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Original article

Mycobacterium tuberculosis persistence in various adipose depots of infected mice and the effect of anti-tubercular therapy

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Abstract

The adipocytes are one of the non-professional phagocytes postulated to be a haven for *Mycobacterium tuberculosis* during persistence in the human host. The adipocyte – *M. tuberculosis* interaction data available to date are ex vivo. The present study was primarily aimed to investigate *M. tuberculosis* infection of adipocytes in course of infection of mouse model. Using primary murine adipocytes, the study first confirmed the infection and immunomodulation of natural adipocytes by *M. tuberculosis.* The bacilli could be isolated form visceral, subcutaneous, peri renal and mesenteric adipose depots of immunocompetent mice infected with *M. tuberculosis* intravenously. The bacilli could be isolated from adipocytes and the stromal vascular fraction, even though the numbers were significantly higher in the latter. The bacterial burden in the adipose depots was comparable to those in lungs in the early phase of infection. But with time, the burden in the adipose depots was either decreased or kept under control, despite the increasing burden in the lungs. Infected mice treated with standard anti tubercular drugs, despite effective elimination of bacterial loads in the lungs, continued to harbour *M. tuberculosis* in adipose depots at loads similar to untreated mice in the late infection phase.
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Keywords: Mycobacterium tuberculosis; Adipocytes; Persistence; Mouse adipose depots

1. Introduction

Mycobacterium tuberculosis is a successful pathogen because of its ability to persist in the host in the absence of clinical disease-a condition termed latent tuberculosis. One third of the world's population is infected with *M. tuberculosis* [1]. Among the infected, a mere 10% develop active disease [2], while the remaining 90% continue to have a latent infection for their life time. The estimted risk of reactivation TB in the latently infected individuals is between 2 and 23% per life time [3], which becomes 10% per year in presence of HIV co-infection [4]. *M. tuberculosis* population retained in the system of latent TB individuals could be called persisters as it continues to survive despite an active host immune system. The term is also used to denote the bacterial population in active tuberculosis that necessitates an extended chemotherapy to prevent relapse [5]. However, even after a successful therapy, reactivation can happen later in life up on immunosuppression [6], which is an indication of drug tolerant persisters.

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The physiological form and anatomical location of persisting *M. tuberculosis* is a topic of debate and active research [7]. It is widely believed that persisters exist as non-replicating dormant forms, induced primarily by hypoxic conditions in the lung granulomas [8]. The bacilli are thought to reach extrapulmonary locations through lympho-haematogenous spread, and can reactivate in latently infected individuals under immunosuppression [9–11]. Unexpected locations like healed fibrotic lesions in the lung and as well as extrapulmonary locations have been revisited in the recent times. In a study published in the year 2000, *M. tuberculosis* DNA was detected in type II pneumocytes, endothelial cells and fibroblasts in apparently healthy location in lungs [12]. Hence the possibility of *M. tuberculosis* persisting in the non-immune cells in and away from the lungs cannot be excluded. Thus, a few years later, the adipose tissue was proposed a niche for *M. tuberculosis* for long term persistence [13]. In a recent study, molecular evidence of viable *M. tuberculosis* was detected in the spleen, liver and kidney of latent TB individuals, while *M. tuberculosis* DNA was detected in the endothelium, Bowman's parietal cells and proximal convoluted tubules of the kidney [14]. Another proposed niche for dormant *M. tuberculosis* is the bone marrow mesenchymal stem cell [15].

Among the various proposed non-professional phagocytes, adipocyte is the most interesting candidate because of its primary physiological role-long term fat storage, that can be advantageous to any dormant microbe surviving on a lipid diet. In addition to the abundant visceral and subcutaneous depots, adipose depots occur throughout the body, associated to lungs, heart, kidneys, bone marrow and the adventitia of major blood vessels [16]. Lymph nodes are always embedded in adipose tissue [17], and *M. tuberculosis* disseminating to the lymph nodes may get access to the adipocytes. In the very first report, *M. tuberculosis* H37Rv was demonstrated to infect 3T3L1 and primary human adipocytes through scavenger receptor, and persist as phenotypically drug tolerant forms [13]. In addition, *M. tuberculosis* presence was detected in different fat depots of active and latent TB individuals using conventional PCR, in situ PCR and/or immune staining.

In the present study, we studied interactions of *M. tuberculosis* H37Rv with murine primary adipocytes and the role of adipose depots in *M. tuberculosis* persistence using the mouse model.

2. Materials and methods

2.1. Mycobacteria

Fluorescent *M. tuberculosis* H37Rv was constructed by introducing the Turbo RFP602 gene (Evrogen, Moscow, Russia) codon optimized for mycobacteria cloned into pMV261 (kind gift from Prof. WR Jacobs Jr., Albert Einstein College of Medicine, NY). The fluorescent and wild type strains were cultured in Middlebrook 7H9 broth supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC) (BD, Sparks, MD) and 0.05% tween 80 with and with out kanamycin (50 µg/ml) respectively.

2.2. Murine primary adipocyte culture

2.2.1. Differentiation and culture of murine predipocytes

Isolation of stromal vascular cells and their differentiation were done as described elsewhere [18]. Inguinal fat pads were collected from 3 weeks old Swiss mice, were digested in buffer (1 ml per 100 mg tissue) containing 1 mg/ml collagenase (C1764, Sigma, St. Louis) as described. The stromal vascular cells in complete culture medium [DMEM/F12 + 10% fetal bovine serum + antibiotic-antimycotic solution] was plated at a concentration of 5×10^4 cells/ml/well in 12-well plate and incubated at 37 °C and 5% CO_2. Upon reaching confluency, the cells were induced to differentiate by adding 5 µg/ml insulin, 0.5 mM isobuthyl-methylxanthine and 1 µM dexamethasone. After 48 h induction, cells were maintained in medium containing 5 µg/ml insulin until 80–90% cells accumulated lipid droplets (~9 days post induction).

2.2.2. Culture of mature adipocytes from mice

Mature adipocytes from the inguinal fat pads of mice were directly harvested and cultured by ceiling culture method described by Sugihara et al. [19].

2.3. Infection of adipocytes

Approximately 1×10^5 adipocytes on coverslips/wells were infected with *M. tuberculosis* H37Rv^{RFP} at a multiplicity of infection (MOI) 1:1 for 18 h. The adipocyte number was determined by performing a cell count of stromal vascular cells at near 100% confluency prior to differentiation. Extracellular bacilli were removed by extensive washing. Adipocytes on coverslip were stained with Lipid-TOX (Invitrogen) and mounted with Fluoroshield with DAPI (Sigma, St. Louis). Infected cells on wells were lysed at different time points with 0.1% triton X- 100, and serial dilutions of lysates were plated on kanamycin (50 µg/ml) containing Middlebrook 7H10 agar + 10% OADC plates to estimate CFUs.

2.4. Cytokine/adipokine estimation

Primary adipocytes were treated with live or heat killed *M. tuberculosis* H37Rv (1:1 MOI) or 100 ng/ml LPS. The supernatants collected after 24 h, were centrifuged at 9168 g for 5 min and stored at −70 °C till various assays were performed. IL-6, TNF-α, MCP-1 and IL-10 were estimated using respective Mouse CBA Flex kits (BD) and further analysis in a FACS Calibur machine (BD). Leptin and adiponectin were estimated by ELISA using Mouse Leptin DuoSet and Mouse Adiponectin/Acrp30 DuoSet (R&D systems) respectively.

2.5. Staining of adipocyte derived M. tuberculosis

Infected adipocytes in 12-well plates were lysed at intervals up to day 7 of infection. Lysates of same time point were pooled and centrifuged at 9168 g for 25 min. The pellet containing bacteria was resuspended in 200 µl of PBS

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