The Effect of Macrophage Dose to Secretion Interleukin 6 (IL-6) on Model of Tuberculosis Granuloma In Vitro

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Abstract: Granuloma is a pathological sign of host response as a system of defense against infection of Mycobacterium tuberculosis (Mtb) causing Tuberculosis (TB) disease in humans estimated to be 8.7 million new cases, 1.4 million deaths, and about 2 billion latent infections. Macrophages are responsible for activating protective immune responses in innate and adaptive immune to control or eliminate infection. The inner body protects against Mtb infection producing various secretions of cytokines interleukin 6 (IL-6) that play the role of activating multinucleated giant cells, differentiation of macrophage T cells and thus stimulating CD4+ and CD8+ T-cells to strengthen macrophage antimicrobial capacity as early response reactions (early phase). The aim of this research is to see the effect of dose of macrophage on secretion of IL-6 levels on granuloma TB model in vitro. Human blood was made by Peripheral Blood Mononuclear Cells (PBMC) and treated with the addition of a dose of macrophages of 1x10^5 cells/well, 2x10^5 cells/well and 3x10^5 cells/well and control (without macrophages). Then the bacterium Mycobacterium tuberculosis was added and then observing the secretion The enzyme-linked immunosorbent assay (ELISA) method of IL-6 cytokine during the 1st, 2nd, 3rd, 4th, and 5th days. The results of IL-6 examination on ELISA obtained p value of 0.7520 (p> 0.05). The conclusion of the study was that there was no effect of adding macrophage dose to cytokine secretion of IL-6 levels on granuloma TB model in vitro.

1 INTRODUCTION

Bacteria Mycobacterium tuberculosis (Mtb) is the cause of Tuberculosis (TB) disease in humans causing death and one of the infectious agents in humans worldwide. There are an estimated 8.7 million new cases, 1.4 million deaths and about 2 billion latent infections caused by Mtb (Fitzgerald et al., 2014). Based on data from the World Health Organization (WHO) in 2014, the cases of TB in Indonesia reach 1 million and the number of deaths due to TB is estimated at 110,000 each year (Kesehatan and Indonesia, 2017).

Granulomas are a pathological sign of the host response to Mycobacterium tuberculosis infection that has innate immunity, inflammation, but has developed into a complex and dynamic structure capable of being mediated by T cell response (Orme and Basaraba 2014). 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 are responsible for the activation of protective immune responses, innate and adaptive immune, thus playing an important role in ongoing cross-cell communication that is needed to control or eliminate host cell infection in the early phase (Murugesan V.S., Rajaram et al., 2015). Pathophysiology begins with internalization of Mtb by host cell macrophages so that activated macrophages secrete various cytokine markers such as IL-8, IFN-γ, and TNF-α (Kapoor et al., 2013). Mycobacterial infected macrophages also release large amounts of IL-6 that play the role of active macrophage differentiation into multinucleated giant cells (Fitzgerald et al., 2014). IL-6 secreted by Mtb infected macrophages suppresses an uninfected...
macrophage response to IFN-γ. Increased levels of IL-6 correlate with elevated levels of IL-1β and IL-11 so that IL-6 can play many roles (pleotropic cytokines) contributing positively or negatively to host control cells against Mtb infection (Romero-Adrian., 2015).

Based on the above phenomenon, this research was conducted to analyze the role of the addition of dose of macrophage to the formation of granuloma TB and the role of cytokine levels of IL-6 in the formation of granuloma TB in vitro so that a granuloma model can be used in latent and active stage with kedapannya done to develop effective diagnosis and develop a vaccine.

2 MATERIALS AND METHODS

Research data is quantitative. The independent variables were the addition of the dose of macrophages as 1x10⁵ (dose 1), 2x10⁵ (dose 2), and 3x10⁵ (dose 3) cells/well and duration of incubation days 1, 2, 3, 4 and 5 on granuloma model in vitro. While the dependent variable is the amount of IL-6 secretion level.

2.1 PBMC

PBMC is made from adult blood, a healthy criterion. After the blood was taken it was immediately isolated. Blood centrifuge blood plasma was taken 1.5 ml conical tube and added 3 ml PBS and 5 ml histopag and then centrifuge 30 minutes/ 1700 rpm taken buffycoat centrifuge plus PBS centrifuge 5 minutes/1300 rpm then transferred to tube 1.5 ml save temperature -30°C.

2.2 Macrophages

Buffycoat is taken 60 ml. 50 ml conical tubes were prepared with a histopag of 15 ml each. Prepared one tube every 10 ml of buffycoat. Histopagues are used at room temperature. Then sterile scissors and tubes for buffycoat with ethanol 70%, cut the ends of the tube and buffycoat poured into tissue culture flask 75 cm². Buffycoat was diluted with 2% FBS and PBS volume and then stirred slowly then centrifuge was taken ring cell on the surface between histopag and plasma cell as much as 50 ml. Discarded supernatant and centrifuge. Plate at 10 cm culture dishes (10 ml / dish) incubated for 1-2 hours at 37°C 5% CO². Observe macrophage cells under a microscope then discard the supernatant and then add PBS and homogenize. Let the cell differentiate for 7 days observed macrophage cells daily.

2.3 Model Granuloma In Vitro

PBMC added media Roswell Park Memorial Institute (RPMI). Plate 96 well (control, treatment day 1, 2, 3, 4, 5 per/well). Bacterial strain Mycobacterium tuberculosis strain H37Rv (obtained from ITD department Tbc) inoculated as much as 1x10⁵ CFU into all wells. Then macrophages were added at doses 1, 2, 3. Plate was incubated at 37°C CO² 5% to 5 days per day granuloma harvested and 50 μl for ELISA examination.

2.4 ELISA

Examination of ELISA content using reagent kit Elabscience Biotechnology Inc. All Rights Reserved, 2017 (Interleukin 6). Preparation of reagent consists of wash buffer, standard working solution, Biotinylated Detection Ab working solution, Concentrated HRP Conjugate working solution. Advanced examination can be seen in the guide book and determine the optical density (OD value) of each well with 450 nm.

3 RESULTS

Granuloma was taken by BTA dye to confirm Mtb. Then haematoxylin-eosin (HE) staining looked at the morphology of the granuloma structure. Before PBMC made media observed macrophage cells under a microscope.
3.1 Direct Granuloma Observation

Granulomas are observed directly in an inverted microscope per day. Seen granuloma morphology, aggregation and cells form granuloma.

Figure 2: PBMC infected M.Tb without macrophage (control). Figure 1, 2, 3, 4 and 5 have not formed granuloma aggregation.

Figure 3: Direct observation of the group with the addition of 1x10^5 macrophage (400x magnification). The brown arrow indicates 2, 3 have formed granuloma aggregations and 4 granulomas are more clustered.

Figure 4: Direct observation of the group with the addition of 2x10^5 macrophage (400x magnification). The brown arrow indicates 2, 4 have formed granuloma aggregation and 3 perfect granulomas are more clustered and dense.
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3.2 The Examination the levels of IL-6

Supernatant samples were then examined for levels of IL-6 secretion using ELISA method and obtained the average result from each treatment, either control or sample.

Results of IL-6 examination by ELISA method from 4 groups were control with the addition of dose of macrophage 1x10^5, 2x10^5, 3x10^5 showed high level. The highest levels of IL-6 secretion occurred on day 5 with a dose of 3x10^5. While the lowest levels of IL-6 secretion occurred on day 2 with a dose of 2x10^5.
The test results using One-Way ANOVA which aims to determine the significance of the price of the proportion (p). In the control group with the addition of dose of macrophage 1x10^5, 2x10^5, and 3x10^5, p value = 0.4624 was higher than 0.05 (p > 0.05) so it showed no significant difference.

4 DISCUSSION

Granulomas create a balance between both preventing the spread of infection in the host and the protection of Mycobacterium tuberculosis from immune reactions (Majeed, Mir, and Sharma, 2015). The advantage of this study is to know the effect of dose of macrophage on the level of IL-6 secretion so as to explain the early phase macrophage mechanism on host cells infected by Mtb. It was also observed that granulomas from the early stages of formation, clustered from granuloma aggregation to rupture, were seen based on the amount of secretion of IL-6 cytokine. The addition of the expected macrophage dose suggests adding to the availability of macrophages as a therapeutic strategy for tuberculosis (Randolph, 2015). The ability of Mtb through the early secreted antigenic target of 6 kDa (ESAT-6) is capable of replicating active Mtb to modulate the number of cells secreted through the mycobacterial type VII secretion system (ESX-1) (V. R. Parasa et al., 2014).

The ability of Mtb to secrete ESAT-6 plays an important role in inducing aggregate aggregation of monocyte and macrophage cells from early stage granuloma formation. This is in line with observations made in the in vivo zebrafish embryo that transparency permits a picture of the life of neutrophil cells against granuloma formation (Je et al., 2016). IL-6 is involved in the differentiation of macrophage and cytotoxic T cells. IL-12 induces IFN-γ to differentiate CD4+ T cells on Th1 effectors. Cytokines can also direct neutrophils, monocytes, lymphocytes to CD4+ and CD8+ T-cell-inducing infections to strengthen macrophage antimicrobial capacity. ESAT-6 and culture filtrate protein 10 (CFP-10) in Mycobacterium tuberculosis are among the candidates of the Tb vaccine because they induce immune-strong T cells in animal models so that some previous studies raised extraordinary problems related to ESAT-6/CFP-10 candidates as an effective vaccine against Tb (Abebe et al., 2017).

Using a model for studying human-like human papillomavirus granulomas is important because: (1) it is difficult to directly study human lung biopsy specimens due to access limitations; (2) biopsy specimens only show static images and should foresee various possibilities for understanding dynamic process in granuloma and (3) M. tuberculosis has no natural host other than humans so it takes an in vitro granuloma model to study the initial steps of granuloma formation and treatment, and has the potential to address more of the translational aspects of human M. tuberculosis infection. These in vitro models are less similar in structures such as the lung and micro-tissue environment (Guirado and Schlesinger, 2013). This granuloma model has the potential to provide insight into host-pathogen interactions at different stages of human granuloma Tb formation (Kapoor et al., 2013) granuloma Tb manusia (Kapoor et al., 2013).

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REFERENCES


