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Original article

Chlamydia pneumoniae induces macrophage-derived foam cell formation by up-regulating acyl-coenzyme A: cholesterol acyltransferase 1

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Abstract

In macrophages, the accumulation of cholesteryl esters synthesized by acyl-coenzyme A: cholesterol acyltransferase 1(ACAT1) plays a crucial role in foam cell formation, a hallmark of early atherosclerotic lesions. It is suggested that *Chlamydia pneumoniae* (*C. pneumoniae*) induces foam cell formation. However, the mechanism of foam cell formation induced by *C. pneumoniae* has not been fully elucidated. In this study, we found that *C. pneumoniae* increased the expression of acyl-coenzyme A: cholesterol acyltransferase 1(ACAT1) mRNA and protein in a dose-dependent manner in THP-1-derived macrophages exposed to low density lipoprotein (LDL). In addition, *C. pneumoniae* dose-dependently suppressed the expression of peroxisome proliferator-activated receptor gamma (PPAR γ) mRNA and protein. Rosiglitazone, a specific PPAR γ agonist, not only dose-dependently alleviated the down-regulation of PPAR γ expression by *C. pneumoniae* infection, but also dose-dependently inhibited the *C. pneumoniae*-induced ACAT1 expression. Furthermore, higher doses of rosiglitazone (10 and 20 μM) suppressed the *C. pneumoniae*-induced foam cell formation from morphological (Oil red O staining) and biochemical (zymochemistry method) criteria. These results first demonstrate that *C. pneumoniae* induces macrophage-derived foam cell formation by up-regulating ACAT1 expression via PPAR γ-dependent pathway, which may contribute to its pro-atherogenic properties.

Keywords: *Chlamydia pneumoniae*; Acyl-coenzyme A: cholesterol acyltransferase 1; Foam cells; Peroxisome proliferator-activated receptor gamma; Atherosclerosis

1. Introduction

In recent decades, there are increasing evidences suggest that infection may be as a novel risk factor for atherosclerosis [1,2]. Various infectious agents have been found in atherosclerotic lesions, and of them, *Chlamydia pneumoniae* (*C. pneumoniae*) has received the most attention [2−4]. *C. pneumoniae* is an obligate intracellular gram-negative pathogen that causes bronchitis, pneumonia, and other respiratory tract disease. *C. pneumoniae* may be transported to the endothelial surface in arterial wall via circulating monocytes or infected macrophages from the respiratory tract, which may contribute to endothelial dysfunction and product a series of pro-inflammatory effects [5,6]. Amounts of epidemiological and clinical studies suggest that *C. pneumoniae* infection aggravates the progression of atherosclerotic lesions [6−8]. Kalayoglu et al. [9,10] have shown that *C. pneumoniae* infection induces human and murine macrophage foam cell formation, partly by stimulating low density lipoprotein (LDL) oxidization and entry, indicating this organism as a causative agent in atherosclerosis.

The formation of cholesteryl esters (CE)-laden foam cells in the arterial wall is a key event in early atherosclerosis. Acyl-coenzyme A: cholesterol acyltransferase (ACAT) is the key and exclusive intracellular microsomal enzyme that catalyses the formation of CE in a variety of tissues [11]. Two ACAT1 genes (ACAT1 and ACAT2) have been identified in mammals [12]. In macrophages, ACAT1 esterifies intracellular excess free cholesterol (FC) and stores lipid droplets form of CE, which plays a vital role in foam cell formation [11−14]. In

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vitro studies have shown that the expression and activity of ACAT1 are increased in the transformation of macrophages into foam cells [14,15]. In vivo studies have demonstrated that ACAT1 expression is abundant in macrophage foam cells of atherosclerotic lesions [13,16].

C. pneumoniae has been reported to induce the expression of peroxisome proliferator-activated receptor gamma (PPAR γ) in human vascular smooth muscle cells [17]. PPAR γ, a ligand-activated nuclear receptor abundant in adipocytes and macrophages, plays an important role in adipocyte differentiation, lipids storage and glucose homeostasis [18,19]. Recent studies reveal that the physiological role of PPAR γ in regulating the expression of key genes involved in macrophage cholesterol metabolism [19,20]. Our earlier study has demonstrated that activation of PPAR γ in macrophages inhibits the expression of ACAT1 [21]. Although ACAT1 and PPAR γ have been shown to be involved in foam cell formation, the roles of ACAT1 and PPAR γ in *C. pneumoniae*-induced foam cell formation have not been fully investigated. In this study, we examined the roles of ACAT1 and PPAR γ in THP-1 macrophage-derived foam cell formation induced by *C. pneumoniae* infection.

2. Materials and methods

2.1. Reagents and antibodies

Human monocytic THP-1 cells and epithelial HEp-2 cells were obtained from Wuhan University, China. *C. pneumoniae* strain AR-39 was purchased from American Type Culture Collection (ATCC, Rockville, MA). RPMI1640 powder and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY). Phorbol 12-myristate 13-acetate (PMA), cycloheximide and rosiglitazone were purchased from Sigma (St. Louis, MO, USA). RT-PCR kit was purchased from TOYOBO (Japan). Fluorescein isothiocyanate (FITC)-conjugated specific anti-chlamydial monoclonal antibody was obtained from Dako (Copenhagen, Denmark). Rabbit polyclonal anti-ACAT1 was obtained from Cayman (USA). Mouse polyclonal anti-PPAR γ, Rabbit polyclonal anti-β-actin, horseredish peroxidase (HRP)-conjugated secondary antibodies were obtained from Santa Cruz (CA, USA).

2.2. Propagation of C. pneumoniae

C. pneumoniae strain AR-39 was propagated in HEp-2 cells by centrifugation-driven infection as described [22]. Infected HEp cells were harvested after incubation for 72 h at 37 °C and 5% CO_2, and disrupted by freezing, thawing and ultrasonication. After centrifugation at 500 × g and 4 °C for 30 min to remove cell debris, the supernatants containing elementary bodies (EBs) were harvested and stored at −70 °C. Chlamydial inclusion forming units (IFU) were determined by counting chlamydial inclusions formed in HEp-2 cells with FITC-conjugated specific anti-chlamydial antibody.

2.3. Low density lipoprotein (LDL) isolation and assessment

Human LDL ($d = 1.006–1.063$ g/mL) was isolated from blood of healthy volunteers by density-gradient ultracentrifugation as described [23]. The isolated LDL was dialyzed in phosphate buffered saline (PBS) for 24 h, and then was condensed with polyoxyl 20,000. The concentration of LDL was measured by the Bradford assay, sterile filtered (0.22 μm) and stored for no longer than 2 weeks in the dark at 4 °C before use. Analysis of LDL on a 4–20% SDS-PAGE gradient gel and Sudan black B staining revealed a single protein.

2.4. Infection and culture of THP-1 cells

THP-1 monocytic cells were seeded into six-well plates at 1×10^6 cells per well in RPMI1640 medium containing 10% fetal bovine serum (FBS) and were differentiated into macrophages by the addition of 160 nM phorbol 12-myristate 13-acetate (PMA) for 48 h. In the presence of LDL (50 mg/L), THP-1-derived macrophages were infected with or without increasing doses of *C. pneumoniae* ($1 \times 10^5–1 \times 10^6$ IFU) for 48 h. In some experiments, pretreatment with the presence or absence of the specific PPAR γ agonist rosiglitazone (1–20 μM) for 2 h, THP-1-derived macrophages were infected or uninfected with *C. pneumoniae* (1×10^6 IFU) in the presence of LDL (50 mg/L) for additional 48 h.

2.5. Oil red O staining

Cells were washed twice with PBS, fixed with 4% paraformaldehyde for 15 min, and stained with 1% Oil red O for 10 min, then after stained with hematoxylin for 3 min. Cells were washed three times with PBS and examined by light microscopy. The intracellular lipid droplets were stained red, and cell nuclei were stained blue. Foam cells of morphological criterion were defined as cells that have ten Oil red O-positive lipid droplets or more (≥10) under a microscope [24].

2.6. Intracellular cholesterol measurement

Cells were harvested and disrupted by ultrasonication. The contents of intracellular total cholesterol (TC) and free cholesterol (FC) were detected by zymochemistry method as described [25]. The contents of TC and FC were determined by the standard curve (1–10 μg/mL standard cholesterol) and expressed as microgram cholesterol per mildigram of protein (μg/mg). The contents of cholesteryl ester (CE) were calculated by subtracting that of free cholesterol (FC) from the total cholesterol (TC). The ratio of CE to TC (≥50%) was defined as foam cells of biochemical criterion [26].

2.7. RT-PCR analysis

Total RNA was extracted by Biozol reagent kit, according to the manufacture's protocol. RT-PCR reactions were carried out in 25 μL volumes containing 1.5 μg RNA. The ACAT1 primers of