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Original article

Effect of *Mycobacterium tuberculosis* infection on adipocyte physiology

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Abstract

Tuberculosis (TB) remains as a major threat to human health worldwide despite of the availability of standardized antibiotic therapy. One of the characteristic of pathogenic *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis is its ability to persist in the host in a dormant state and develop latent infection without clinical signs of active disease. However, the mechanisms involved in bacterial persistence and the establishment of latency is not well understood. Adipose tissue is emerging as an important niche that favors actively replicating as well as dormant Mtb during acute and latent infection. This also suggests that Mtb can disseminate from the lungs to adipose tissue during aerosol infection and/or from adipose tissue to lungs during reactivation of latent infection. In this study, we report the interplay between key adipokine levels and the dynamics of Mtb pathogenesis in the lungs and adipose tissue using a rabbit model of pulmonary infection with two clinical isolates that produce divergent outcome in disease progression. Results show that markers of adipocyte physiology and function were significantly altered during Mtb infection and distinct patterns of adipokine expression were noted between adipose tissue and the lungs. Moreover, these markers were differentially expressed between active disease and latent infection. Thus, this study highlights the importance of targeting adipocyte function as potential target for developing better TB intervention strategies.

Keywords: Tuberculosis; Latency; Adipose tissue; Adiponectin; Inflammation

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is a top infectious disease worldwide. According to the World Health Organization (WHO, 2016), 10.4 million people contracted TB (new cases) and 1.4 million died from the disease in 2015 [1]. About one-third of the world's population has latent TB (LTBI), which is asymptomatic and non-communicable [2]. Nevertheless, in these individuals the pathogen is alive and over 10% of this population ultimately develop active and communicable TB. Reactivation occurs under conditions of immune system compromise, which explains why individuals infected with HIV and those with type 2 diabetes (T2DM) [3–5] are at exceptionally high risk for reactivation [6–8]. The WHO estimates that by 2030 there will be a substantial proportion of TB reactivation cases attributable to HIV or T2DM comorbidity [5]. Therefore, understanding the mechanisms involved in the pathogenesis of TB reactivation is of prime importance to control TB reactivation.

Mtb has shown a remarkable ability to persist in the infected host in a non/semi-replicating dormant stage [9]. Recent studies suggest that the dormant bacteria most likely exist in host cells at both pulmonary and extra-pulmonary sites [10]. Adipose tissue, a nutritionally rich organ, provides a suitable environment for dormant Mtb [11–13]. Many

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pathogens, including Mtb and *Trypanosoma cruzi,* Rickettsia, HIV and SIV utilize adipose tissue as a reservoir for their survival [10−17]. Recent findings have shown the presence of Mtb in various adipose tissue depots during acute and chronic phases of Mtb aerosol infection [10−12] suggesting that Mtb disseminates from lungs to distant adipose depots. Similar to the observations made in *T. cruzi* infection, Mtb can disseminate to the lungs from adipose depots [10−15].

Adipose tissue is not only a storage site for triglycerides, but also acts as an endocrine organ contributing to energy homeostasis, inflammation and immune response to infection. It constitutes 15−25% of the total body mass and is broadly distributed throughout the body [18,19]. Adipose tissue is composed of various cell types including fibroblasts, endothelial cells, leukocytes, skeletal, and smooth muscle cells in addition to adipocytes [15]. Mtb infection and persistence may have a dynamic effect on adipose tissue physiology and pathology which regulate metabolic and energy homeostasis [18,19].

We have established a rabbit model of pulmonary Mtb infection using clinical strains HN878 and CDC1551 that mimic most of the pathological features in humans with active TB or latent infection (LTBI) [20]. Although the rabbit model is more expensive and has stringent regulatory and facility requirements compared to mouse and guinea pig models, it is an excellent animal model to study host−pathogen interactions during LTBI and active disease. Aerosol infection of rabbits with the hypervirulent clinical Mtb isolate HN878 leads to progressive, active pulmonary TB marked with elevated bacterial growth, inflammation and formation of granulomas that undergo central necrosis, caseation/liquefaction; some of these granulomas ultimately develop cavitation [21]. In contrast, pulmonary infection of rabbits with the hyperimmunogenic clinical Mtb isolate CDC1551 results in protracted bacillary growth in the lungs early during infection that is controlled effectively upon the onset of adaptive immunity, resulting in significant reduction in bacillary load until no viable bacteria could be cultured from the lung homogenates. The kinetics of bacillary growth is consistent with the loss of disease pathology in the lungs [22]. Thus, infection with CDC1551 results in non-progressive latent infection (LTBI) in rabbits. Importantly, upon immune suppression treatment, these rabbits can reactive bacillary growth and disease pathology in the lungs [22].

Here, we investigated the effect of Mtb infection on the key adipokine levels using our rabbit model of pulmonary active TB and LTBI to elucidate a link between adipose tissue physiology and the lung pathology during TB infection. We hypothesize that adipokine levels are differentially altered in LTBI and active TB, which distinctly affect respective lung TB pathogenesis.

2. Materials and methods

2.1. Ethics statement

All rabbit procedures were performed in accordance with Animal Welfare Act guidelines and approved by the Institutional Animal Care and Use and Institutional Biosafety Committees of Rutgers University.

2.2. Bacteria and chemicals

Mtb CDC1551 and HN878 were obtained from Dr. Shinnick at the Centers for Disease Control and Prevention (CDC), Atlanta, GA and Dr. Musser at Houston Methodist, TX, respectively. Stock Mtb cultures were prepared by growing the bacilli in Middlebrook 7H9 medium with supplements (BD, Sparks, MD) as described earlier and banked frozen at −80 °C [23]. To prepare bacterial inoculum for infection, frozen cultures were thawed just before use as previously described [23]. All chemicals were obtained from Sigma−Aldrich (St Louis, MO), unless otherwise mentioned.

2.3. Aerosol infection of rabbits

Female New Zealand White rabbits (Covance Inc, MI, USA) weighing between 2.2 and 2.5 kg (n = 16) were exposed to HN878 or CDC1551 aerosols, as described [22]. Uninfected rabbits served as controls (n = 4). Briefly, rabbits were exposed to Mtb-containing aerosols using a nose-only delivery system (CH Technologies, NJ). At 3 h after exposure, a group (n = 4) of rabbits was euthanized, and serial dilutions of the lung homogenates were cultured on Middlebrook 7H11 (Difco BD, Franklin Lakes, NJ) agar plates to enumerate the number of initial (time = 0) bacterial CFUs implanted in the lungs. At 12 weeks after infection (p.i.), groups of infected rabbits (n = 4) were euthanized and lung and visceral white adipose tissues (WAT) were harvested for protein analysis and total RNA isolation. As expected, we observed a significant decrease in the body weight of HN878-infected, compared CDC 1551-infected, rabbits at this time point (data not shown).

2.4. Mtb infection of cultured adipocytes

3T3-L1 murine preadipocytes purchased from Zen-bio inc., were propagated and differentiated to adipocytes in culture plates as previously described [24]. Cells were used between day 8 and 12 post-induction of differentiation. The cultured adipocytes were infected by Mtb (HN878 or CDC1551) at a multiplicity of infection of 3:1 (bacteria:adipocyte) for 48 h as described previously [25].

2.5. Preparation of cell and tissue protein lysates

After 48 h post infection, the adipocytes were washed four times with phosphate buffered saline (pH 7.4) and lysed in 1 ml lysis buffer containing 50 mmol/l Tris pH 7.5, 1% NP-40, and 150 mmol/l sodium chloride plus protease inhibitor cocktail. Adipose tissues were homogenized in TNET buffer lacking Triton X-100 (150 mm NaCl, 5 mm EDTA, 50 mm Tris−HCl, pH 7.5), supplemented with complete protease inhibitor cocktail and phosphatase inhibitor cocktail (Thermofisher). This was followed by low-speed centrifugation

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