Cholesterol acquisition by *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (Mtb), the aetiological agent of Human Tuberculosis (TB), is an intracellular pathogen. According to the World Heath Organisation, 1.8 billion people are infected and approximately 1.3 million people die from Tuberculosis annually. The global prevalence of TB is sustained by the ongoing HIV-AIDS pandemic and the alarming increase of antibiotic resistant Mtb isolates(1). As a successful intracellular pathogen targeting macrophages, nutrient acquisition is a key feature to establish infection within the phagosome, a nutritionally-constrained environment (2, 3), and in this issue of Virulence, Ramón-Garcia et al demonstrate that the P55 efflux pump is required for optimal mycobacterial growth on cholesterol(4).

Most bacteria are subject to catabolite repression which is a widespread regulatory trait by which bacteria maximize growth by consuming individual carbon substrates in a preferred sequence. However Mtb does not follow the same rules. de Carvalho et al demonstrated that Mtb has the ability to co-catabolize different carbon sources such acetate, dextrose and glycerol, and that each component carbon source has a distinct metabolic fate, leading to enhanced monophasic growth(5). Effectively, by using a combination of stable isotope labelling and state-of-art liquid-chromatography accurate mass-spectrometry, de Carvalho et al demonstrated that during growth on carbon sources mixtures, Mtb metabolized each component carbon source to a distinct metabolic fate. For example, during growth on an equimolar mixture of dextrose and acetate, Mtb preferentially metabolized dextrose into glycolytic and pentose phosphate pathway intermediates while directing acetate into intermediates of the tricarboxylic acid cycle (TCA cycle). Appelberg et al noted that intracellular pathogens like Mtb must acquire nutrients while surrounded by host-derived lipids(6), and it has been proposed that they may be utilized as carbon source(7). Several lines of evidence suggest that pathogenic mycobacteria primarily use fatty acids rather than carbohydrates as carbon substrates during infection(8). It is important to stress that cholesterol is a major structural component of animal cell membranes. Host cholesterol is thought to be involved in the development of Mtb infection(9), with a high level in the diet shown to significantly enhance bacterial burden in the lung(10) and impair immunity to Mtb(11). Further work indicates that cholesterol is not required for establishing infection but rather appears to be essential for persistence in the lungs and for growth within IFN-γ activated macrophages. Cholesterol acquisition and metabolism has been the topic of several investigations. The *mce4* operon (mammalian cell entry 4

transport system), is crucial for virulence in animal models and cholesterol uptake(12). The *mce4* locus is one of four homologous regions in Mtb genome and consists of several genes predicted to encode a multi-subunit ABC-like transporter system(13). Senaratne et al demonstrated that *mce4*-mutants were attenuated in mice(12). Pandey and Sassetti performed a synthetic lethality screen and identified a number of genes located near the *mce4* operon that appeared to function in concert with this transporter(13), demonstrating that deletion of the *mce4* operon led to a marked growth defect when cholesterol was sole carbon source, as well as severely reduced accumulation of cholesterol compared to the wild-type(14). In addition, cholesterol is not only taken up by Mtb, but also catabolised. Pandey and Sassetti provides evidence of cholesterol degradation by monitoring from both 4- and 26-carbons of the molecule, with C-4 converted to CO_2 and C-26 becoming incorporated to membrane lipids, including a major virulence-associated lipid, phthiocerol dimycocerosate(14, 15). This last finding has been supported by Griffin et al who describe that metabolic alterations observed during cholesterol catabolism are centred on propionyl-CoA and pyruvate pools(16), leading to transcriptional regulation of propionyl-CoA-assimilating methylcitrate cycle enzymes *via* Rv1129c regulatory proteins. The growth defect of methylcitrate cycle mutants is largely attributable to degradation of host-derived cholesterol(16). More recently, using an unbiased chemical screen to identify chemical compounds that inhibit Mtb metabolism within macrophages VanderVen et al isolated a compound that inhibits PrpC (Rv1131), the 2-methylcitrate synthase, which is required for assimilation of cholesterol-derived propionyl-CoA into the TCA cycle(17).

In this issue of Virulence, Ramón-Garcia et al demonstrate the requirement for the mycobacterial P55 efflux pump for optimal growth on cholesterol(4). Previous work by Joshi et al reported that the gene encoding the P55 efflux pump (*rv1410c*) interacted positively with *mce1* and *mce4* gene clusters *in vivo*(13). This suggested a potential shared function for this efflux pump. P55 is a mycobacterial efflux pump belonging to the Major Facilitator Superfamily (MFS) of membrane transporters required for growth, maintenance of cellular redox balance, and export of some antibiotics, including first-line antibiotics(18, 19). It is important to stress that P55 is part of an operon with the LprG lipoprotein, encoded by the gene rv1411c. Bigi et al reported that deletion of the lprG-rv1410 operon leads to a strong attenuation of Mtb in mice, suggesting a crucial role for these two genes in Mtb virulence(20). Gaur et al confirmed and defined that LprG is essential for Mtb virulence via mediating surface exposition of lipoarabinomannan (LAM)(21). LAM is critical for several important immunological process such as cell entry, inhibition of phagosome-lysosome fusion, and intracellular survival(22).

Altogether, this article provides the first evidence for an efflux pump being involved in cholesterol uptake. However, defining the mechanism how P55 interacts synergistically with mce4 will provide a better understanding of this complex and essential process, new routes for antibiotics development and a better understanding of antimicrobial resistance.

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