

The Influence of Euphorbia Milii Flower Extract in the Activity of Th17 through IL-17 Secretion in *Mycobacterium tuberculosis* Infected Mice

Ni Made Linawati¹, Dewa Made Sukrama², Made Mertaniasih³

¹Department of Histology, Medical Faculty Udayana University Denpasar-Bali, Indonesia (0361) 222510

²Department of Microbiology, Medical Faculty Udayana University Denpasar-Bali, Indonesia (0361) 222510

³Department of Microbiology Medical, Faculty of Airlangga University

Abstract: *The present study was conducted to study Euphorbia milii flower extract (EM) in increasing the activity of Th17 cell through IL-17 secretion. This was an experimental study with Post Test Only Control Group design. 24 BalbC, male, were divided into 6 groups of treatment which were observed at first and third week. Group K1 and K2 are negative controls; P1 and P3 were given OAT and M.tb infected; while the P2 and P4 were infected M.tb, given OAT and EM. Terminations of K1, P1 and P2 were in the first week, while K2, P3 and P4 were terminated in the third week. Pulmonary organs were removed for examination of IL-17 by ELISA. The result showed mean of level of IL-17 in K1, P1, P2, K2, P3 and P4 respectively 81.18; 90.00; 88.65; 87.53; 75.45 and 87.53 pg/ml. EM extract increased the concentration of IL-17 in the third week higher than the group that did not receive the extract but the difference was not significant. It happened probably because a lot of factors that affect the stability of IL-17, thus affecting the outcome. It is necessary for further research on the mRNA expression of IL-17, so that the influence factors can be minimized.*

Keywords: Euphorbia milii, Mycobacterium tuberculosis, Th17, IL-17

1. Introduction

Mycobacterium tuberculosis has caused 1.4 million deaths each year in which Indonesia now ranked fifth among countries with the highest tuberculosis burden in the world¹. Transmission occurs after inhaling droplets containing *Mycobacterium tuberculosis* and are deposited on the distal alveoli. In which alveolar macrophages and dendritic cells that phagocytose mycobacteria, forming fusion phagolysosomes though in a state of optimal activation of macrophages, NK, dendritic then Mycobacterium tuberculosis often unable to cope with preventing fusion phagolysosome². Research over the years acquired the role of Th1 CD4⁺ through the secretion of IFN- γ were able to increase the activation of macrophages to kill intracellular mycobacteria. But the last few years found a new subset of CD4 + Th cells that Th17 are not related to the secretion of IFN- γ . Th17 cells that secrete IL-17 proved to have a protective role of Th1 and Th2 subsets currently unable to fight extracellular bacteria. Th17 role through the secretion of IL-17 in the pathogenesis of tuberculosis shows protective capability against Mycobacterium tuberculosis infection equaled IFN- γ but without effect induced damage to lung tissue^{3,4}. Also found a decrease in the population of Th17 cells and the secretion of IL-17 in patients with active pulmonary tuberculosis⁵. This shows that the optimal activity of Th17 cells through the secretion of IL-17 is important in the fight against Mycobacterium tuberculosis infection to prevent active disease manifestations. So it is necessary to find drugs from natural materials which have the ability to modulate the optimal activation of Th17 through the secretion of IL-17. This study is a continuation of research in the first year that showed EM was able to increase NK cell activity in mice infected with Mycobacterium tuberculosis through Nkp46 expression⁶. In the second year of this study aims to prove

that EM able to increase the expression of Th17 through the IL-17 in mice infected with Mycobacterium tuberculosis.

2. Material and Methods

This research is an experimental post-test only control group design. A total of 24 BalbC mice, 8 weeks old are placed randomly in 6 cages, each cage containing 4 mice. They are adapted for 2 weeks. The first day after the adaptation, the group K1 and K2 is given only sterile distilled water; group P1, P2, P3 and P4 M.tb infection strain H37Rv given intranasally by 60 μ L with a concentration of germs 10⁵. Then next day, the group K1 and K2 as control are given only sterile distilled water of 1 ml/day, the group P1 and P3 are given standard medication INH 0.5 mg / 20 grbw; P2 and P4 group given standard drugs INH 0.5 mg / 20 grbw and EEM dose of 10 mg / 20 grbw. Giving done every day for 21 days. On day 8 carried the termination of each 4 mice in group K1, P1 and P2, while on day 21 the termination of 4 mice performed again in the K2 group, P3, and P4, take pulmonary organs and prepared as standard procedure for measuring the secretion of IL -17 by ELISA (R & D Systems).

3. Result

The Secretion of IL-17 in groups of study

The concentration of IL - 17 from lung samples obtained by the formula slot -intercept of the standard OD values and samples. Average concentration of IL - 17 in group K1, P1, P2, K2, P3 and P4 are respectively 81.18; 90.00; 88.65; 87.53; 75.45 and 87.53 pg / ml . Data analysis indicates that the data is normally distributed, One Way Anova result there was no significant difference between groups in IL- 17 secretion (p = 0.134). However, if seen IL-17 level curve in

all groups of treatment (Figure 1) appears there were differences secretion of IL-17 in the research groups .

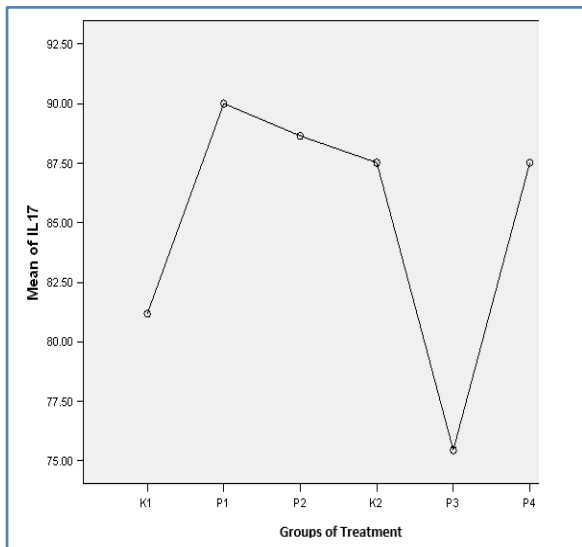


Figure 1: Interleukin 17 level (pg/ml) curve in all groups of treatment

4. Discussion

In the first week the concentration of IL-17 highest in P1 while the third week the concentration of IL-17 in group P4 (OAT and EM) equaled the control group. The test results One Way Anova indicating no significant difference between groups. This due to cytokines such as IL-17 stability is influenced by various factors that require peak right time for measuring the secretions. As also described by Korn 2009 that the differentiation of T cells into Th17 cells is influenced by many factors and chemokine such as differentiation factors (TGF- β , IL-6, IL-21), growth factors and stabilization (IL-23), as well as transcription factors (STAT3, ROR γ t, and ROR α)⁷. The curve showed the secretion of IL-17 in third week on P4 group equaled with group of control (K2) and also higher than P3. This shows that the extract was given for 3 weeks in group of intervened M.tb and granted OAT able to increase IL-17 equaled with the normal group. While EM intervention provided only within 1 week (P2) was not able to increase the secretion of IL-17. It is characteristics of immunostimulatory from natural materials in which to work optimally it requires a fairly large doses and long enough time, but the side effects are smaller than a synthetic immunostimulatory⁸.

5. Conclusion

From the above results it can be concluded that The ethanol extract of *Euphorbia milii* (EM) increase the secretion of IL-17 in the third week, but the increase was not shown significantly.

References

[1] Anonim 1. Kenmenkes RI. 2011. Strategi Nasional Pengendalian TB di Indonesia 2010-2014. P. 1-80.
 [2] Philips JA, Ernst JD. 2012 Tuberculosis pathogenesis and immunity. *Annu Rev Pathol* 7: 353-84

[3] Roldan, C, P., Barba, J., Valdes, M, A, F. Th17: A New Player to be Considered in Tuberculosis Studies. *J Bacteriol Parasitol.* 3(-) : 9
<http://dx.doi.org/10.4172/2155-9597.1000e115>
 [4] Li, Q., Li, J., Tian, J., Zhu, B., Zhang, Y. 2012. IL-17 and IFN- γ production in peripheral blood following BCG vaccination and *Mycobacterium tuberculosis* infection in human. *European Review for Medical and Pharmacological Sciences.* 16 (-) : 2029-36
 [5] Chen, X., Zhang, M., Liao, M., Graner, M, W., Wu, C. 2010. Reduced Th17 Response in Patients with Tuberculosis Correlates with IL-6R Expression on CD4⁺ T Cells. *American Journal of Respiratory and Critical Care Medicine.* 181 (7): 734-42.
 [6] Linawati, N, M., et al. 2014. The Optimal dose of *Euphorbia milii* extracts in NKp46 expression against mice infected with *Mycobacterium tuberculosis*. *Journal of US-China Medical Science.* 2014. 11(2): 68-73.
 [7] Korn, T., Bettelli, E., Oukka, M., Kuchroo, V, K. 2009. IL-17 and Th17 cells. *Annu Rev immunol.* 27 (-): 485-517
 [8] Tamioka H. 2004. Adjunctive Immunotherapy of Mycobacterial Infections. *Current Pharmaceutical Design* 10: 3297-3312.