 10).

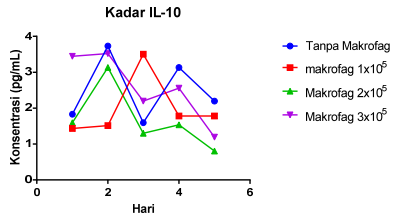

Figure 10. IL-10 levels on day variations

The data obtained are then analyzed by two-way ANOVA which aims to determine the significance of the price of the proportion (p). In groups without macrophages with a group of 1x105, 2x105, and 3x105 macrophages, the value p = 0.3197 was greater than 0.05 (p> 0.05). Likewise with day variations of groups without macrophages with groups of macrophages added 1x105, 2x105, and 3x105 obtained value p = 0.2407 value is greater than 0.05 (p> 0.05) so it shows no difference.

3.3 Discussion

Tuberculosis granuloma is a hallmark of mycobacterial infection (Heemskerk et al., 2015). Characteristic granuloma formation originates from the presence of inflammation Specifically caused by doubling the Mycobacterium tuberculosis bacilli in any place (Elorriaga et al., 2015). Granuloma is a pathological sign host response to Mycobacterium tuberculosis infection. Development Granuloma is a defense tool designed as a wall and contains pathogens (Orme and Basaraba, 2014). Granuloma formation facilitates host in accommodating Mycobacterium tuberculosis bacilli and prevent spread of bacteria, but can also be used by bacteria for breed (Heemskerk et al., 2015). Granulomas have a dynamic process because the more cells move in, the size structure will increase (Orme and Basaraba, 2014).

M. tuberculosis IL-10 is an immunoregulatory cytokine with activity immunosuppressive is potent against APC and Th1 cells. IL-10 production level by Macrophages and dendritic cells are infected, although promoted by Th1 ability, IL-10 is secreted more by dendritic cells than macrophages. IFN-γ decreases IL-10 production from macrophages infected with M. tuberculosis. In this microenvironment, IFN-γ secreted by activated T cells can synergize with M. tuberculosis to reduce IL-10 production (Hickman et al, 2002).

IL-10 is not always produced by T cells that make other pro-inflammatory cytokines, although checking granulomas in a small subset, there is a small population of T cells (1.2%) that make IL-10 and IL-17. T cells with this phenotype have been associated with control of several bacterial infections rather than with autoimmune diseases (Gideon et al., 2015).

4 CONCLUSIONS

There w no effect of adding macrophages to IL-10 levels in tuberculosis granuloma models in vitro.

ACKNOWLEDGEMENTS

The authors would like to thank the technicians of the Stem cell Research Centre and Tuberculosis and Leprosi Laboratory of Tropical Diseases (ITD) of Airlangga University and all those who have assisted in the completion of this research.

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