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Review

Cytosolic lipid inclusions formed during infection by viral and bacterial pathogens

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Abstract

Lipid inclusions play an important role in several pathological processes. Intracellular bacterial pathogens, such as members of the *Mycobacterium* and *Chlamydia* species are able to trigger the formation of lipid-laden foamy macrophages. Lipid droplet accumulation in the host constitutes a reservoir used by the bacilli for long-term persistence. Viruses need lipid droplets as assembly platform. We present the current knowledge about structural, functional and regulatory aspects of lipid inclusions.

Keywords: Lipid droplets; Lipid body; Diacylglycerol acyltransferase; Latent tuberculosis; Triacylglycerols (TAG); *Mycobacterium tuberculosis*

1. Introduction

Lipid inclusions in cells have been described since the 19th century and were also designated as lipid droplets, lipid bodies, fat bodies, fat droplets, and adiposomes. In plants, they are often called oil bodies. Lipid inclusions are present in virtually all organisms, including plants, yeast, prokaryotes and animal cells. Lipid inclusions have been seen largely as non-structured inert fat particles, which have been largely ignored by many scientists. For a long time they were only regarded as simple passive storage depots for fats in energy homeostasis and for membrane and lipid hormone precursors [1]. However, more recently, lipid inclusions have been recognized as functionally active organelles [2]. They play an important role in the development and progression of atherosclerosis [3], for virus replication and recognition and as nutrient source for intracellular pathogens [4].

The formation of lipid inclusions during infection occurs in the host as well as in the pathogen, such as reported for *Mycobacterium tuberculosis* infection [5]. It has become apparent that pathogens induce the accumulation of lipids in the host cells and use them as energy and carbon source. This review summarizes the current understanding of the interplay between lipid accumulation of the pathogen and its host. Subsequently, we will focus on the lipid metabolism of *M. tuberculosis*, the causative agent of tuberculosis, but we will also highlight the important role of host lipid droplets in virus replication. Unfortunately, there is no uniform naming system for the discrimination of lipid inclusions in the host and the pathogen. In this review we will use the term “lipid droplets” for lipid-rich inclusions in the host and “lipid bodies” for lipid-rich inclusions in the pathogen.

2. Biogenesis of lipid inclusions in bacteria and eukaryotes

The molecular mechanisms of lipid inclusion formation remain still somehow elusive. The current models of lipid droplet biogenesis are still hypothetical and have been reviewed extensively by Murphy in 1999 and Ohsaki in 2009 [6,7]. The most common model supposes that the membrane protein diacyltransferase DGAT1 produces triacylglycerols

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(TAG), which accumulate between the two membrane leaflets of the endoplasmic reticulum (ER) to be finally released by budding. The lipids are covered by a phospholipid monolayer from the ER membrane.

The formation of lipid bodies in bacteria has been even less characterized. Wältermann et al. suggested in 2005 that a bifunctional wax ester synthase/acyl-CoA:diacylglycerol acyltransferase (WS/DGAT) synthesizes TAG for lipid body formation. WS/DGAT is an integral membrane protein and synthesizes a growing globule around the cytoplasmic portion of the enzyme. Finally the lipid body is released to the cytoplasm. The origin of the surface phospholipid monolayer is not known [6,8].

3. Lipid droplets in the host

Lipid droplets (LDs) are intracellular organelles that store neutral lipids within cells. They regulate the storage and hydrolysis of TAG and/or cholesterol esters. Probably all cell types are able to store fat in LDs, but LDs are usually highly enriched in specialized cell types such as the mammalian adipocytes, where they are the major reservoir of TAG in the body.

The accumulation of lipid droplets occurs also in several infectious, and inflammatory conditions, including in atherosclerosis [3], bacterial sepsis [9], viral infections [10], and in mycobacterial infections [11–13]. LDs are observed in various cells of the immune system including macrophages, neutrophils, and eosinophils. LDs can vary greatly in size, from sub-μm to 200 μm in diameter. The structure and composition of LDs is highly conserved. They contain a core of neutral lipid esters typically TAG, but also sterols and sterol esters [1,14]. The surface is covered by a phospholipid monolayer, which is composed at least in some cells by unique fatty acids [15].

4. Lipid bodies in the pathogen

Prokaryotes do not generally produce lipid bodies containing TAG. Accumulation of TAG in intracellular lipid-bodies is a property of only a few bacteria, mostly belonging to the actinomycetes group [16]. Anyway most prokaryotes produce inclusion bodies, but they generally accumulate polymeric lipids such as poly(3-hydroxybutyrate) (PHB) or other polyhydroxyalkanoates (PHA) [8,17].

M. tuberculosis and *Mycobacterium leprae* accumulate considerable amounts of TAG during infection [8,18]. Already in the 1940s to 1960s, the formation of lipid bodies had been described in various species of the genus *Mycobacterium* but no further investigations were performed [19]. *M. tuberculosis* is an intracellular pathogen that can survive up to decades in a phenotypically non-replicating dormant state, primarily in hypoxic granulomas in the lung [20]. The otherwise drug-susceptible dormant mycobacteria develop drug resistance within the granulomas of the host. These nonreplicative drug-resistant bacteria within the host's tissues are called persisters [21].

It has been observed that persisters store large amounts of intracellular triacylglycerol lipid bodies (LBs) [11,12,22–24]. *M. tuberculosis* uses TAG from the lipid bodies as energy and carbon source under conditions such as starvation [25], oxygen depletion [26], and pathogen reactivation [27]. The observation that sputum from tuberculosis patients contains lipid body-laden bacilli, proves the importance of lipids for the survival of the bacterium in the host [24].

5. *M. tuberculosis*-induced lipid biogenesis in macrophages

M. tuberculosis is an intracellular pathogen and infects primarily alveolar macrophages, which reside within alveoli. The infected macrophage leaves the alveoli and migrates then toward the next lung draining lymph node. *M. tuberculosis* inhibits the generation of the phagolysosome and the bacteria begin to multiply within the macrophage [28]. The host's immune response to *M. tuberculosis* seems to be unable to clear the bacillus from the infected macrophages. But anyway the infected macrophage induces a strong immune response by producing TNF-α and chemokines that recruits systemic monocytes. The macrophages start to enlarge and accumulate TAG in lipid droplets. These lipid-filled foamy macrophages (FM) are surrounded by an outer layer of lymphocytes. Within the foamy macrophages the bacteria resist in phagosomes, packed with lipid droplets. Enclosed by lipid droplets the bacteria establish a state of dormancy and induced drug resistance. These persisters are nearly bullet-proof to the host's immune response. Finally the host's immune system and the pathogen reach an equilibrium state, where the infected macrophages are surrounded by additional differentiated macrophages, T lymphocytes, B lymphocytes, dendritic cells, neutrophils, fibroblasts and an extracellular matrix [13,29]. The aggregate of immune cells is called a granuloma, which is the hallmark of tuberculosis infection [13,30]. The induction of foamy macrophages seems to be a general host response upon infection with several other intracellular pathogens such as *M. leprae*, *Chlamydia* [31], and *Toxoplasma* [32].

The development and composition of a human tuberculosis granuloma is depicted in Fig. 1.

5.1. M. tuberculosis *produces lipid bodies in the foamy macrophage*

Induction of foamy macrophages appears to be a key event in both sustaining persistent bacteria and contribution, that leads to cavitation and release of infectious bacilli [11].

After being taken up into macrophage phagosomes the bacteria are located in the close vicinity of cellular lipid drops. *M. tuberculosis*-containing phagosomes engulf cellular lipid droplets and finally the bacteria are completely enclosed by cellular lipid droplets. Only within the lipid droplets the bacteria form lipid bodies and cell replication comes to a halt and finally the bacteria enter the state of dormancy [5,12]. When the pathogen goes into the nonreplicative state several bacterial genes involved in lipid metabolism such as