RESPONS IMUN TERHADAP PROTEIN REKOMBINAN ANTIGEN 85A DAN 85B *M. tuberculosis* PADA MENCIT *BALB/C*

INTISARI

Vaksin BCG (*Bacillus Calmette-Guerin*) konvensional masih digunakan sebagai vaksin utama dalam perlindungan dini terhadap *M. tuberculosis*. Proteksi vaksin ini kurang optimal mengatasi penularan dan prevalensi kasus tuberkulosis secara global sehingga diperlukan vaksin baru yang efektif dan efisien terhadap tuberkulosis. Protein kompleks antigen 85 *M. tuberculosis* merupakan salah satu target sistem imun dan berperan dalam menginduksi proliferasi sel T dan B. Tiga anggota protein Ag85 dengan berat molekul 30–32 kDa, meliputi Ag85A, Ag85B dan Ag85C. Ekspresi protein Ag85 diperlukan untuk kelangsungan hidup *M. tuberculosis* dalam makrofag dan diduga sebagai faktor virulensi. Penelitian ini bertujuan untuk melakukan amplifikasi, kloning dan mengekspresi gen penyandi Ag85A dan Ag85B *M. tuberculosis*, serta memprediksi epitop sel T dan sel B dan menguji respon imun humoral dan selulernya pada mencit *Balb/c*. Penelitian ini merupakan penelitian *eksperimental* yaitu melakukan kloning dan ekspresi gen penyandi Ag85 *M. tuberculosis MDR* untuk diujikan imunogenitasnya pada mencit *Balb/c*. Analisis perbandingan imunogenitas Ag85 menggunakan desain *The Static Group Comparison Group*. Pada rancangan ini, gen penyandi protein Ag85A dan Ag85 B di amplifikasi, dikloning dan diekspresikan pada sel kompeten *E. coli BL 21 (DE3*). Respon imun Ag85A dan Ag85B di uji secara *in vivo* menggunakan mencit *Balb/c* dan secara *in vitro* menggunakan kultur sel dengan berbagai perlakuan. Hasil amplifikasi *gen fbpA* dan *fbpB* adalah 1130 bp dan 978 bp dengan BM 36 kDa dan 34 kDa. Prediksi epitop Ag85A dan Ag85B terhadap sel T menggunakan GENETYX ver 8.0 di peroleh masing-masing 7 epitop, prediksi epitop sel B dengan *ABCPred score* diatas 0.83 diperoleh masing-masing 11 dan 9 epitope. Prediksi epitop MHC I (HLA-A*0201) dengan *score* diatas 59 dan MHC II (HLA DR17/DRB1*0301) dengan *score* di atas 5.0 menggunakan *RANKPEP prediction* ditemukan 15 dan 13 posisi epitop MHC I, serta 11 dan 8 posisi epitop MHC II. Uji MTT sel T menunjukkan aktivitas proliferasi paling tinggi dengan paparan Ag85B 10 µg/ml (OD 0.358), sedangkan sel B dengan pemberian gabungan protein Ag85A dan Ag85B 5 µg/ml (OD 0.346). Jumlah sel NK dan sel T $CD8^+$ menggunakan *flowcytometri* paling besar di peroleh dari gabungan BCG dan Ag85B yaitu masing-masing 4.23% dan 1.03%. Konsentrasi *granzyme B* dan *perforin* ditemukan paling tinggi pada kelompok yang diberikan paparan BCG dan Ag85B, yaitu 34.45 pg/ml dan 6.19 pg/ml, sedangkan IL-1β dari gabungan BCG, Ag85A dan Ag85B yaitu 141.9 pg/ml. Uji statistik *One-Way ANOVA* dengan tingkat kepercayaan 95 % menunjukkan perbedaan signifikant kelompok perlakuan di banding kontrol dengan nilai $p<0,05$. Protein Ag85 *M. tuberculosis* MDR isolat klinik dapat dikombinasikan dengan vaksin BCG untuk meningkatkan proteksi terhadap infeksi *M. tuberculosis* dan sebagai bahan untuk membuat alat diagnostik untuk uji *screening M. tuberculosis*.

Kata kunci: *M. tuberculosis*, protein Ag85, vaksin, imunitas humoral, imunitas seluler

IMMUNE RESPONSE TO RECOMBINANT PROTEINS OF ANTIGEN 85A AND 85B of *M. tuberculosis* IN THE BALB/C MICE

ABSTRACT

Conventional Bacillus Calmette-Guerin (BCG) vaccine is still used as the main vaccine in early protection against *M. tuberculosis* infections. The protection of this vaccine is low efficiency for overcoming transmission and epidemics of tuberculosis cases globally. therefore effective and efficient a new vaccine against tuberculosis is required. Antigen complex of proteins 85 of *M. tuberculosis* is one of the immune system targets and plays a role in inducing proliferation of T and B cells. There are three members of the Ag85 proteins, consist of Ag85A, Ag85B and Ag85C. Expression of their proteins are reqiured for the survival of *M. tuberculosis* in macrophages and is considered a virulence factor. This study aims to amplify, clone and expression genes encoding of proteins Ag85A and Ag85B of *M. tuberculosis*, and predict of T cell and B cell epitopes and examine humoral and cellular immune responses in *Balb/c* mice. This research is a pre-exprimental study, that is cloning and expression of the encoding gene of Ag85 multi-drug resistant (MDR) of *M. tuberculosis* to be tested it immunogenicity in *Balb/c* mice. Comparative analysis of Ag85 immunogenicity using the design of The Static Group Comparison Group. In this design, the genes encoding Ag85A and Ag85 B proteins are amplified, cloned and expressed on competent *E. coli BL 21 (DE3) cells*. The immune responses of Ag85A and Ag85B proteins were tested in vivo using *Balb/c* mice and in vitro using cell cultures with various treatments. The results of *fbpA* and *fbpB* gene amplification were 1130 bp and 978, molecular weight of 36 kDa and 34 kDa respectively. Predictions of Ag85A and Ag85B to T cell epitopes using *GENETYX ver 8.0* were obtained for each of 7 epitopes, prediction of B cell epitopes using *ABCPred* prediction with score above 0.83 were obtained 11 and 9 epitope respectively. MHC I epitope prediction (HLA-A*0201) with a score above 59 and MHC II (HLA DR17/DRB1*0301) with a score above 5.0 using *RANKPEP* prediction was found 15 and 13 epitope positions (MHC I), and 11 and 8 epitope positions (MHC II). In MTT test obtained that T cell showed the highest proliferation activity with exposure by 10 µg/ml Ag85B protein (OD 0.358), while B cell by exposure a combination of 5 µg/ml Ag85A and Ag85B proteins (OD 0.346). The highest number of NK cells and CD8+ T cells using flowcytometri was obtained from a combination of BCG and Ag85B, which were 4.23% and 1.03%, respectively. The concentration of granzyme B and perforin was found to be highest in the group given BCG and Ag85B exposure, were 34.45 pg/ml and 6.19 pg/ml respectively, whereas IL-1β from the combination of BCG, Ag85A and Ag85B (141.9 pg/ml). The One Way Anova statistical test with a 95% confidence level showed a significant difference in the treatment groups compared to the control with a value of $p<0.05$. The protein Ag85 of MDR *M. tuberculosis* from clinical isolates can be combined with the BCG vaccine to increase protection against *M. tuberculosis* infection and used to make diagnostic tools for *M. tuberculosis* screening tests.

KeyWord: *M. tuberculosis*, protein Ag85, vaccine, humoral immunity, cellular immunity