Effect of Macrophage Addition and Incubation Time to Interferon Gamma (IFN-γ) Levels on Tuberculosis Granulomas In Vitro Models

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Abstract: Tuberculosis (TB) is an infectious disease caused by stem bacteria Mycobacterium tuberculosis (Mtb) which can cause latent infection. Mtb enters through aerosolization which then infects and activates macrophages and dendritic cells present in the lungs. The active dendritic cells of coumadin present an antigen which has been processed in peptide form into the lymphocyte cell which later becomes granuloma. Granulomas are compounds of tissue composed of infected macrophages and multinucleated giant cells, known by aggregates of new monocytes or macrophages, and neutrophils and lymphocytes. Macrophages have a duty to kill Mtb germs. Different types of macrophage phenotype in granulomas with various functions, including anti-mycobacterial effector action, produce cytokines ie IFN-γ. In humans, IFN-γ, released by activated Th1 cells, is the major lymphokine to activate the search process and antimicrobial activity that eliminates mycobacteria. This study aims to determine the relationship between IFN-γ and In-vitro method using PBMC infected with Mtb germ with the addition of Macrophage with concentration 1,2,3x105 with incubation time 1,2,3,4 and 5 days to determine the level of IFN -γ using ELISA test. With use with day variables of macrophages 1x105, 2x105, and 3x105 analized with One-Way ANOVA obtained values p = P = 0.7201 The value is greater than 0.05 (p> 0.05). There is no meaningful difference.

1 INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by stem bacteria Mycobacterium tuberculosis (Mtb). TB is a disease with the highest rates of morbidity and mortality especially in developing countries and is also a problem of chronic infection in the world (Santoso et al., 2017). In addition to causing active disease, Mtb can cause latent infection. Latent infection results in one third of the world's population carrying asymptomatic infections that can produce 8 million new TB cases and 2 million deaths annually (WHO, 2011; Birkness et al., 2007).

Mtb enters through aerosolization which then infects and activates macrophages and dendritic cells present in the lungs. The active dendritic cells of kemuadin present treated antigens in peptide form to CD4 T cells. The activated lymphocytes and infected macrophages, in the inflammatory response of cytokines and chemokines migrate to the infection section and form an arrangement called granuloma, where Mtb is in the dorman phase (Kapoor et al., 2013).

Granulomas are tissue compounds consisting of infected macrophages and multinucleated giant cells, surrounded by aggregations of new monocytes or macrophages, and neutrophils and lymphocytes (Parasa, 2014). Macrophages have an important role of granuloma. Macrophages serve as early cells of granuloma formers. Macrophages have a duty to kill Mtb germs. Different types of macrophage phenotype in granulomas with various functions, including anti-mycobacterial effector mechanisms, produce proinflammatory and anti-inflammatory cytokines, chemokine secretions and proteins associated with tissue remodeling. These cells play a major role in infection control in granulomas (Flynn et al, 2011).

IFN-γ is a major cytokine involved in the immune response to mycobacteria, and its primary function is the activation of macrophages, enabling them to use their microbicide role function (Khan et

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al., 2016). IFN-γ along with IL-12, IL-6, and TNF-α stimulate the production of oxidative explosions, thus mediating the function of tuberculostatic macrophages, as well as stimulating immune cell migration to the site of infection, contributing to granuloma formation, which controls disease progression (Khan et al., 2016).

Based on this background, researchers wanted to know the role of adding macrophages to IFN-y levels in granuloma models using ELISA.

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 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 10^6 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. Comparison of PBMC and bacteria concentrations used in this study was MOI 1: 0.1.

2.4 Macrophage Issolation

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% CO2. Observe macrophage cells under a microscope and then make doses 1,2 and $3x10^5$.

2.5 Procedures

Divided into 4 groups. 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 1x105 M. tuberculosis strain H37Rv bacterial isolates into all wells. Added macrophages as much as $1x10^5$, $2x10^5$ and $3x10^5$ cells into group II, III and IV. Plate was incubated at 37°C with 5% CO2 condition. Observed on days 1,2, 3, 4 and 5. Every day 100uL of supernatant was taken to test IL-10 levels using the ELISA test at 450nm wavelength.

3 RESULTS

3.1 Direct Granuloma Observation

The method used for direct observation is performed directly under an inverted microscope using specimens of living cell cultures in the plate / well with the above lighting system followed by the lens system on the microscope base. The direct observational images present in this journal against the control group (no macrophage), macrophage addition of 1,2 and $3x10^5$ and then incubate for 1 until 5 days in 37ºC.

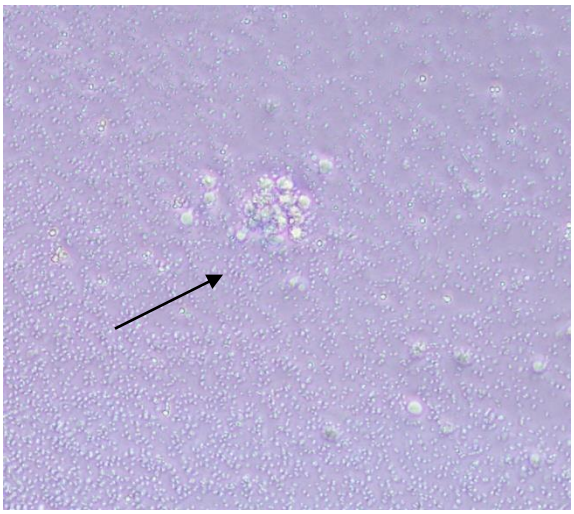

Figure 1: PBMC infected M.Tb without macrophage

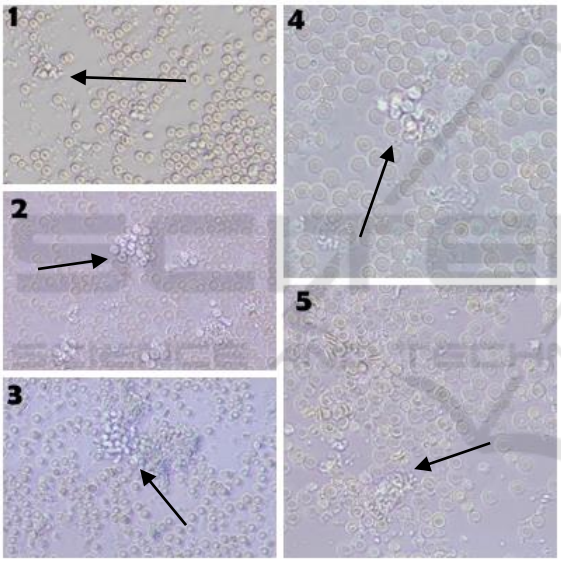

Figure 2: Direct observation of the group with the addition of $2x10^5$ macrophage (400x magnification

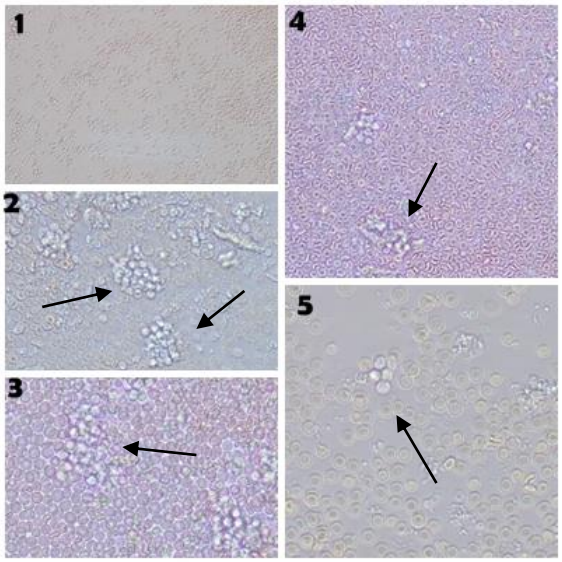

Figure 3: Direct observation of the group with the addition of $2x10^5$ macrophage (400x magnification)

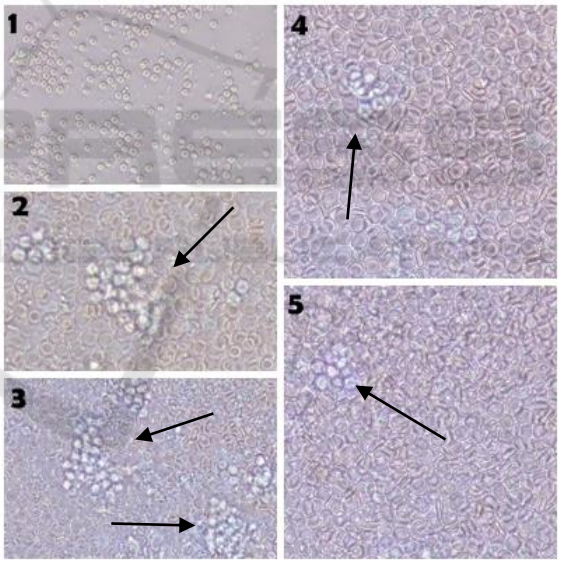

Figure 4: Direct observation of the group with the addition of $3x10^5$ macrophage (400x magnification)

Day three (each dose) is the culmination of granuloma formation. It is clearly visible from the solid structure, with the many number of cells. Cell aggregation appears larger than the first and second days. Immune cell cells on the third day begin to respond to Mtb for further elimination.

3.2 Examination the levels of IFN-γ

Samples in the form of supernatant were then examined by IFN-g using ELISA method and got the average result from each treatment either control or sample.

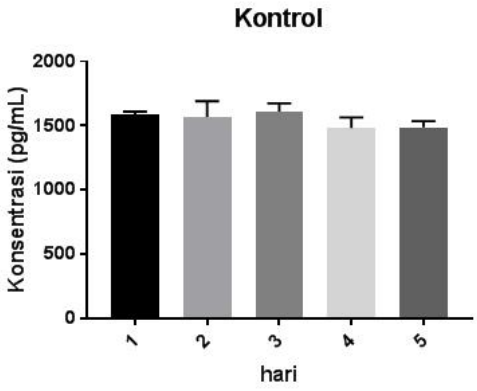

Figure 5: IFN-γ secretion control without the addition of macrophages by day variation (pg / mL)

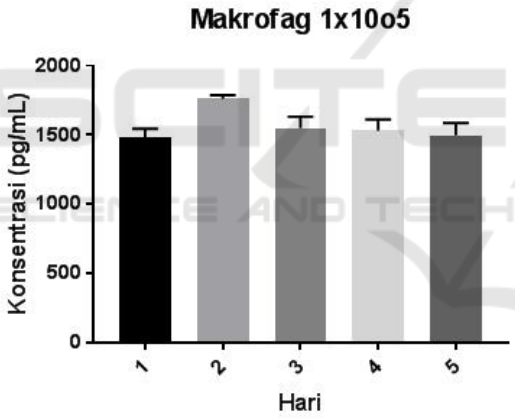

Figure 6: IFN-y secretion with the addition of $1x10^5$ macrophages based on day variation (pg / mL)

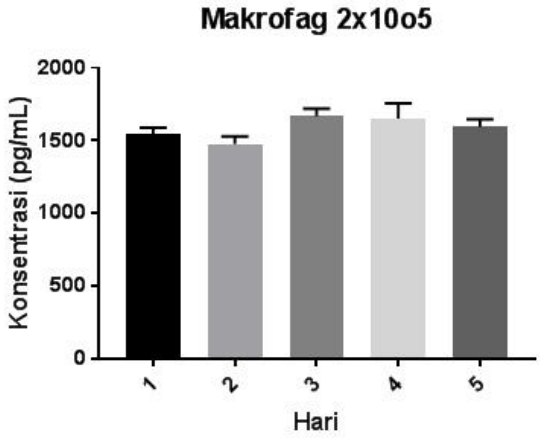

Figure 7: IFN-y secretion with the addition of $2x10^5$ macrophages based on day variation (pg / mL)

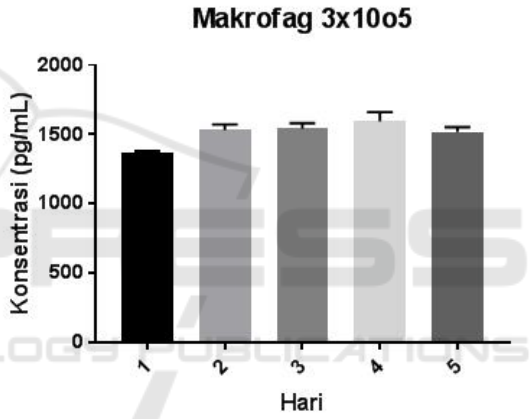

Figure 8: IFN-y secretion with the addition of $3x10^5$ macrophages based on day variation (pg / mL)

IFN-y examination results with ELISA method of 4 groups ie without the addition of macrophages with the addition of macrophages $1x10^5$, $2x10^5$, $3x10^5$ showed high levels. The highest level occurred on day 2 at concentrations of 3x105. On the 3rd day showed an increase in levels which then tend to fall on days 4 and 5.

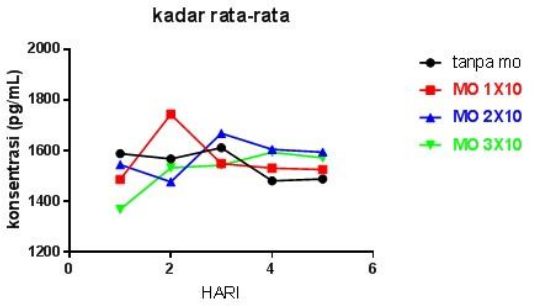

Figure 9: average IFN-y levels

The test results using One-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, p = 0.451 was obtained. the value is greater than 0.05 (p> 0.05) thus indicating no significant difference.

3.3 Discussion

The formation of granulomas is a dynamic process that begins immediately after infection and continues to develop over time. Typically, granulomas can be divided into three distinct phases: (1) "congenital granuloma," a loose aggregate consisting of macrophages and recruited neutrophils; (2) "immune granuloma" is formed after the emergence of antigen-specific T cells; and (3) "chronic granulomas," resulting from different morphological changes in granuloma structures (Shaler et al., 2013).

After innate activation, APC cells are recruited to the lungs and transport mycobacteria to mediastinal lymph nodes. APC activates antigen-specific T cells. Because of the nature of M.tb infection, the majority of bacilli and antigen are in the endosome, and most efficiently loaded into the major histocompatibility complex (MHC) class II. Class II MHC loading facilitates priming of the interferon gamma TH1 (IFN-γ) which is T cell discretion, which rapidly returns the lung. While the dominant subset of T cells is CD4 +, the cross presentation also allows strong induction of CD8 + T cells, collectively resulting in a polarized type 1 adaptive immune response

Macrophages are important effector cells in immunity against intracellular bacteria. In infection, macrophages (MO) recognize mycobacteria with Toll Like Receptor (TLR) involvement (mainly TLR1 / 2 and TLR2 / 6) followed by phagocytosis and mycobacterial growth control. In addition, macrophages and dendritic cells also secrete cytokines such as IL-12 and IL23 to induce IFN-produksi production by T and NK cells, which, in turn, increase phagocytosis, fagolososomes fusion, oxidative bursts (Khan et al., 2016).

The addition of macrophages with different doses does not affect the levels of IFN-y. this is because IFN-y levels tend to be produced by T cells, especially Th1 to stimulate macrophages more actively in phagocytosis mtb. In this case the T cell in generating IFN-y is independent.

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