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**Pengaruh Pemberian Outer Membran Protein
Mycobacterium tuberculosis terhadap ekspresi
sel T CD4 dan Interferon γ**

Tahun 1(Satu)ke dari rencana 2 (Dua) tahun

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ABSTRAK

PENGARUH PEMBERIAN OUTER MEMBRAN PROTEIN TERHADAP EKSPRESI SEL TCD4 DAN INTERFERON γ

Tuberkulosis merupakan penyakit yang disebabkan *Mycobacterium tuberculosis*. Jumlah kasus dan kematian akibat penyakit ini semakin meningkat. Pencegahan tuberkulosis saat ini dengan pemberian vaksin BCG yang efektifitasnya bervariasi 0-80%. Walaupun BCG dapat mencegah penyebaran penyakit tuberkulosis pada anak-anak tetapi efek protektif tidak dapat mencegah terjadinya tuberkulosis pada paru orang dewasa. Tuberkulosis pada paru merupakan manifestasi klinis terbanyak pada orang dewasa dan penyebab terbanyak kematian pada usia produktif, sehingga masih diperlukan pengembangan vaksin terhadap tuberkulosis.

Pengembangan vaksin pada penelitian ini berdasarkan protein yang disolasi dari bagian bakteri *Mycobacterium tuberculosis* yaitu Outer Membrane Protein (OMP) 70,87kDa. Pemberian OMP *Mycobacterium tuberculosis* 70,87kDa bersama dengan adjuvan pada mencit melalui injeksi secara subkutan. Setelah diinduksi, diamati pengaruh pemberian OMP *Mycobacterium tuberculosis* 70,87kDa tersebut terhadap respons imun seluler protektif dalam hal ini jumlah dan aktivasi sel TCD4 dan pengeluaran sitokin Interferon- γ pada paru dan limpa mencit. Hasil induksi dievaluasi dengan flowcitometri dan Elispot.

Pada penelitian ini, dilakukan SDS-PAGE dan ujiwestern blotting. Didapatkan protein OMP berat molekul 70,87 kDa yang bereaksi dengan serum penderita. Dari hasil penelitian didapatkan bahwa protein OMP 70,87kDa bersama adjuvan yang diimunisasikan dapat mengaktivasi sel NK dan sel limfosit T. Setelah dilakukan analisis dengan Statistik menunjukkan perbedaan perlakuan imunisasi serta organ yang diperiksa, secara umum memberikan pengaruh yang signifikan terhadap rata-rata jumlah sel NK, sel limfosit TCD4⁺ dan TCD8⁺ dan sel tersebut teraktivasi ($p < 0,05$) serta sekresi IFN- γ .

Kesimpulan Protein OMP 70,87kDa *Mycobacterium tuberculosis* dapat menginduksi respons imun seluler pada paru dan limpa mencit

Kata kunci: *Mycobacterium tuberculosis*, OMP, sel limfosit TCD4⁺.

ABSTRACT

EFFECT OF OUTER MEMBRANE PROTEIN EXPRESSION OF CELL TCD4 and INTERFERON γ

Tuberculosis is a disease caused by *Mycobacterium tuberculosis*. The number of cases and deaths from the disease is increasing. The current tuberculosis prevention with BCG vaccine effectiveness varies 0-80 %. Although BCG can prevent the spread of tuberculosis in children but can not prevent the protective effect of the occurrence of pulmonary tuberculosis in adults. Tuberculosis of the lungs is the largest clinical manifestations in adults and the most common cause of death in the productive age, so it still requires the development of a vaccine against tuberculosis.

Development of the vaccine in this study based protein electrically insulated from the bacterium *Mycobacterium tuberculosis* that is the Outer Membrane Protein (OMP) 70.87KDa. Provision of 70.87 kDa OMP *Mycobacterium tuberculosis* together with the adjuvant in mice via subcutaneous injection. Once induced, observed the effect of 70.87 kDa OMP *Mycobacterium tuberculosis* is the protective cellular immune response in this case and the amount of TCD4 cell activation and cytokine interferon - γ expenditure in the lungs and spleen of mice. The results were evaluated with flowcitometri induction and ELISPOT.

In this study, performed SDS - PAGE and ujiwestern blotting. Obtained OMP protein molecular weight 70.87 kDa that react with patient serum. The result showed that the protein is 70.87 kDa OMP immunized with adjuvant can activate NK cells and T lymphocytes. After analyzing the statistics in immunization and treatment differences were examined organs, generally have a significant influence on the average number of NK cells, lymphocytes and TCD8 TCD4 + and these activated cells ($p < 0.05$) and IFN - γ secretion.

Conclusion 70.87 kDa OMP proteins of *Mycobacterium tuberculosis* induces cell - mediated immune response in the lungs and spleen of mice

Keywords : *Myc*

obacterium tuberculosis, OMP, lymphocytes TCD4

RINGKASAN

Tuberkulosis (TB) merupakan penyakit menular yang disebabkan oleh bakteri *Mycobacterium tuberculosis*. Diperkirakan sepertiga penduduk dunia telah terinfeksi oleh *M.tuberculosis*, 5-10% akan berkembang menjadi tuberkulosis dengan manifestasi klinis. Pencegahan tuberkulosis sampai saat ini dilakukan dengan pemberian vaksin BCG (*Bacille Calmette- Guerine*). Tampaknya pemberian imunisasi BCG, belum dapat melindungi terjadinya infeksi TB pada orang dewasa. Oleh karena itu, masih diperlukan pengembangan terhadap vaksin tuberkulosis, mengingat efek proteksi BCG yang diberikan pada kejadian tuberkulosis paru orang dewasa bervariasi sekitar 0-80 %.

Pengembangan vaksin berdasarkan materi permukaan bakteri yang disebut adhesin sebagai target untuk pengembangan vaksin mulai banyak diteliti. Proses adhesi merupakan salah satu sifat virulensi dari bakteri yang berperan penting untuk terjadinya kolonisasi sampai timbulnya infeksi. *Mycobacterium tuberculosis* memiliki Outer Membran Protein (OMP) yang merupakan bagian bakteri yang berperan pada pelekatan dengan sel epitel host, dengan dasar mekanisme ini OMP dapat dipakai sebagai kandidat vaksin

Manfaat penelitian ini secara teoritis diharapkan temuan penelitian ini dapat menjadi dasar pengembangan Ilmu Pengetahuan dan Teknologi, mengenai OMP *Mycobacterium tuberculosis* dalam menginduksi respons imun seluler protektif. Manfaat Praktis diharapkan temuan penelitian ini dapat dilanjutkan dan digunakan sebagai pengembangan bahan vaksin yang dapat digunakan untuk membantu menurunkan terjadinya infeksi tuberkulosis.

Metode penelitian dilakukan dalam 3 tahap, yaitu: isolasi OMP *Mycobacterium tuberculosis strain H37Rv* dan SDS-PAGE (Tahap-1); Uji reaktivitas OMP terhadap serum penderita TB (Tahap-2); Uji imunogenitas OMP *Mycobacterium tuberculosis strain H37Rv* dalam menginduksi respons imun seluler (Tahap-3).

Hasil penelitian tahap-1 dan 2 menunjukkan bahwa OMP *Mycobacterium tuberculosis strain H37Rv* 70.87 Kda memberikan reaksi positif terhadap serum penderita tuberkulosis.

Pada penelitian tahap-3 untuk menganalisis kemampuan OMP *Mycobacterium tuberculosis strain H37Rv* 70,87kDa dalam menginduksi respons imun seluler. Mencit dibagi dalam empat kelompok, yaitu kelompok perlakuan pemberian PBS steril, perlakuan pemberian OMP *Mycobacterium tuberculosis strain H37Rv* 70,87kDa, perlakuan pemberian ajuvan dan perlakuan pemberian OMP *Mycobacterium tuberculosis strain H37Rv* 70.87 kDa bersama ajuvan. Dosis OMP *Mycobacterium tuberculosis strain H37Rv* 70,87kDa yang diberikan 100µg. Hasil pemeriksaan respons imun seluler dengan menggunakan *flowcitometry* dan sekresi IFN γ menggunakan *Elispot* mouse IFN- γ . Setelah dilakukan isolasi sel dalam bentuk *singgle cell suspension* maka sel tersebut dilakukan *multiple staining* dengan antibodi monoklonal selanjutnya diinkubasi dan diperiksa dengan *flowcitometry*.

Hasil pemeriksaan sel limfosit TCD4⁺ dan TCD4⁺CD69 rata-rata prosentase jumlah sel tertinggi secara keseluruhan didapatkan pada perlakuan pemberian imunisasi dengan protein OMP *Mycobacterium tuberculosis strain H37Rv* 70,87 kDa bersama adjuvan masing-masing sebesar $26.757 \pm 2,808$ dan $12.004 \pm 1,323$ (limpa), serta $18.361 \pm 1,828$ dan $14,804 \pm 1,578$ (jaringan paru). Analisis statistik menunjukkan perbedaan perlakuan imunisasi serta organ yang diperiksa, secara umum memberikan pengaruh yang signifikan terhadap rata-rata jumlah sel limfosit TCD4⁺ dan TCD4⁺ CD69 ($p < 0,05$).

Hasil pemeriksaan limfosit teraktifasi TCD8⁺ dan TCD8⁺CD69 rata-rata prosentase jumlah sel tertinggi secara keseluruhan didapatkan pada perlakuan pemberian imunisasi dengan protein OMP *Mycobacterium tuberculosis strain H37Rv* 70,87kDa bersama adjuvan masing-masing sebesar 11.833 ± 2.162 dan $6,946 \pm 1.575$ (limpa), 25.36 ± 6.87 dan 14.477 ± 1.687 dan 2.384 ± 0.785 (jaringan paru). Berdasarkan analisis statistik menunjukkan perbedaan perlakuan imunisasi serta organ yang diperiksa, secara umum memberikan pengaruh yang signifikan terhadap rata-rata jumlah sel limfosit TCD8⁺ dan TCD8⁺CD69 ($p < 0,05$).

Hasil pemeriksaan sel NK1.1 dan NK1.1CD69 rata-rata prosentase jumlah sel pada organ limpa dan paru didapatkan pada perlakuan pemberian imunisasi dengan protein OMP *Mycobacterium tuberculosis strain H37Rv* 70,87kDa bersama adjuvan masing-masing sebesar 1.830 ± 0.556 dan 1.664 ± 0.519 (limpa) dan 0.996 ± 0.561 dan 0.052 ± 0.018 (paru). Berdasarkan analisis statistik menunjukkan perbedaan perlakuan imunisasi serta organ yang diperiksa, secara umum memberikan pengaruh yang signifikan terhadap rata-rata jumlah sel NK1.1 dan sel NK1.1CD69 ($p < 0,05$).

Hasil pemeriksaan spot IFN- γ rata-rata jumlah spot tertinggi pada semua organ didapatkan pada perlakuan pemberian imunisasi dengan protein OMP *Mycobacterium tuberculosis strain H37Rv* 70,87kDa OMP *Mycobacterium tuberculosis strain H37Rv* 70,87kDa ditambah adjuvan, masing-masing sebesar 31.3 ± 2.35 (Limpa) dan 28.0 ± 2.44 (paru). Berdasarkan analisis statistik menunjukkan perbedaan perlakuan imunisasi serta organ yang diperiksa, secara umum memberikan pengaruh yang signifikan terhadap rata-rata jumlah spot IFN γ ($p < 0,05$).

Dapat disimpulkan bahwa pemberian imunisasi protein OMP *Mycobacterium tuberculosis strain H37Rv* 70,87kDa dapat menginduksi respons imun seluler .

SUMMARY

EFFECT OF OUTER MEMBRANE PROTEIN EXPRESSION OF CELL TCD4 and INTERFERON γ

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* bacteria. It is estimated that one-third of the world's population has been infected with *Mycobacterium tuberculosis*, 5-10 % will develop into clinical manifestations of tuberculosis. Prevention of tuberculosis has so far been done with the BCG vaccine (*Bacille Calmette-Guerine*). Apparently BCG immunization has failed to provide protection against tuberculosis infection to adults. Therefore, the development of a vaccine against tuberculosis, is still requires considering that the protective effect of BCG given on adult pulmonary tuberculosis incidence varies around 0-80 %.

The development of vaccines based on bacterial surface material called adhesin as a target for vaccine development is beginning to be widely studied. The process of adhesion is one of the virulence properties of bacteria playing an important role for the occurrence of colonization until the onset of infection. *Mycobacterium tuberculosis* has outer membrane protein that are part of bacteria that play a role in adhesion to host epithelial cells and pili can induce an immune response, on the basis of this mechanism it can be used as a vaccine candidate.

The significance of this research is that theoretically it is expected that the findings of this study can be a basis for the development of Science and Technology, regarding the Outer membrane protein of *Mycobacterium tuberculosis* in inducing protective cellular immune responses. The practical benefit is that it is expected that the findings of this study can be further pursued and used as vaccine development materials that can be used to help reduce the occurrence of tuberculosis infection.

Method of research covered 3 phases: isolation of proteins outer membrane of *Mycobacterium tuberculosis* strain H37Rv and SDS-PAGE (Phase1); Reactivity outer membrane protein testing of *Mycobacterium tuberculosis* strain H37Rv (Phase-2); Outer membrane protein immunogenicity testing of *Mycobacterium tuberculosis* H37Rv strain in inducing cellular immune response (Phase-3) ;

The phase 1 and 2 research results showed that the outer membrane protein of 70,87 kDa *Mycobacterium tuberculosis* strain H37Rv was able reactivity with serum patient tuberculosis.. In phase-3 research analyzed the ability of Outer membrane protein of 70,87kDa *Mycobacterium tuberculosis* strain H37Rv in inducing cellular immune responses. Mice were divided into four groups, namely the provision of sterile PBS treatment, OMP protein of 70,87Da *Mycobacterium tuberculosis* strain H37Rv, adjuvant, and OMP of 70,87kDa *Mycobacterium tuberculosis strain* H37Rv with adjuvant. Dosage of OMP of 70,87 kDa *Mycobacterium tuberculosis* strain H37Rv was 100 μ g.

The cellular immune response was examined result using flowcitometry and IFN- γ secretion using IFN- γ mouse ELISpot. After isolating cell in the form of

single cell suspension , then multiple staining of cells was conducted with monoclonal antibodies, then incubated and examined with flow cytometry.

The examination result on TCD4⁺ and TCD4⁺ CD69 lymphocyte cells was that the average percentage of the highest number of the cells obtained in the treatment of immunization with OMP *Mycobacterium tuberculosis* strain H37Rv of 70.87 kDa with adjuvant was $26.757 \pm 2,808$ dan $12.004 \pm 1,323$ (spleen), serta $18.361 \pm 1,828$ dan $14,804 \pm 1,578$ (lung tissue). respectively. The statistical analysis showed a difference in MANOVA treatment immunization and organ being examined, in general, had a significant influence on the average percentage number of TCD4⁺ and TCD4⁺ CD69 lymphocytes cells ($p < 0.05$).

The examination result on the lymphocytes activated TCD8⁺ and TCD8⁺ CD69 showed that average percentage of the highest number of cells as a whole was found in the treatment of immunization with the OMP *Mycobacterium tuberculosis* strain H37Rv of 70,87kDa with adjuvant amounting 11.833 ± 2.162 dan $6,946 \pm 1.575$ (spleen), 25.36 ± 6.87 dan 14.477 ± 1.687 dan 2.384 ± 0.785 (lung tissue). Based on the statistical analysis, it showed that the differences in treatment immunization and organ being examined, in general had a significant effect on the percentage of the average number of TCD8⁺ CD69 lymphocytes cells ($p < 0.05$).

The examination result on cells NK1.1 and NK1.1CD69 showed that the highest average percentage of the number of the cells in the spleen and blood obtained at treatment immunization using OMP protein of 70,87 kDa *Mycobacterium tuberculosis* strain H37Rv with ajuvan was 1.830 ± 0.556 dan 1.664 ± 0.519 (spleen) dan 0.996 ± 0.561 dan 0.052 ± 0.018 (lung tissue). respectively. Based on the statistical analysis, it showed the differences in treatment immunization and organ being examined, in general had a significant effect on the average number of NK1.1 and NK1.1CD69 cells ($p < 0.05$).

The result of IFN- γ spot examination showed that the average number of the highest spots in all organs obtained at treatment immunization with OMP of 70,87kDa *Mycobacterium tuberculosis* strain H37Rv with adjuvant, was 31.3 ± 2.35 (spleen) dan 28.0 (lung) respectively. Based on the statistical analysis, it showed the differences in treatment immunization and organ being examined, in general had a significant effect on the average number of IFN- γ spots ($p < 0.05$).

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