RINGKASAN

URUTAN ASAM AMINO DARI EPITOP REGIO N-TERMINUS ANTIGEN ESAT-6 SEBAGAI MARKA DIAGNOSTIK PENYAKIT TUBERKULOSIS PARU AKTIF

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Penyakit tuberkulosis (TB) paru masih merupakan masalah kesehatan, baik di Indonesia maupun di seluruh dunia. Vaksin BCG, sekalipun telah berhasil menurunkan prevalensi TB primer, tetapi belum berhasil mengurangi prevalensi TB pasca-primer.

Seiring dengan kemajuan di bidang teknologi, penelitian di bidang diagnostik dan vaksinasi untuk penyakit TB telah maju pesat. Urutan genom dari *Mycobacterium tuberculosis* sebagai agen penyebab TB telah diketahui dengan lengkap, maka informasi mengenai seluruh bahan genetik dari kuman ini telah dapat diperoleh. Antigen ESAT-6 dengan berat molekul 6 kDa, merupakan protein antigenik yang disekresikan oleh kuman *M. tuberculosis.*

Ada banyak bagian protein kuman yang potensial imunogenik yang telah berhasil diidentifikasi. Beberapa protein antigenik yang terpenting di antaranya adalah ESAT-6, 38 kDa, hsp 65, hsp 70 dan complex Ag85.

ESAT-6 merupakan antigen penting yang dikenali oleh sel T protektif baik pada hewan coba maupun pada manusia. ESAT-6 ini disandi oleh gen RD-1 (*Region of difference*) yang terdapat pada *M. tuberculosis* dan *M. bovis* yang patogen, dan tidak terdapat pada *M. bovis BCG strain* vaksin yang avirulen. Bila gen yang menyandi ESAT-6 dimasukkan ke *M. bovis BCG* maka didapatkan peningkatan daya proteksi pada percobaan hewan yang mendapat *challenge* dengan kuman *M. tuberculosis.* Antigen ESAT-6 terdiri dari 95 asam amino dan mempunyai beberapa epitop di antaranya terdapat pada bagian N-terminus yang bersifat hidrofilik dan terpapar di bagian permukaan. Di bagian ini tentu ada satu atau lebih epitop yang reaktif dengan antibodi dalam sera subyek populasi Indonesia yang menderita TB paru aktif dan ada pula *overlapping common sequence* yang reaktif hanya pada sera perawat sehat yang merawat penderita TB.

Pemecahan masalah ini dilakukan dengan penelitian jenis observasional dengan rancangan *cross-sectional.* Variabel penelitian yang diperiksa adalah banyaknya frekuensi *overlapping common sequence* asam amino (epitop) dari residu asam amino 1-35 antigen ESAT-6 yang reaktif terhadap antibodi (IgG) dalam sera subyek penderita TB aktif, subyek kontrol sehat dan subyek perawat sehat. Rancangan analisis data dilakukan dengan uji Z.

Analisis dari residu asam amino 1-35 dari antigen ESAT-6 *M.tuberculosis* yang dipotong dengan panjang 9 mer, saling tumpang tindih 8 mer serta *offset* 1 mer, dan diuji reaktivitasnya dengan antibodi dalam sera ke tiga kelompok subyek penelitian menggunakan metode *B-cell epitope scanning.* Analisis dari hasil penelitian menunjukkan bahwa telah ditemukan dua jenis epitop. Yang pertama adalah epitop yang reaktif terhadap sera penderita TB paru aktif dan perawat sehat yang terpapar *M. tuberculosis,* dan berbeda bermakna dengan

sera kontrol sehat. Epitop ini disebut epitop infeksi TB berat yaitu IHSLLD (25-30). Jenis kedua adalah epitop protektif dengan *overlapping common sequence* asam amino (epitop) EAAASA (12-17) yang reaktif terhadap antibodi spesifik pada kelompok sera subyek perawat sehat yang terpapar *M. tuberculosis* dan hanya sedikit berbeda pada orang sehat dan penderita TB dengan perbedaan frekuensi yang bermakna.

Epitop protektif ini ditemukan *overlapping* dengan daerah yang merupakan epitop sel T pada populasi Jerman dan India, dan diperkirakan juga pada populasi Indonesia sehingga mungkin juga dapat menimbulkan respons imun seluler yang protektif.

Berpijak pada hasil analisis data yang diperoleh dalam penelitian ini dapat disimpulkan sebagai berikut.

1. Epitop infeksi TB berat IHSLLD (25-30) didapatkan baik pada penderita TB maupun pada perawat sehat yang terpapar *M. tuberculosis.*
2. Epitop protektif EAAASA (12-17) didapatkan hanya pada perawat sehat yang terpapar *M. tuberculosis.*

Beberapa hal yang disarankan untuk diteliti lebih lanjut adalah sebagai berikut :

1. Perlu diteliti tentang epitop sel-T pada manusia Indonesia yang khas pada antigen ESAT-6 *M. tuberculosis.*
2. Perlu diteliti epitop sel-B seperti pada penelitian ini dengan jumlah sampel yang lebih besar atau memeriksa regio yang lain dari urutan asam amino ESAT-6.
3. Perlu diteliti penggunaan uji diagnostik dengan menggunakan kedua epitop antigen ESAT-6 ialah epitop protektif dan epitop infeksi TB berat, pada populasi di Indonesia.
4. Perlu diteliti lebih lanjut tentang kemungkinan pengembangan epitop protektif *M. tuberculosis* untuk dijadikan vaksin DNA.

Saran tersebut di atas diperlukan untuk memecahkan beberapa permasalahan yang belum dapat dipecahkan dalam penelitian ini.

SUMMARY

The amino acid sequence of epitope from N-terminal region of ESAT-6 antigen as a diagnostik marker of active pulmonary tuberculosis.

Pulmonary TB is still a major health problem in Indonesia as well as in other countries world wide. Although BCG vaccine has succeded to decrease the prevalence of primary TB, it has not yet succeded to decrease the prevalence of post-primary TB.

In concodance with the advancement in the technology field, research in TB diagnostics and vaccination has been much improved. The genome sequence of *Mycobacterium tuberculosis* as causative agent for TB, has been completely determined so information about all genetic material of this microorganism can be obtained. ESAT-6 antigen with 6 kDa molecular weight is an antigenic protein which is secreted by *M. tuberculosis.*

There are many parts from this microorganism which are potentially immunogenic. Some important antigenic proteins are ESAT-6, 38 kDa, hsp65, hsp 70 and Ag85.

ESAT-6 as an important antigen, can be recoqnized by protective T-cells in experimental animals as well as humans. ESAT-6 is encoded by the RD-1 gene (Region of Difference) which is present in pathogenic *M. tuberculosis* and *M. bovis,* but is absent in the avirulent vaccine strain *M. bovis var.BCG.* By inserting gene encoding ESAT-6, an enhancement of protective immune response can be obtained in experimental animal models challenged with *M.tuberculosis.* ESAT antigen consists of 95 amino acids and has many epitopes, some of the epitopes are found in the N-terminal region which is hydrophilic and exposed to outer side. This N-terminal region may have one or more epitopes which are specifically reactive with antibody in sera from Indonesian active pulmonary TB patients, and epitopes which are specifically reactive with antibody in sera from healthy nurses working in the pulmonary disease ward.

This problem solving is done by an observational research approach with a *cross sectional design.* The research variable investigated is the frequency of amino acid overlapping common sequence (epitope) from amino acid residue 1-35 ESAT-6 antigen, which is specifically reactive with antibody (IgG) in sera of active pulmonary TB patients, healthy control and healthy nurse working in the pulmonary ward. Statistic analysis is performed using Z-test.

Amino acid residue 1-35 of antigen ESAT-6 is cut into 27 peptides, with 9-mer long, 8 mer overlapping and 1 mer offset. Reactivity with the sera from the 3 research groups was examined using B-cell epitope scanning methode. The analysis of research results revealed two kinds of epitopes. The first epitope is a heavy TB infection epitope with overlapping common sequence: IHSLLD (25-30). This epitope is specifically reactive with antibody found in pulmonary TB patients and pulmonary ward nurses. The second epitope is a protective epitope with overlapping common sequence EAAASA (12-17) which is reactive specifically with antibody found in the pulmonary ward nurses group.

This protective B-cell epitope is also overlapping with a part of T-cell epitope, found in the German and Indian population and suspected also of overlapping with T-cell epitope in the Indonesian population. Therefore this B-cell epitope can also elicit a protective cellular immune response.

Conclusion from result of data analysis is as follows:

1. Heavy TB infection epitope IHSLLD (25-30) is found to be reactive with TB patients group and pulmonary-ward nurse group.
2. Protective epitope EAAASA (12-17) is reactive only with the pulmonary ward nurse group.

Some suggestions to continue the investigation are as follows:

1. It is necessary to investigate specific T-cell epitope from ESAT-6 antigen found in the Indonesian population.
2. It is necessary to investigate another region of ESAT-6 with more samples to find other epitopes.
3. It is necessary to investigate the usefulness of diagnostic tools using the two epitopes of ESAT-6 antigen namely protective epitope and heavy TB infection epitope in the Indonesian population.
4. It is necessary to investigate the possible development of a DNA vaccine based on a protective epitope of *M. tuberculosis.*

The above suggestions are needed to solve some problems that cannot yet be accomplished by this research.

ABSTRACT

The amino acid sequence of epitope from N-terminal region of ESAT-6 antigen as a diagnostic marker of active pulmonary tuberculosis
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There is an urgent need for reliable diagnostic tools for patients exposed to *Mycobacterium tuberculosis* in the developing countries. The ESAT-6 is a secreted antigen which is absent in *M. bovis BCG* and many environmental mycobacteria. The ESAT-6 gene is located in the RD-1 region, which in not found in *M. bovis BCG* and environmental mycobacteria. There is potential use of this antigen as a diagnostic marker.

A study to find the specific B-cell epitope of N-terminal region of antigen ESAT-6 was carried out on 10 sera of active pulmonary TB patients, 10 sera of healthy individuals and 10 sera of healthy pulmonary nurses. For this purpose the amino acid sequence of residues 1 to 35 of the N-terminal region of ESAT-6 antigen was cut into a series of 27 peptides, 9 mer overlapping peptides with an overlapping of 8-mer and an offset of one amino acid. The series of 27 peptides were synthesized on the surface of polyethylene pins by Chiron Technologies, Clayton, Victoria, Australia, in the form of an epitope scanning kit, and screened using sera of the 30 subjects entered in this study using an indirect ELISA method.

The results ot the study revealed that 2 types of reactive overlapping common sequence of amino acids were present, as follows:

1. TB heavy infective epitope, corresponding to peptides with the amino acid sequence IHSLLD (residue 25 to 30).
2. TB protective epitope, corresponding to peptides with the amino acid sequence EAAASA (residue12 to17).

This study was also revealed that the TB infection marker was also reactive to the antibodies found in the healthy nurse group exposed to *M. tuberculosis*. Further more this TB infective epitope can still be used as a diagnostic tool using combination of two epitopes, namely TB infective and protective epitopes, by using immunochromatographic assay method.

The results are in concordance with those of other studies in that the response to ESAT-6 antigen was more intensive in individuals who were in close contact with TB patients, compared to those who were less in close contact. This findings may contribute to the application of reagents such as ESAT-6 in the diagnostic of tuberculosis in the enormous reservoir of latent human tuberculosis, particularly in developing countries.

Keywords : ESAT-6, B-cell epitope mapping, *M.tuberculosis*,diagnostic marker, protective marker.