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Expression profile of Rab5, Rab7, tryptophan aspartate-containing coat protein, leprae lipoarabinomannan, and phenolic glycolipid-1 on the failure of the phagolysosome process in macrophages of leprosy patients as a viability marker of *Mycobacterium leprae* ☆

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ABSTRACT

Objective/Background: Phagolysosome process in macrophage of leprosy patients' is important in the early phase of eliminating *Mycobacterium leprae* invasion. This study was to clarify the involvement of Rab5, Rab7, and trytophan aspartate-containing coat protein (TACO) from host macrophage and leprae lipoarabinomannan (Lep-LAM) and phenolic glycolipid-1 (PGL-1) from *M. leprae* cell wall as the reflection of phagolysosome process in relation to 16 subunit ribosomal RNA (16S rRNA) *M. leprae* as a marker of viability of *M. leprae*.

Methods: Using a cross sectional design study, skin biopsies were obtained from 47 newly diagnosed, untreated leprosy at Dr Soetomo Hospital, Surabaya, Indonesia. RNA isolation and complementary DNA synthesis were performed. Samples were divided into two groups: 16S rRNA *M. leprae*- positive and 16S rRNA *M. leprae*-negative. The expressions of Rab5, Rab7, TACO, Lep-LAM, and PGL-1 were assessed with an immunohistochemistry technique.

Result: Using Mann-Whitney *U* analysis, a significant difference in the expression profile of Rab5, Rab7, Lep-LAM, and PGL-1 was found ($p < .05$), but there was no significant difference of TACO between the two groups ($p > .05$). Spearman analysis revealed that there was a significant correlation between the score of Rab5, Rab7, Lep-LAM, and PGL-1 and the score of 16S rRNA *M. leprae* ($p < .05$).

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Conclusion: In *M. leprae* infection, Rab5, Rab7, and Lep-LAM play important roles in the failure of phagolysosome process via a membrane trafficking pathway, while PGL-1 plays a role via blocking lysosomal activities. These inventions might be used for the development of an early diagnostic device in the future.

Introduction

Leprosy is a chronic infection caused by *Mycobacterium leprae* infection that may cause damage to skin tissues, nerves, eyes, or systemic damage. If it continues, it may lead to disabilities with the impact of reduced quality of life of the individuals and society [1]. Indonesia is a country with the third highest number of leprosy patients in the world after India and Brazil, with the discovery of new cases increasing from year to year [2]. The most important cause of the high incidence rate and dominance of the multi-bacillary type of leprosy, which is potentially infectious in Indonesia, is the high transmission caused by the difficulty of early detection.

M. leprae cannot be cultured in an artificial medium, so the study of the bacteria is often difficult. Viability of *M. leprae* can be detected by morphological index of acid fast bacilli using Ziehl Nielsen staining. This examination has low sensitivity and specificity that affect the quality of viability detection. The detection of 16 subunit ribosomal RNA (16S rRNA) of *M. leprae* using the method of reverse transcriptase-polymerase chain reaction (RT-PCR) can be used as a marker of viability of *M. leprae* [3]. The 16S rRNA will be destroyed after *M. leprae* dies, thus the study of 16S rRNA using the RT-PCR method can reflect the viability of *M. leprae* with high sensitivity and specificity [4,5]. Although this method is relatively expensive and requires special facilities and infrastructure, it is relatively easier to perform than radiorespirometric methods [6–8].

When viable *M. leprae* in the body of an individual is present, theoretically the individual's phagocytic function has failed. Phagocytic function failure can occur due to the failure of the phagolysosome process so that *M. leprae* remains persistent. Phagolysosome process failure should be identified early because there are differences in the profiles of a variety of compounds derived from *M. leprae*, as well as from the host [9,10]. Based on the extrapolation of research that has been done with other mycobacteria, some proteins are related to the phagolysosome failure process and the most important is Rab5 that has been shown to play a role in early endosome fusion with the phagosome of *Mycobacterium tuberculosis* and *Mycobacterium bovis* bacille Calmette–Guerin (BCG). Rab7 has been shown to play a role in late endosome fusion with *Mycobacterium marinum* phagosome and *M. bovis* BCG. Tryptophan aspartate-containing coat protein (TACO), has been shown to play a role in resisting the movement of *M. bovis* BCG phagolysosome. These three proteins are present in the host's macrophage cell membrane [11–13]. In addition, the cell wall components of *M. leprae*, the leprae lipoarabinomannan (Lep-LAM) and phenolic glycolipid-1 (PGL-1), are also thought to inhibit the phagolysosome by extrapolating from the research performed on the cell wall of *M. tuberculosis* [14,15]. However, to date, there remains uncertainty in the expression profile of Rab5, Rab7, TACO, Lep-LAM, and PGL-1 related to the effectiveness of the phagolysosome process of *M. leprae* infection as reflected by the viability of *M. leprae*.

Knowing the mechanisms of the host macrophage response to *M. leprae* infection, particularly in the phagolysosome process, provides an opportunity to solve the problems of early detection of *M. leprae* viability. It has been reported that immunohistochemical examination has good prospects for early detection of leprosy, especially in endemic countries, with methods that can be done with simple laboratory equipment [16]. The rationale of immunohistochemical examination for the detection of *M. leprae* viability originates from facts showing that failed phagolysosome process causes *M. leprae* persistence.

The purpose of this study was to elaborate Rab5, Rab7, and TACO (host components) expression profiles and Lep-LAM and PGL-1 (cell wall components of *M. leprae*) as a reflection of the effectiveness processes of the phagolysosome associated with the presence or absence of 16S rRNA *M. leprae* as the marker of *M. leprae* viability.

Materials and methods

This study was an observational cross-sectional analytic study to prove that there are differences in the expression profile of Rab5, Rab7, TACO, Lep-LAM, and PGL-1 in macrophages of new leprosy patients based on the presence or absence of 16S rRNA *M. leprae* expression, as well as a correlation between 16S rRNA of *M. leprae* expression with the expression of Rab5, Rab7, TACO, Lep-LAM, and PGL-1 in new leprosy patients' macrophages.

The samples were taken from new leprosy patients over 3 months at the Dermatovenereology Clinic, Dr. Soetomo Hospital, Surabaya, Indonesia, who met the sample inclusion criteria. Leprosy diagnosis was made clinically and bacteriologically. The sample inclusion criteria were new leprosy (diagnostic criteria of World Health Organization), aged 15–40 years, and willing to join the study by signing an informed consent.

Examination of the 16S rRNA of *M. leprae* with real time PCR was performed to obtain more sensitive and specific results of the examination compared with conventional PCR [17]. To date, there have been no scientific publications on the primer used in the examination of the 16S rRNA of *M. leprae* with real time PCR. Therefore, the primer and probe design were done using the Primer Express Software program: Primer express software version 3.0 Applied Biosystems. At the P2–P3 (69–239 bp) region there were 50 combinations of