

# Mycobacteria and the Greasy Macrophage: Getting Fat and Frustrated

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**In the current issue of *Infection and Immunity*, Caire-Brändli and coworkers (Infect. Immun. 82:476–490, 2014, doi:10.1128/IAI.01196-13) describe a novel cell system for studying mycobacterial interactions with foamy macrophages and provide a magnificent series of electron microscopy-based observations providing major insight into the microbiology and cell biology of these interactions.**

With nearly 9 million new cases and 1.3 million deaths each year, as indicated by the World Health Organization (WHO) in its 2013 global report (1), tuberculosis (TB) remains a leading cause of mortality due to a single bacterial pathogen, namely *Mycobacterium tuberculosis*. The development of new tools for controlling, and possibly even eradicating TB, such as new antimicrobial drugs, a more effective vaccine than *Mycobacterium bovis* BCG, and innovative host-targeted strategies (2), is hampered primarily by the incomplete nature of our understanding of the molecular and cellular aspects of host-pathogen interactions in TB.

Pulmonary macrophages are the primary host cells for the TB bacillus (3, 4). *M. tuberculosis* thrives in these cells during the first few weeks of infection, until adaptive immunity kicks in, helping the body to control the proliferation of the pathogen through cytokine-mediated macrophage activation and other mechanisms (5). The continued recruitment of T and B lymphocytes to the site of infection and the concomitant death of infected macrophages leads to the formation of a complex structure with a well-organized architecture, the granuloma, a hallmark of TB (6–8). *M. tuberculosis* can persist for decades within the granuloma, in a dormant state referred to as “latency.” It has been estimated that up to 2 billion individuals may carry such latent infection and that the disease will reactivate in about 10% of these individuals, at some point in their lifetime.

One of the most striking features of the TB granuloma is the presence around the lesion of a specific population of histiocytes/macrophages enriched in lipid droplets (LD) and known as foamy macrophages (9, 10). Foamy macrophages have been observed in a number of inflammatory conditions, including infectious and noninfectious diseases, such as natural and experimental TB in particular (11–16). *M. tuberculosis* slows its multiplication rate in foamy macrophages and accumulates LD in its own cytoplasm. It has been suggested that foamy macrophages (i) constitute a reservoir of nutrients for the pathogen during latency, supplying carbon and energy, in particular, via various lipids, including triacylglycerol (TAG) (9, 10), and (ii) participate in the formation of the caseous center of the granuloma after the death of the macrophages, releasing their lipid content (10, 17). The mechanisms by which mycobacteria induce foam cell differentiation and by which LD accumulate in the bacterial cytoplasm within infected cells remain interesting areas of investigation. In particular, the way in which the intracellular mycobacterial vacuole, or phagosome, interacts with the macrophage LD, and the roles of such interactions in mycobacterial latency, are particularly fascinating issues that

have yet to be resolved. However, such investigations are largely hampered by the lack of a reliable, reproducible, and reversible cellular model of mycobacterium-infected foamy macrophages.

In the current issue of *Infection and Immunity*, Caire-Brändli and coworkers (18) describe a novel cell system for studying mycobacterial interactions with foamy macrophages and provide a magnificent series of electron microscopy-based observations providing major insight into the microbiology and cell biology of these interactions. Caire-Brändli and coworkers used mouse bone marrow-derived macrophages treated with purified very-low-density lipoproteins (VLDL) to trigger the differentiation of the macrophages into foamy macrophages. One of the key features of this cell system is the reversible nature of the foamy phenotype following VLDL removal. Using this system, the authors report the following. (i) The mycobacteria within the foamy macrophages accumulate large LD, referred to as intracytoplasmic lipid inclusions (ILI), within their own cytoplasm and stop dividing. The reversion of the foamy phenotype of the host cells upon VLDL removal leads to the progressive depletion of ILI and the resumption of bacterial growth. (ii) ILI within bacteria consist largely of TAG, rather than cholesterol. (iii) Lipid processing by host cell lysosomal lipases is a prerequisite for ILI formation within intracellular mycobacteria. (iv) Most importantly, the mycobacterial phagosome can fuse with the host cell LD, delivering the TAG content of the LD to the lumen of the phagosome. This system provides a sound, reliable model for studying mycobacterial interactions with foamy macrophages and opens up a series of new avenues of research for the development of novel strategies for either killing mycobacteria or keeping them in the dormant state.

The biology of LD formation in macrophages and other cell types in a number of infectious and noninfectious inflammatory settings has been reviewed extensively (15, 19, 20). In their study (18), Caire-Brändli and coworkers made use of the well-known ability of LDL to induce foam cell differentiation (21). Several microbial compounds have been reported to induce differentia-

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tion of foamy macrophages. In the context of mycobacterial infection, the pathogen is known to reshape the host cell transcriptional landscape profoundly (22), reprogramming its lipid metabolism in particular (17, 23). LD biogenesis in macrophages during mycobacterial infection has been reported to be dependent on Toll-like receptor 2 (TLR2) and TLR6 (24, 25), to require the peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) and testicular receptor 4 (TR4) transcription factors (26), and to be dependent upon the mycobacterium-mediated inhibition of hormone-sensitive lipase (HSL) (27). Mycobacterial molecules, mostly derived from the pathogen envelope, such as lipoarabinomannan, mycolic acids, and the secreted virulence factor ESAT-6 (*M. tuberculosis* early secreted antigenic target of 6 kDa), have been shown to trigger the formation of foamy macrophages *in vitro* (9, 24, 28, 29). A unified view of the process of LD formation in mycobacterium-infected cells is still lacking, but it seems likely that, in addition to direct cell interactions with bacteria or bacterial products, it involves bystander effects between infected and uninfected cells, mediated by cytokines and other immune mediators for example (13, 30) and/or mycobacterial lipids released from the infected cells by exocytosis (31–33). Further studies are required to improve our understanding of this process.

Within foamy macrophages, the mycobacteria accumulate large ILI and stop dividing (9). ILI formation in mycobacteria *in vitro* was first reported decades ago (34–36) and has also been observed in bacilli expectorated in sputum samples from patients with TB (37, 38). In prokaryotes, ILI are composed of several lipids, including TAG, and several topological models have been proposed for their biogenesis (39). In their article (18), Caire-Brändli and coworkers report that ILI formation in intracellular mycobacteria is dependent on host cell lipases. They also found that the number of bacteria (in terms of CFU) remained stable during macrophage treatment with VLDL but that the bacteria continued to elongate, reaching lengths up to eight times their normal size. This observation is important, because it indicates that mycobacterial metabolism remains active within the foamy macrophages, despite frustrated bacterial cell division. When the VLDL was removed, the ILI gradually disappeared, and mycobacterial growth resumed, mirroring reactivation from latency.

The presence of bacterial ILI in infected LD-enriched foamy macrophages raises questions about the physical and functional links between the two lipid structures. One of the most striking findings reported by Caire-Brändli and coworkers (18), illustrated in their article by a highly convincing electron microscopy-based observation, is that the mycobacterial phagosome can readily fuse with host cell LD, delivering the content of the LD to the phagosome. This finding is reminiscent of previous results obtained in my laboratory, in which mycobacteria were shown to persist in a vegetative state within human adipocytes and to accumulate ILI within these cells; we also observed fusion-like events between the mycobacterial vacuole and adipocyte LD (40). These observations raise several questions about possible fusion mechanisms. The prevailing consensus is that LD originate from the endoplasmic reticulum (ER), in which diacylglycerol *o*-acyltransferases (DGAT1 and DGAT2) produce TAG, which accumulates between the two membrane leaflets of the ER before being released after budding (15). LD are thus formed as a lipid core surrounded by a phospholipid monolayer, and the topology of fusion events between this monolayer structure and the phospholipid bilayer comprising the phagosomal membrane remain unclear. It will

also be important to decipher the molecular machinery involved in phagosomal fusion with LD. A number of proteins have been detected in eukaryotic LD, including Rab GTPases, such as the early endosomal Rab5, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, and ADP-ribosylation factor (ARF)-related proteins (13, 15, 41). This is consistent with the general notion that LD are dynamic platforms involved in protein trafficking and prone to fusion/fission events with other cellular organelles, rather than passive lipid storage structures. It remains to be determined whether and how LD-associated proteins participate in phagosome-LD fusion.

The observations of Caire-Brändli and coworkers (18) also demonstrate that the mycobacteria within foamy macrophages have direct access to the TAG content of the host cell LD, potentially metabolizing this TAG to generate their own TAG-enriched ILI. This might explain previous observations of direct transfers of host cell fatty acids from LD to ILI within mycobacterium-infected foamy macrophages and the almost identical TAG compositions of these two entities (42). Mycobacteria are unlikely to take up TAG directly from the surrounding environment. The most plausible scenario would therefore appear to be that host TAG are first hydrolyzed by secreted or surface-exposed lipases, such as the secreted lipase LipY, to generate fatty acids (43), which are then imported into the bacterial cytoplasm and used for the *de novo* synthesis of bacterial TAG by TAG synthases, such as Tgs1 (44). ILI probably provide a unique source of carbon and energy for bacterial metabolism during latency and reactivation, and it has been reported that mycobacterial reactivation from hypoxia-induced dormancy involves TAG hydrolysis by lipases (45). The *M. tuberculosis* genome contains more than 20 lipase/esterase-encoding genes (46); identification of the enzymes involved in mycobacterial latency and reactivation in foamy macrophages will open up fascinating new avenues of research, potentially leading to the development of novel antimicrobial drugs.

Collectively, the data reported by Caire-Brändli and coworkers (18), and the simple and easily controllable cell model they have established, provide us with a fantastic opportunity to investigate further the interactions of mycobacteria with foamy macrophages and, more generally, to improve our understanding of the cellular and molecular mechanisms at work during mycobacterial latency and reactivation, with the ultimate aim of developing novel intervention tools to control TB.

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