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Mini Review

The Role of Lipids in the Intracellular Parasitization of *Mycobacterium leprae*: Mini-Review

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ABSTRACT

Mycobacterium leprae (M. leprae), an obligate intracellular pathogen, is the causative agent of leprosy by parasitizing skin macrophages (histiocytes) in the dermis and Schwann cells in the peripheral nerves. Host cells hijacked by *M. leprae* accumulate large amounts of lipid droplets, which appear foamy in typical lepromatous leprosy tissue sections. In these cells, lipid synthesis is promoted and its degradation suppressed by changes in the gene expression profile. We have recently reported that *M. leprae* infection increases the accumulation of triacylglycerol in infected cells, which is important for the survival of bacilli. In this mini-review, we briefly summarize the mechanism of lipid accumulation in *M. leprae*-infected cells in relation to its intracellular parasitization and discuss the possibility of altering lipid metabolism as a novel therapeutic strategy for leprosy.

Keywords: Leprosy; *Mycobacterium leprae*; Lipid; Triacylglycerol; Macrophage

ABBREVIATIONS

M. leprae: *Mycobacterium leprae*; TAG: Triacylglycerol; GPAT3: Glycerol-3-Phosphate Acyltransferase 3; ADRP: Adipose Differentiation-Related Protein; HSL: Hormone-Sensitive Lipase; BCG: Bacille Calmette-Guerin; MDT: Multi-Drug Therapy; TLR2: Toll-Like Receptor 2; LDL: Low-Density Lipoprotein; ACSL: Acyl-CoA Synthetase Long-Chain; FABP4: Fatty Acid-Binding Protein 4; MOI: Multiplicity of Infection; HPTLC: High-Performance Thin-Layer Chromatography; PPAR: Peroxisome Proliferator-Activated Receptor; PGN: Peptidoglycan.

INTRODUCTION

Hansen's disease, or leprosy, is a chronic skin disorder caused by *Mycobacterium leprae* (*M. leprae*). The disease was documented in an Egyptian mummy from the first century C.E., and in 2019, about 200,000 new cases were reported from more than 100 countries worldwide [1]. *M. leprae* has a long incubation period, and sometimes the disease manifests more than 30 years after exposure, while others may not develop the disease in their lifetime [2]. After manifestation, the disease primarily affects the skin and peripheral nerves, as *M. leprae* mainly infects skin macrophages (histiocytes) and Schwann cells in peripheral nerves. Thus, in addition to skin lesions, *M. leprae* causes motor nerve damage that leads to disability and sensory nerve damage that results in numbness and analgesia, which allows repeated injuries and the possible loss of limbs.

Leprosy is classified into lepromatous leprosy and tuberculoid leprosy, according to the difference in the cell-mediated immune response against *M. leprae*. Lepromatous leprosy lacks cellular immunity and is a progressive, disseminated disease characterized by widespread skin lesions with numerous bacilli observable within the accumulated lipids of foamy histiocytes. Tuberculoid leprosy occurs when a strong cellular immune response forms granulomas in which it is difficult to demonstrate the presence of bacilli.

M. leprae CELL-WALL LIPIDS

Mycobacteria have a very thick cell wall that is composed of many unique lipids, which constitute approximately 60% of the cell-wall dry weight. It is covered with components such as arabinan, galactan, and peptidoglycan (PGN), which are the core cell-wall components, and phenolic glycolipids, which compose the capsules [3]. The recognition of *M. leprae* cell-wall lipids by host cells induces phagocytosis, the activation of immune responses, and phagosomal

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maturation (phagosome-lysosome fusion). To synthesize and maintain such complex and essential cell-wall lipids, mycobacteria have evolved several genes necessary for lipid metabolism [4]. The entire *M. leprae* genome was sequenced in 2001, which revealed missing functional genes as well as a high proportion of pseudogenes and non-coding regions [5]. Compared to *Mycobacterium tuberculosis* (*M. tuberculosis*), many genes that contribute to lipid metabolism, energy metabolism, and amino acid synthesis are absent from the *M. leprae* genome. Although *M. tuberculosis* has 66 genes involved in lipid metabolism, approximately 50% of the corresponding *M. leprae* genes were lost [5, 6]. This evidence makes it plausible to speculate that *M. leprae* has lost its ability to metabolize lipids for creating a cell wall or using them as an energy source. Therefore, *M. leprae* must largely rely on host-cell lipid synthesis for their survival. By this logic, *M. leprae* probably evolved to parasitize host cells and modify host-cell gene expression to create an intracellular environment suitable for maintaining their cell-wall lipids. This would also explain why efforts to grow *M. leprae* in culture media have been unsuccessful.

M. leprae INDUCES LIPID ACCUMULATION IN HOST CELLS

A diffuse infiltration of foamy histiocytes that contain *M. leprae* (as evidenced by acid-fast staining) is the characteristic, histological feature of skin lesions of lepromatous leprosy [7]. Macrophages infected with *M. leprae* show enlarged, lipid-filled phagosomes that contain many bacilli [7, 8]. Transmission electron microscopy also allowed the observation of an amorphous material indicative of accumulated lipid within the cytoplasmic vacuoles formed in Schwann cells by *M. leprae* infection [9].

Live *M. leprae* is rapidly taken up by human pre-monocytic THP-1 cells into phagosome, where they stay longer than heat-killed bacilli [10]. When live *M. leprae* is phagocytosed, gene expression profile of the host cell is significantly modified, whereas the effect of heat-killed bacilli is transient [8,11]. In addition, live *Mycobacterium bovis* Bacille Calmette-Guerin (*M. bovis* BCG) infection showed that bacilli-containing phagosomes resist lysosomal fusion, while heat-killed bacilli undergo lysosomal degradation [12]. Tryptophan aspartate-containing coat protein (TACO; also known as CORO1A or coronin-1), a phagosome coat protein, has been shown to inhibit phagosome maturation in *M. bovis* BCG infection [12]. We have observed in cultured macrophages that CORO1A was recruited from the plasma membrane to the phagosomal membrane following *M. leprae* infection and that CORO1A suppresses Toll-Like Receptor (TLR)-mediated innate immune activation [7, 13]. In accordance, we have shown that CORO1A localizes on phagosomal membranes containing *M. leprae* in tissue sections [7]. *M. leprae* activates host genes essential for lipid accumulation and maintenance, which induces significant lipid droplet formation [7,8,10,11,14]; however, clofazimine, a therapeutic agent for leprosy, suppresses these events [15]. This evidence suggests that the lipid droplets observed in *M. leprae*-infected cells are essential for successful infection and intracellular survival.

To clarify the effect of an *M. leprae* infection on the change in host-cell lipid composition, we examined the lipid components of *M. leprae*-infected THP-1 cells using High-Performance Thin-Layer Chromatography (HPTLC) optimized for separating neutral lipids. The results showed a sustained increase in triacylglycerol (TAG) levels upon *M. leprae* infection that was only transient when with dead *M. leprae* [10]. Other studies that used human Peripheral Blood Mononuclear Cells (PBMCs) and HPTLC showed an increase in cellular cholesterol levels upon *M. leprae* infection [9]. These results indicate that *M. leprae* induces lipid droplet formation in host cells and that these droplets are mainly composed of TAG and cholesterol.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS CONTRIBUTE TO LIPID DROPLET FORMATION IN *M. leprae*-INFECTED THP-1 CELLS

After infection, *M. leprae* induces dramatic changes in host gene expression [8,10,11,14]. Peroxisome Proliferator-Activated Receptors (PPARs), consisting of PPAR-α, PPAR-β/δ and PPAR-γ, regulate the expression of genes that are closely associated with lipogenesis and lipid metabolism. We showed that live *M. leprae* infection induced PPAR-δ and PPAR-γ expression in THP-1 cells in a multiplicity of infection (MOI)-dependent manner [14]; the PPARs then underwent nuclear translocation to perform their functions as transcription factors. Among others, PPAR-δ and PPAR-γ induced the genes that encode for CD36, which mediates macrophage uptake of oxidized Low-Density Lipoproteins (LDL); the Acyl-CoA Synthetase Long-Chain family (ACSL), which participates in *de novo* TAG synthesis from intracellular fatty acids; and Fatty Acid-Binding Protein 4 (FABP4), which contribute to foam-cell formation by transporting fatty acids [14]. These results suggest that *M. leprae* activates PPARs to regulate the expression of host genes that contribute to lipid droplet formation; however, the components of *M. leprae* that trigger these cellular responses are unknown.

ESSENTIAL ROLES OF TAG AND CHOLESTEROL IN *M. leprae* INFECTION

There are two intracellular TAG synthesis pathways: the glycerol phosphate pathway, which synthesizes TAG from a glycerol precursor, and the monoacylglycerol pathway, which reuses TAG metabolites [16]. Since Glycerol-3-Phosphate Acyltransferase (GPAT) is the rate-limiting enzyme for *de novo* TAG synthesis, we investigated the potential involvement of GPATs in *M. leprae*-infected THP-1 cells. Like TAG accumulation, the expression of only one GPAT (GPAT3) out of four was significantly increased in *M. leprae*-infected cells in a time- and MOI-dependent manner [10]. CRISPR-Cas9 genome editing of GPAT3 in THP-1 cells dramatically reduced the accumulation of TAG following an *M. leprae* infection [10]. In addition, it was also reported that *M. leprae* stimulates glucose uptake and activates the pentose phosphate pathway that contributes to TAG synthesis in Schwann cells [17]. These results suggest that the survival rate of *M. leprae* depends on the amount of TAG that accumulates in infected host cells.

M. leprae also induces the expression of Adipose Differentiation-Related Protein (ADRP) and perilipin, both of which reduce TAG hydrolysis, while decreasing the expression of Hormone-Sensitive Lipase (HSL), which contributes to TAG degradation [8,11]. However, PGN, a cell-wall component of *M. leprae*, suppresses the expression of ADRP and HSL. Since PGN is recognized by TLR2, which is on the cell surface of macrophages, we hypothesized that activating innate immunity may suppress lipid accumulation to eliminate *M. leprae* [7, 13].

An *in vivo* study reported that the skin lesions of lepromatous

leprosy contain more cholesterol compared to tuberculoid leprosy lesions [9]. *M. leprae*, however, has lost the mce4 operon that would enable it to use cholesterol as a direct nutrient source. To survive, *M. leprae* may convert cholesterol to cholestenone using an endogenous cholesterol oxidase (ML1492), an essential catabolic pathway for the *M. leprae* pathogenicity [9,18]. Furthermore, cholesterol synthase (HMG-CoA reductase) expression was higher in the skin lesions of lepromatous leprosy than tuberculoid leprosy, and a Fourier Transform ion Cyclotron Resonance Mass Spectrometry Analysis (FT-ICR MS) demonstrated an increase of cholesterol metabolites, i.e. (S)-squalene-2,3-epoxide, lanosterol, and zymosterol. In vitro experiments revealed that inhibiting *de novo* cholesterol synthesis by lovastatin reduced *M. leprae via*bility [9]. From these data, TAG and cholesterol seem to be essential for *M. leprae* survival within the host cell.

THE ROLE OF MYCOLIC ACID FOR MYCOBACTERIA

Mycolic acids, long-chain fatty acids, are the major constituents of the mycobacterial cell wall. Mycolic acid primarily exists in combination with trehalose, glucose, and glycerol; its subtypes have been identified as alpha-, keto-, and methoxy-mycolic acid in *M. tuberculosis*, while *M. leprae* has alpha- and keto-mycolic acids [19]. This structure confers important characteristics including the resistance to chemical injury and dehydration, lower antibiotic permeability, and the ability to survive within phagosomes [20]. *Mycobacterium smegmatis (M. smegmatis)* synthesis of mycolic acid uses an extension reaction from acyl-CoA *via* fatty acid synthase I and II (FASI and FASII) as well as a pathway that uses TAG-metabolized fatty acids [21]. The inhibition of TAG degradation by anhydrotetracycline significantly reduced mycolic acid biosynthesis, suggesting that *M. smegmatis* stores lipids that could serve as an intracellular fatty acid reservoir to biosynthesize complex lipids [21]. As we have reported, the TAG accumulated in *M. leprae*-infected macrophages contains a complex mixture of fatty acids and may be used to synthesize mycolic acid. Further studies are needed to examine whether *M. leprae* uses mycolic acid to escape from host-derived exclusion systems, thus allowing intracellular parasitization.

LIPID METABOLISM AS A POTENTIAL BIOMARKER FOR DISEASE PHENOTYPE AND TREATMENT EFFICACY

As an obligate intracellular pathogen, *M. leprae* is almost always found in lipid-filled phagosomes. We previously showed that mRNA levels of *ADRP*, *perilipin*, and *HSL* in slit-skin smear samples are significantly different between patients and change during Multi-Drug Therapy (MDT) [11,14]. We also reported that clofazimine, one of the drugs in MDT, modulates lipid metabolism in *M. leprae*-infected macrophages by modulating the levels of ADRP and HSL [15]. The metabonomic analysis of patient sera revealed significantly higher levels of potential fatty acid metabolites in lepromatous leprosy than tuberculoid leprosy, and MDT treatment reduced these metabolites in both groups [22]. Other studies similarly demonstrated that serum levels of cholesterol and TAG were significantly higher in lepromatous leprosy than tuberculoid leprosy [23]. These data suggest that monitoring TAG and its metabolites as well as the expression of lipid metabolism genes could provide useful indicators of disease phenotype, prognosis, and therapeutic efficacy.

CONCLUSION

The Global Leprosy Strategy 2021-2030 "Towards zero leprosy" was collaboratively developed by the World Health Organization during 2019 and 2020. Although the numbers of new cases in Southeast Asia, East Africa, and Brazil have declined, these areas still account for a large proportion of the new cases reported worldwide. The prevalence of obesity has also increased in these endemic regions over the last few decades. Since host-cell lipids seem to be essential factors for *M. leprae* survival, therapeutics that treats dyslipidemia might be effective against leprosy when combined with conventional MDT regimens. In addition, drugs to reduce cellular lipid might also be effective to treat household contacts with subclinical infection with *M. leprae* in dormant state, although antibiotics may not be fully effective in such cases. Further studies on lipid metabolism and its role in *M. leprae* infections are expected to investigate these methodologies.

AUTHOR CONTRIBUTIONS

Conceptualization, K.T.; writing-original draft preparation, K.T.; writing-review and editing, K.T., Y.H., A.K., M.K., Y.N., Y.F., Y.L., M.M., K.K., K.S.; supervision, K.S. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

CONSENT FOR PUBLICATION

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