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Chlamydia pneumoniae exploits adipocyte lipid chaperone FABP4 to facilitate fat mobilization and intracellular growth in murine adipocytes

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ABSTRACT

Fatty acid-binding protein 4 (FABP4), a cytosolic lipid chaperone predominantly expressed in adipocytes and macrophages, modulates lipid fluxes, trafficking, signaling, and metabolism. Recent studies have demonstrated that FABP4 regulates metabolic and inflammatory pathways, and in mouse models its inhibition can improve type 2 diabetes mellitus and atherosclerosis. However, the role of FABP4 in bacterial infection, metabolic crosstalk between host and pathogen, and bacterial pathogenesis have not been studied. As an obligate intracellular pathogen, *Chlamydia pneumoniae* needs to obtain nutrients such as ATP and lipids from host cells. Here, we show that *C. pneumoniae* successfully infects and proliferates in murine adipocytes by inducing hormone sensitive lipase (HSL)-mediated lipolysis. Chemical inhibition or genetic manipulation of HSL significantly abrogated the intracellular growth of *C. pneumoniae* in adipocytes. Liberated free fatty acids were utilized to generate ATP via β-oxidation, which *C. pneumoniae* usurped for its replication. Strikingly, chemical inhibition or genetic silencing of FABP4 significantly abrogated *C. pneumoniae* infection-induced lipolysis and mobilization of liberated FFAs, resulting in reduced bacterial growth in adipocytes. Collectively, these results demonstrate that *C. pneumoniae* exploits host FABP4 to facilitate fat mobilization and intracellular replication in adipocytes. This work uncovers a novel strategy used by intracellular pathogens for acquiring energy via hijacking of the host lipid metabolism pathway.

1. Introduction

Adipocyte fatty acid-binding protein (FABP4, also known as aP2/A-FABP) is an intracellular lipid chaperone abundantly expressed in adipocytes and macrophages [1]. By binding with long-chain fatty acids, FABP4 can affect the uptake, transportation, esterification, and β-oxidation of fatty acids and regulate the energy balance and lipid signal transduction within cells [1,2]. Evidence suggests that FABP4 is central to the generation of meta-inflammation, especially upon exposure to lipids, and couples lipotoxicity to organelle function and endoplasmic reticulum (ER) stress; furthermore, FABP4 inhibition improved type 2 diabetes mellitus and atherosclerosis in mouse models [3–7]. In humans, elevated circulating FABP4 levels are associated with obesity and metabolic diseases [8,9]. Thus, targeting FABP4 offers a novel therapeutic approach for the treatment of many metabolic diseases [10]. However, the roles of FABP4 in bacterial infection, metabolic crosstalk between host and pathogen have not been studied.

Chlamydia pneumoniae is an obligate intracellular pathogen that needs to obtain nutrients, such as nucleotides (ATP), amino acids, and lipids, from host cells for its replication [11,12]. To meet their bioenergetic and biosynthetic demands for replication, intracellular pathogens modulate host metabolism. However, how the pathogen

Abbreviations: ATGL, adipose triglyceride lipase; HSL, hormone sensitive lipase; MGL, monoglyceride lipase; FABP4, fatty acid binding protein 4; FFAs, free fatty acids; LD, lipid droplet; CPT-1, carnitine palmitoyltransferase 1; FAO, fatty acid oxidation; OCR, oxygen consumption rate.

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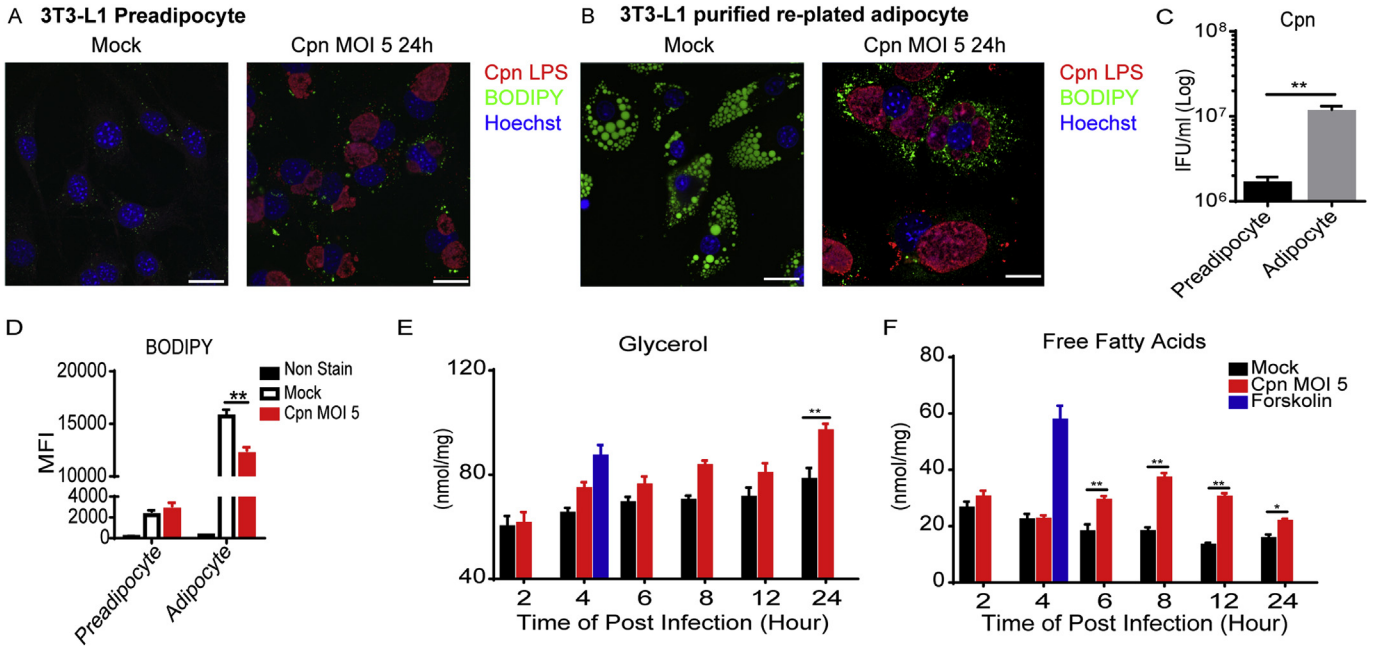

Fig. 1. *C. pneumoniae* infection induces lipolysis in murine adipocytes. 3T3-L1 pre-adipocytes (A) or 3T3-L1 adipocytes (B) were mock-infected or infected with *C. pneumoniae* (Cpn) MOI 5 for 24 h and examined for chlamydial inclusions (red), lipid droplets (green), and DNA binding (blue). Scale bar: 20 μm. (C) The number of infectious elementary body (EB) progeny of 3T3-L1 pre-adipocytes or adipocytes at 24 h after Cpn infection was determined using an inclusion forming unit (IFU) assay. (D) Mean fluorescent intensity (MFI) of BODIPY in 3T3-L1 pre-adipocyte or adipocytes after mock or Cpn infection at 24 h. Secretion of glycerol (E) and free fatty acids (F) were measured in the cultured medium of 3T3-L1 adipocytes after mock or Cpn infection for 2–24 h. A 4-h incubation with forskolin (20 μM) served as the positive control for lipolysis. n = 3 per group (C–F). $^{*}p < 0.05$, $^{**}p < 0.01$ by Student *t*-test (C) or two-way ANOVA (D–F).

modulates host metabolism to meet its bioenergetic demands for growth is largely unknown. Adipose tissues may provide a niche or reservoir for intracellular pathogens like *Mycobacterium tuberculosis* [13], *Rickettsia prowazekii* [14], and *Trypanosoma cruzi* [15]. Adipocytes may provide a suitable host for obligate intracellular pathogens, which require various nutrients or energy source from host cells [16]. It has been demonstrated that *C. pneumoniae* can infect murine pre- and post-differentiated adipocytes and, through a TNF-α mediated inflammatory mechanism, can impair differentiation and insulin signaling [17]. However, how *C. pneumoniae* proliferates in adipocytes and how the abundance of lipid stores in adipocytes may favor its survival, growth, and persistence remains unexplored topics.

Herein, we demonstrate that *C. pneumoniae* successfully infects mature adipocytes and induces hormone-sensitive lipase (HSL)-mediated lipolysis. In addition, we demonstrate that liberated free fatty acids (FFAs) undergo β-oxidation and generate ATP in host adipocytes, which *C. pneumoniae* usurps for its intracellular propagation. Collectively, our data demonstrate that *C. pneumoniae* reprograms lipid metabolism by hijacking FABP4 for its own benefit and sheds new light on the etiological link between *C. pneumoniae* infection and metabolic syndrome.

2. Materials and methods

2.1. Microbes

C. pneumoniae (strain AR39, ATCC53592) and *C. muridarum* (mouse pneumonitis strain Nigg II, ATCC VR-123) were obtained from the ATCC and propagated as previously described [18].

2.2. 3T3-L1 adipocyte differentiation

3T3-L1 preadipocytes (ATCC CL-173) or stable shRNA-knockdown (control EGFP, HSL) preadipocytes were seeded in 24-well plates and allowed to reach confluence for 2 days. On day 0, adipocyte differentiation was induced by adding 2.5 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylexanthine (IBMX), and 10 μg/mL insulin. On day 2 and thereafter, the medium was replaced with DMEM-10% FCS containing only 10 μg/mL insulin.

2.3. In vitro infection of adipocytes

Cultured adipocytes were infected at days 4–6 of differentiation. To initiate infection, *C. pneumoniae or C. muridarum* was added to plates at MOI = 5, and plates were centrifuged at 900 × *g* and 25 °C for 1 h. Next, the inoculum was removed, and cells were cultured in DMEM containing 10% FCS and streptomycin (100 μg/mL). Inhibitors were added 60 min after inoculation with *C. pneumoniae or C. muridarum*.

2.4. IFU assay

Infected cells were collected, frozen and thawed, serially diluted 10-fold in SPG medium, and reseeded into 24-well plates containing a HeLa-cell monolayer. After centrifugation at 900 × *g* and 25 °C for 1 h, and cells were cultured in DMEM containing 10% FCS and 1 μg/mL cycloheximide. After 24–48 h, cells were fixed with ice-cold methanol for 10 min and stained with a FITC-conjugated anti-*Chlamydia* LPS-specific monoclonal antibody (PROGEN).

2.5. Immunofluorescence

Chlamydia-infected preadipocytes or adipocytes were fixed with 4% paraformaldehyde, blocked and permeabilized with 1% BSA and 1% Triton X-100 in PBS for 10 min at RT and incubated with avidin/biotin blocking buffer (abcam). Next, the cells were incubated with primary antibodies against biotin conjugated anti-chlamydial LPS

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