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Essential role of hormone-sensitive lipase (HSL) in the maintenance of lipid storage in *Mycobacterium leprae*-infected macrophages

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ABSTRACT

Mycobacterium leprae (M. leprae), the causative agent of leprosy, parasitizes within the foamy or enlarged phagosome of macrophages where rich lipids accumulate. Although the mechanisms for lipid accumulation in the phagosome have been clarified, it is still unclear how such large amounts of lipids escape degradation. To further explore underlying mechanisms involved in lipid catabolism in *M. leprae*-infected host cells, we examined the expression of hormone-sensitive lipase (HSL), a key enzyme in fatty acid mobilization and lipolysis, in human macrophage THP-1 cells. We found that infection by live *M. leprae* significantly suppressed HSL expression levels. This suppression was not observed with dead *M. leprae* or latex beads. Macrophage activation by peptidoglycan (PGN), the ligand for toll-like receptor 2 (TLR2), increased HSL expression; however, live *M. leprae* suppressed this increase. HSL expression was abolished in the slit-skin smear specimens from patients with lepromatous and borderline leprosy. In addition, the recovery of HSL expression was observed in patients who experienced a lepra reaction, which is a cell-mediated, delayed-type hypersensitivity immune response, or in patients who were successfully treated with multi-drug therapy. These results suggest that *M. leprae* suppresses lipid degradation through inhibition of HSL expression, and that the monitoring of HSL mRNA levels in slit-skin smear specimens may be a useful indicator of patient prognosis.

1. Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* (*M. leprae*). Although its prevalence has declined over the last several decades due to the introduction of multi-drug therapy (MDT), leprosy still remains a major public health problem in many developing countries. In 2009, 244,796 new cases were registered worldwide [1]. *M. leprae* is a typical intracellular pathogen that parasitizes tissue macrophages (histiocytes) and Schwann cells of the peripheral nerves of the dermis. In 1966 Ridley and Jopling used clinical, histological and immunological criteria to classify leprosy patients across the spectrum, and suggested five member groups: Tuberculoid (TT), Borderline Tuberculoid (BT), Borderline (BB), Borderline Lepromatous (BL) and Lepromatous (LL) [2]. Lepromatous leprosy is a stable condition (patient status does not shift from these polar positions), while borderline lepromatous leprosy is immunologically unstable. Lepromatous leprosy is characterized by widespread skin lesions that form due to an impaired cellular immune response. The lesions consist of numerous bacilli that live in the foamy or enlarged lipid-filled phagosome within macrophages. Although lipid-laden macrophages are observed in other mycobacterial infections, including tuberculosis [3,4], the amount of lipid and the number of infected macrophages are most prominent in cases of lepromatous leprosy.

The PAT protein family is named after perilipin, adipophilin/adipose differentiation-related protein (ADRP) and the tail-interacting protein of 47 kDa (TIP47). Members of the PAT family are responsible for lipid transportation and lipid droplet formation in a variety of tissues and cultured cell lines, including adipocytes [5–8]. We previously reported that ADRP and perilipin play important roles in lipid accumulation in *M. leprae*-infected macrophages [9]. ADRP and perilipin localized to the phagosomal membrane of histiocytes, which contained numerous *M. leprae*, in the skin lesions of patients with lepromatous leprosy. *M. leprae* infection increased mRNA and protein expression of ADRP and perilipin in cultured human THP-1 monocytes. The results suggested that ADRP and perilipin contribute to the creation of

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a lipid-rich environment that is favorable for *M. leprae* parasitization and survival in the host.

However, accumulated lipids are supposed to undergo degradation and reutilization by cells over time. In fact, fatty acids mobilized from stored triacylglycerols (TAG) are a major energy source in humans. Mobilization occurs through the consecutive action of three lipases: adipose triglyceride lipase (ATGL), monoacylglycerol lipase (MGL) and hormone-sensitive lipase (HSL) [10]. Among these, HSL was the first enzyme identified in the induction of lipo-catabolic action initiated by hormones and is the predominant lipase effector of catecholamine-stimulated lipolysis in adipocytes [11]. Therefore, ADRP/perilipin and HSL have opposing functions, *i.e.* lipid accumulation vs. its degradation. In addition to adipocytes, HSL is expressed in the cytoplasm of macrophages, pancreatic β cells, skeletal muscle cells, steroid producing cells, the intestine, and spleen [10]. HSL serine residues are phosphorylated by enzymes such as protein kinase A (PKA), 5′ AMP-activated protein kinase (AMPK) and mitogen-activated protein kinase (MAPK) to regulate the process of hormone-induced lipolysis [11].

To date, the molecular mechanism(s) that allows the phagosome of *M. leprae*-infected macrophages to escape lipolytic activities is not known. In this study, we investigate the expression and phosphorylation of HSL in *M. leprae*-infected cultured macrophages. We also examine clinical samples from leprosy patients and explore the impact of *M. leprae* on lipid metabolism in infected host cells.

2. Materials and methods

2.1. M. leprae isolation and cell culture

Hypertensive nude rats (SHR/NCrj-*rnu*), in which the Thai53 strain of *M. leprae* was actively grown [12,13], were kindly provided by Dr. Y. Yogi of the Leprosy Research Center, National Institute of Infectious Diseases, Japan. *M. leprae* was isolated as previously described [14,15]. The human premonocytic cell line THP-1 was obtained from the American Type Culture Collection (ATCC; Manassas, VA). The cells were cultured in 10 cm tissue culture dishes in RPMI medium supplemented with 10% charcoal-treated fetal bovine serum (FBS), 2% non-essential amino acids and 50 mg/ml penicillin/streptomycin at 37 °C in 5% CO_2 [9,16]. Typically, 3×10^7 bacilli were added to 3×10^6 THP-1 cells, for a multiplicity of infection (MOI) of 10. Peptidoglycan (PGN) and lipopolysaccharide (LPS) were purchased from Sigma (St Louis, MO) and added at final concentrations of 2 μg/ml and 1 μg/ml, respectively. TLR2 antibody (sc-21759; Santa Cruz Biotechnology, Santa Cruz, CA) was used at a final concentration of 5 μg/ml.

2.2. Immunohistochemistry and lipid staining

THP-1 cells were grown on glass coverslips in 24-well plates for 24 h before the culture medium was exchanged with RPMI 1640 containing *M. leprae*. Control and *M. leprae*-infected THP-1 cells were fixed in 10% paraformaldehyde for 10 min. They were then washed with Dulbecco's phosphate buffered saline (DPBS) containing 0.4% Triton-X 100 (DPBST), incubated with anti-HSL antibody (Cell Signaling Technology, Danvers, MA) diluted to 1:100 for 24 h at 4 °C and washed again with DPBST. The signal was detected using peroxidase-labeled streptavidin-biotin (LSAB2 Kit; DAKO, Carpinteria, CA) and 3,3-diaminobenzidine tetrahydrochloride (DAB) [9]. Cells were counterstained with methylene blue. Lipid staining was performed with oil red O (Muto Pure Chemicals, Tokyo, Japan) for 10 min, and counterstained with hematoxylin for another 5 min.

2.3. RNA preparation and RT-PCR

RNA from cultured cells was prepared using RNeasy Mini Kits (Qiagen Inc., Valencia, CA) as described previously [9,16]. RNA preparation from slit-skin smear samples was performed as described [9]. Briefly, stainless steel blades (Feather Safety Razor Co., LTD, Osaka, Japan) used to obtain slit-skin smear specimens were rinsed in 1 ml of sterile 70% ethanol, then the tube was and centrifuged at 20,000 × g for 1 min at 4 °C. After removing the supernatant, RNA was purified with the same protocol used for cultured cells. RNA was eluted in 20 μl of elution buffer and treated with 0.1 U/μl of DNase I (TaKaRa Bio, Kyoto, Japan) at 37 °C for 60 min in order to degrade any contaminating genomic DNA. RNA concentration and purity were assessed using a NanoVue spectrophotometer (GE Healthcare, Little Chalfont, UK). Total RNA from each sample was reverse-transcribed to cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) [9]. The following primers were used to amplify specific cDNAs: HSL: 5′-CTCCTCATGGCTCAACTCCTTCC-3′ (forward) and 5′-AGGGGTTCTTGACTATGGGTG-3′ (reverse); ADRP: 5′-TGTGGAGAAGACCAAGTCTGTG-3′ (forward) and 5′-GCTTCTGAACCAGATCAAATCC-3′ (reverse); and actin: 5′-AGCCATGTACGTAGCCATCC-3′ (forward) and 5′-TGTGGTGGTGAAGCTGTAGC-3′ (reverse). Touchdown PCR was performed using a PCR thermal cycler DICE (TaKaRa Bio) as previously described [9]. The products were analyzed by 2% agarose gel electrophoresis.

Slit-skin smear samples from leprosy patients were used according to the guidelines approved by the National Institute of Infectious Diseases, Tokyo, Japan.

2.4. Protein preparation and Western blot analysis

Cellular protein was extracted and analyzed as previously described [9,17]. Briefly, cells were lysed in a lysis buffer containing 50 mM HEPES, 150 mM NaCl, 5 mM EDTA, 0.1% NP40, 20% glycerol, and protease inhibitor cocktail (Complete Mini, Roche, Indianapolis, IN) for 1 h. After centrifugation, the supernatant was transferred and 10 μg of protein was used for analysis. Samples were heated in SDS sample loading buffer at 95 °C for 5 min and loaded on a polyacrylamide gel. After electrophoresis, proteins were transferred to a PVDF membrane using a semi-dry blotting apparatus (Bio-Rad, Hercules, CA). The membrane was washed with PBST (PBS with 0.1% Tween 20), blocked in blocking buffer (PBST containing 5% nonfat milk) overnight, and then incubated with either anti-HSL, anti-phospho-HSL (Ser^{563}) or anti-phospho-HSL (Ser^{565}) antibody (Cell Signaling Technology, 1:2000 dilution). After washing with PBST, the membrane was incubated for 1 h with biotinylated donkey anti-rabbit antibody (GE Healthcare, 1:2000 dilution) and streptavidin-HRP (GE Healthcare, 1:10,000 dilution) according to the manufacturer's protocol. The signal was developed using ECL Plus Reagent (GE Healthcare).

3. Results

3.1. HSL expression is suppressed in macrophages infected with M. leprae

To confirm the possible relationship between lipid accumulation and HSL expression in macrophage, we infected *M. leprae* in THP-1 cells and performed oil red O staining and HSL and ADRP immunostaining. Lipid droplets were not evident in control THP-1 cells (Fig. 1A), but accumulation was clearly demonstrated in cells 24 h after *M. leprae* infection (Fig. 1B). ADRP expression, which contributes to lipid intake, was not evident in control cells, but was significantly increased following *M. leprae* infection as previously reported (Fig. 1C and D, respectively) [9]. Conversely, HSL expression