**EXPRESSION OF IMMUNOGLOBULIN, GRANZYME-B AND PERFORIN AGAINST Ag85A AND Ag85B PROTEINS OF MYCOBACTERIUM TUBERCULOSIS IN BALB/C MICE**

***Fihiruddin1,2*,***Tri Wibawa3, Ni Made Mertaniasih4, Wayan Tunas Artama5,6,\**

1Doctoral Program, Research Center of Biotechnology, Universitas Gadjah Mada, Yogyakarta. Indonesia

2Department of Medical Laboratory Technology, Politeknik Kesehatan Mataram, Indonesia

3Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta. Indonesia

4Department of Clinical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya. Indonesia

5Department of Biochemistry, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta. Indonesia

6One Health/Ecohealth Resource Center, Universitas Gadjah Mada, Yogyakarta. Indonesia

\*Corresponding Author’s E-mail: artama@ugm.ac.id

**ABSTRACT**

Ag85 is a protein that may maintain survival of *M. tuberculosis* in intracellular parts of host cells and is considered as a virulence factor. The expression of Ag85 protein can stimulate proliferation and differentiation of B- cells and T-cells in patients with tuberculosis. This research aimed to determine the ability of Ag85A and Ag85B proteins in activating the response of antibodies, granzyme-B and perforin in Balb/c mice. Twenty-five male Balb/c mice were assigned into five groups. Group I was treated with adjuvant, group II with Bacillus Calmette-Guerin (BCG) vaccine, group III with a combination of BCG and Ag85A, group IV with a combination of BCG and Ag85B and group V with a combination of BCG, Ag85A and Ag85B. Concentrations of immunoglobulin G, granzyme-B and perforin were examined using ELISA and the number of CD8+ T-cells and NK T-cells were checked by flow cytometry. The highest concentration of immunoglobulin G was found in group V with 62.49±5.4327 ng/ml. The highest mean number of CD8+ T-cells, NK T-cells, granzyme-B and perforin was found in group IV with 4.32%, 1.03%, 35.11±1.7789 pg/ml and 6.19±0.2235 pg/ml, respectively. The results of One-Way ANOVA test showed that there were significant differences in immunoglobulin responses, with p<0.05. The expressions of granzyme-B and perforin were higher in mice treated with combination of BCG and recombinant proteins. Ag85 protein can be combined with the BCG vaccine to improve protection against M. tuberculosis infection.

**Keywords:** Ag85A and Ag85B proteins, Immunoglobulin, granzyme B and perforin, TB Vaccine