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Review Article

Role of cholesterol in *Mycobacterium tuberculosis* infection

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Mycobacterium tuberculosis (MTB) acquisition and utilization of nutrients within the host cell is poorly understood, although it has been hypothesized that host lipids probably play an important role in MTB survival. Cholesterol has recently been identified as an important lipid for mycobacterial infection. The *mce4* transport system is required for cholesterol import into bacterial cells, and deletion of *mce4* locus resulted in severe attenuation in a chronic mouse model of infection. However, it has remained unclear what additional bacterial functions were required for utilization of this sterol. We have found that the *igr* locus, which was previously found essential for intracellular growth and virulence of MTB, is required for cholesterol metabolism: *igr*-deficient bacteria cannot grow using cholesterol as a primary carbon source. The growth-inhibitory effect of cholesterol *in vitro* depends on cholesterol import, as the Δ*igr* mutant growth defect during the early phase of disease is completely suppressed by mutating *mce4*, implicating cholesterol intoxication as the primary mechanism of attenuation. We conclude that *M. tuberculosis* metabolizes cholesterol throughout the course of infection, and that degradation of this sterol is crucial for bacterial persistence.

Keywords: Cholesterol, Δ*igr,* Lipid metabolism, *mce4, Mycobacterium tuberculosis*

Mycobacterium tuberculosis (MTB) infection is one of the leading causes of death worldwide. It kills an estimated 2 million people annually, and approximately 2 billion (one third of the world's population) are infected with this organism. MTB resides in a modified phagosome of host macrophages and, like other intracellular pathogens, has adapted unique ways of thriving in this harsh environment. While several genes and virulence factors have been studied extensively, very little is known about the ability of MTB to infect a host and cause latent infections for years and sometimes decade[1-5].

The most fundamental challenge MTB faces in the host is the need to acquire nutrients from host cells. It is not clear what nutrients MTB obtains from the host in order to survive in the harsh surroundings of the nutrient-deficient vacuole, although host lipids have been implicated as an important source of carbon. This was first suggested by the observation that fatty acids but not carbohydrates stimulate respiration of MTB isolated from mouse lung[6]. Subsequently, sequencing of the MTB genome revealed at least 250 genes potentially involved in lipid metabolism[7]. Many of these genes are transcriptionally induced during intracellular growth, and a few are known to be required for infection[8-12]. However, the complexity of MTB lipid metabolism has made it difficult to determine if any individual gene is genuinely required for host lipid catabolism, as opposed to the synthesis or modification of an endogenous bacterial lipid.

The array of lipids an MTB bacterium might encounter during the course of infection is currently unknown. MTB likely interacts with fatty acids in the macrophage vacuolar membrane. Phagosomal membranes are complex structures in themselves, the bacteria most likely acquire nutrients from cytosolic molecules as well. The role of these fatty acids affecting MTB growth, and the ability of the pathogen to cause infection and disease, is currently of great interest. Major questions are a) *which* lipids are MTB exposed to during infection and b) *how* are those lipids utilized by the mycobacteria?

The link between cholesterol and tuberculosis disease has been observed by an array of methods. Epidemiologic analysis has revealed a link between patient cholesterol levels and the outcome of pulmonary tuberculosis. These studies have concluded that higher serum cholesterol levels

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correlated with reduced radiologic signs of disease and faster sputum sterilization following the initiation of chemotherapy[13,14]. Using molecular genetics and microbiology, the MTB-cholesterol connection has been further elucidated by Pieters *et al.*[15], showing that cholesterol is required for at least one route of mycobacterial entry into macrophages. It was further shown that cholesterol is required for retention of the host protein coronin 1 (also called P57 or TACO) on mycobacteria-containing phagosomes and that retention of coronin 1 is associated with a block in phagosome-lysosome fusion[16]. Altogether these studies illustrate the importance of host cholesterol for MTB in the context of host macrophages and lung infection. However, the bacterial utilization of cholesterol during infection remained unresolved.

Cholesterol import: the mce4 transport system—The *mce4* locus is one of four homologous regions in the MTB genome and consists of several genes predicted to encode a multi-subunit ABC-like transport system[17] (Fig. 1). Pandey and Sassetti recently identified cholesterol as a substrate for the *mce4* transporter[18]. Upon deletion of the *mce4* operon, MTB showed a marked growth defect in media containing cholesterol as the sole carbon source, as well as severely reduced accumulation of cholesterol compared to wild type. Cholesterol degradation by MTB can be monitored from both the 4- and 26-carbons of the molecule, with C-4 converted to CO_2 and C-26 becoming incorporated into cell membrane lipids[18]. The Δ*mce4* strain was unable to convert radiolabeled cholesterol to CO_2 or incorporate radiolabeled cholesterol into the cell membrane, consistent with the hypothesis that *mce4* is required for MTB cholesterol import. This block in import resulted in the attenuation of Δ*mce4* bacteria in activated but not naïve macrophages, and severe attenuation in the mouse model of infection during late but not early time-points[18]. These studies show that cholesterol import is necessary for MTB during chronic infection, a time when host macrophages become INF-γ activated, possibly limiting nutrient availability.

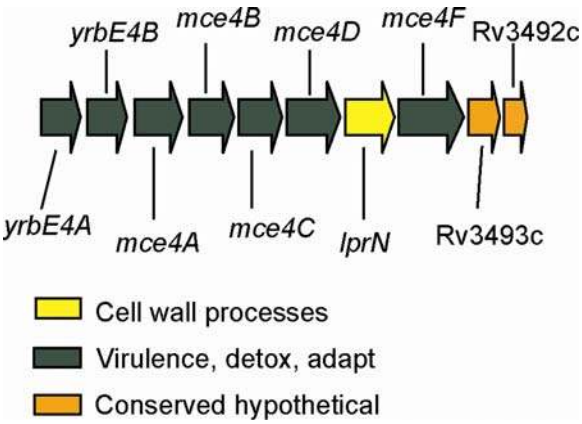

Fig. 1—The *mce4* locus of *M. tuberculosis*. [Gene annotation and predictive function are based on Tuberculist—yellow box – cell wall processes; green box – virulence, detox and adaptation; orange box – conserved hypothetical]

In a separate study of *mce4*, Joshi *et al.* used TraSH (Transposon Site Hybridization) to identify genes that genetically interact with the *mce4* locus. Among the genes genetically linked to this transport system was the operon Rv3540-5c[17]. Bioinformatics suggested that these genes were involved in lipid metabolism[9].

The igr operon—After the TraSH analysis report[17], another study showed that the Rv3540-5c operon was required for MTB growth in THP-1 cells in a tissue culture model of infection[9]. Due to the inability of transposon mutants with disruptions in Rv3540-5c to grow in macrophages, the operon was named *igr* for intracellular growth. Expression of this operon was previously found to be up-regulated in macrophages where it plays an important role, and these genes were found to be required for survival in mouse spleens following intravenous infection[10,19,20]. The six genes of the *igr* operon are annotated to suggest some role in lipid metabolism: a putative cytochrome p450 (Rv3545c, *igrA*), two acyl-coA dehydrogenases (Rv3543-4c, *igrBC*), two conserved hypothetical proteins (Rv3541-2c, *igrDE*), and a lipid carrier protein (Rv3540c, *igrF*) (Fig. 2).

It has been hypothesized that the *igr* deletion mutant could be defective in some aspect of lipid metabolism and therefore might grow atypically in the

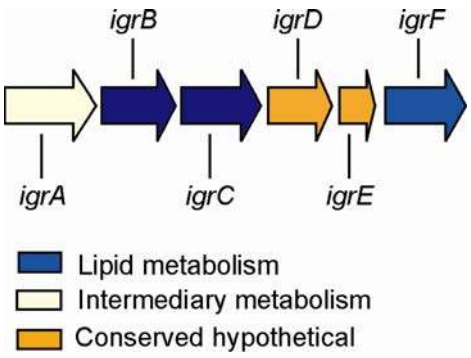

Fig. 2—The *igr* locus of *M. tuberculosis*. [Gene annotation and predictive function are based on Tuberculist—blue box – lipid metabolism (*igrB*: *fadE28*, *igrC*: *fadE29*, *igrF*: *ltp2*); beige box – intermediary metabolism (*igrA*: *cyp125*); and orange box – conserved hypothetical (*igrD*: Rv3542c, *igrE*: Rv3541c)]

presence of one or more lipids. The *igr* deletion mutant grew identically to wild type strains in media containing a variety of fatty acids, including pyruvate, succinate, butyrate, valerate, isovalerate, dodecanoate, palimitate, and Tween 80 (Ref 9). In addition, the *igr* deletion mutant showed no discernable difference from wild type in the presence of iron chelators, acidified nitrite or hydrogen peroxide, mechanisms used by macrophages to control MTB infection (Chang & Sherman, unpublished observations). Despite the similarities of Δ*igr* and wild type in all these conditions, the Δ*igr* strain was powerfully attenuated *in vivo*. Aerosol infection of mice revealed a severe lag in growth in the lungs and delayed dissemination to the spleen[9] (Fig. 3). While colony forming units (CFUs) eventually reached wild type levels, the histopathology of lungs from Δ*igr* infected mice showed considerably less damage than those infected with wild type[9]. The unusual delayed growth phenotype in mice showed that the *igr* locus is important during infection, but provided few clues as to the role that these genes play.

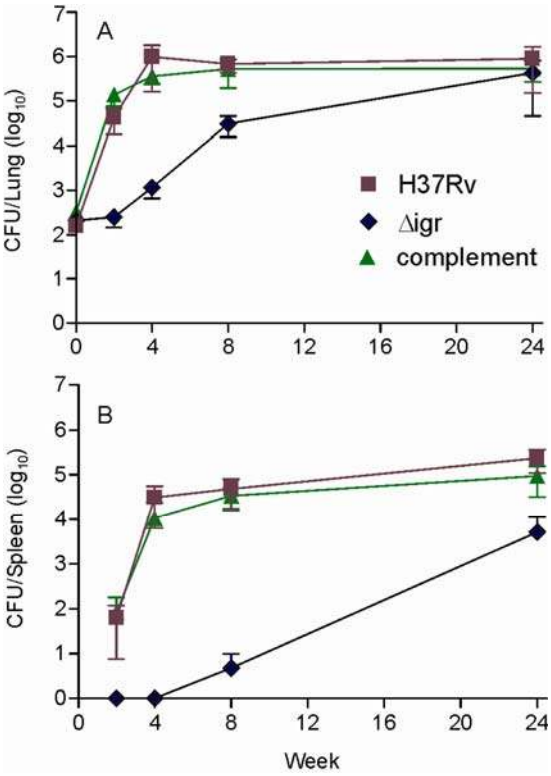

Fig. 3—Δ*igr* attenuation in immunocompetent mice.—Female C57BL/6 mice were infected via aerosol with approximately 200 CFU of H37Rv (WT) (□), Δ*igr* (◊), or complement (Δ). At the times indicated, bacterial burdens in (A) lungs or (B) spleens were determined by plating homogenized tissues. [The data shown represent the average mean SD for 4–5 mice/strain/time point. Adapted from Chang *et al.*[9]]

igr and cholesterol—While the lipid substrate for the *igr* system remained a mystery, a breakthrough came from a publication describing a cholesterol-catabolism gene cluster in the MTB-related actinomycete Rhodococcus strain RHA1[21]. The authors identified a 51-gene cluster of RHA1 whose transcription was specifically induced in the presence of cholesterol. This region corresponds to an 82-gene cluster in the genome of MTB that includes both the *igr* operon as well as the *mce4* transport system[21]. In addition, conditions were identified in which MTB could grow, albeit slowly, with cholesterol as the sole carbon source. Considering the genetic link between *igr* genes and the *mce4* cholesterol transport system, these data seemed to suggest the *igr* operon might be involved in cholesterol metabolism during MTB intracellular growth.

Using the growth conditions described by Van der Geize *et al.*[21] we compared growth of wild type and *igr* deletion strains in the presence of cholesterol. In preliminary experiments, the phenotype was striking: the *igr* deletion mutant was unable to grow in the presence of cholesterol. In addition, the growth arrest could not be rescued by the addition of a preferred carbon source, such as dextrose or glycerol (Chang *et al.*, submitted). These data suggest that cholesterol metabolism is incomplete in the *igr* mutant bacilli and that cholesterol or one of its metabolites is toxic to these cells.

To determine if a block in cholesterol import could rescue the growth arrest of Δ*igr* in cholesterol-containing media, an MTB mutant disrupted in both the *igr* and *mce4* loci was constructed. In preliminary experiments, this double knock-out (KO) strain displayed a partial rescue of the *igr*-mutant phenotype, with improved growth kinetics in the presence of cholesterol or cholesterol + glycerol. In addition, the double mutant was able to grow in and kill THP-1 cells close to levels of wild type bacteria. Complementation was also evident *in vivo*. In mice infected with the double KO, deletion of the *mce4* cholesterol transport system restored bacterial growth in the lungs and spleens to wild type levels out to 3 weeks post-infection (Chang *et al.*, submitted). These data are consistent with a model in which attenuation of Δ*igr* results from the inability to degrade cholesterol properly along with the subsequent build-up of toxic intermediates (Fig. 4).

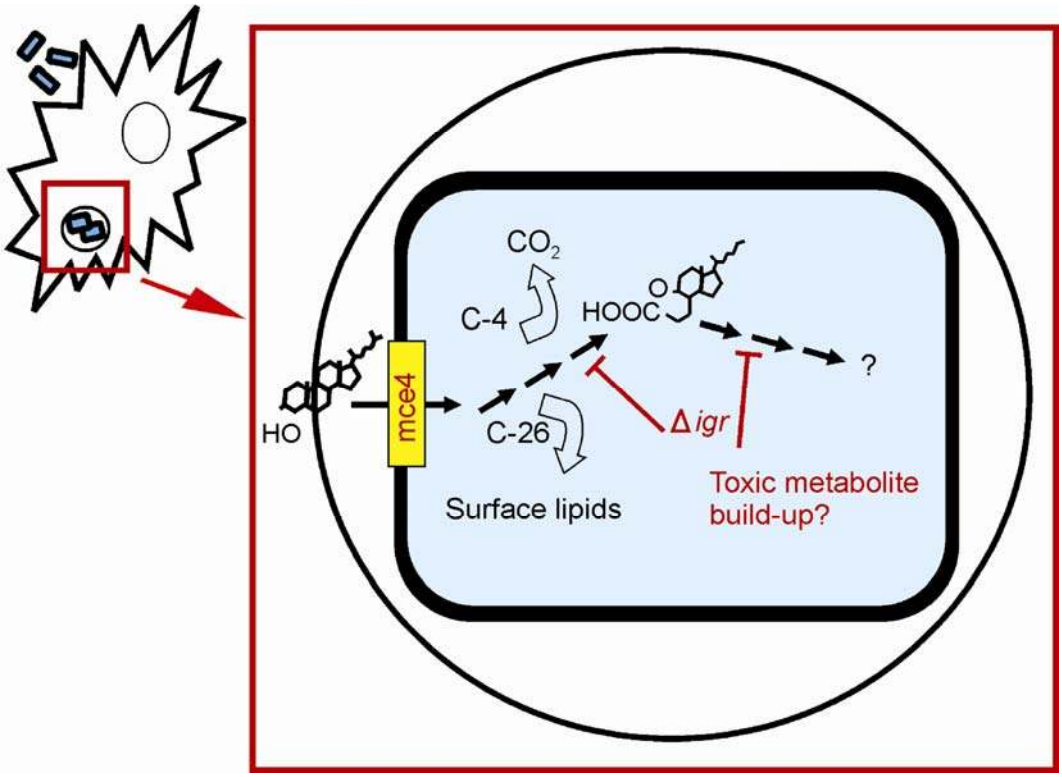

Fig. 4—Role of *mce4* and *igr* in cholesterol metabolism. —Model depicting the possible roles of the *mce4* and *igr* operons in cholesterol uptake and metabolism. MTB infects host macrophages and resides in a modified vacuole (inset). Cholesterol is transported into MTB via the Mce4 transport system. C-4 carbon is metabolized to CO_2 and C-26 carbon is incorporated into bacterial cell lipids. At some point during cholesterol metabolism, *igr* genes are necessary for further degradation. Lack of *igr*-dependent metabolism could result in the build-up of a toxic metabolite. Multiple arrows represent multiple steps. Final products of cholesterol metabolism in MTB are unknown (shown as question mark).

Analysis of the Δ*igr* and Δ*mce4* strains is helping to elucidate new roles for cholesterol during MTB infection. The Δ*igr* strain shows marked attenuation at early time-points following infection of mice, and subsequently bacterial CFUs approch wild type level. In contrast, strains deficient in the ability to import cholesterol (Δ*mce4*) are only attenuated in mice at late time-points, indicating that cholesterol metabolism becomes limiting for MTB growth/survival only during chronic infection, after adaptive immunity has altered the environment in which MTB resides. Taken together, these data suggest that cholesterol is available to MTB throughout the course of infection, but becomes a key nutrient during chronic infection when the host environment has been altered by the adaptive immune response. Of course, the precise manner in which cholesterol is utilized by MTB *in vivo* remains unclear. One possibility is that cholesterol may become a major carbon source for ATP production and macromolecular biosynthesis. Alternatively, cholesterol metabolism by MTB may contribute to production of a specific virulence factor and/or a disruption of normal host cell signalling. Exploring these possibilities will be an area of active investigation in future.

References

1 Walburger A, Koul A, Ferrari G, Nguyen L, Prescianotto-Baschong C, Huygen K, Klebl B, Thompson C, Bacher G & Pieters J, Protein kinase G from pathogenic mycobacteria promotes survival within macrophages, *Science*, 304(5678) (2004) 1800.

2 Fortune, S M, Jaeger A, Sarracino D A, Chase M R, Sassetti C M, Sherman D R, Bloom B R, & Rubin E J, Mutually dependent secretion of proteins required for mycobacterial virulence, *Proc Natl Acad Sci USA*, 102(30) (2005) 10676.

3 Houben E N, Nguyen L & Pieters J, Interaction of pathogenic mycobacteria with the host immune system, *Curr Opin Microbiol*, 9(1) (2006)76.

4 Cosma C L, Sherman D R & Ramakrishnan L, The secret lives of the pathogenic mycobacteria, *Annu Rev Microbiol*, 57 (2003) 641.

5 Ferrari G, Langen H, Naito M & Pieters J, A coat protein on phagosomes involved in mycobacterial survival, *Cell*, 97(4) (1999) 435.

6 Bloch H & Segal W, *J Bacteriol*, Biochemical differentiation of *Mycobacterium tuberculosis* grown *in vivo* and *in vitro*, 72(2) (1956) 132.

7 Cole S T, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon S V, Eiglmeier K, Gas S, Barry C E, 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail M A, Rajandream M A, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston J E, Taylor K, Whitehead S, & Barrell B G, *Nature*, Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence, 393(6685) (1998) 537.

8 Munoz-Elias E J M J D, *Mycobacterium tuberculosis* isocitrate lyases 1 and 2 are jointly required for *in vivo* growth and virulence*, Nature Med*, 11(6) (2005) 638.

9 Chang J C, Harik N S, Liao R P & Sherman D R, Identification of mycobacterial genes that alter growth and pathology in macrophages and in mice, *J Infect Dis*, 196(5) (2007) 788.

10 Schnappinger D, Ehrt S, Voskuil M I, Liu Y, Mangan J A, Monahan I M, Dolganov G, Efron B, Butcher P D, Nathan C & Schoolnik G K, Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: Insights into the phagosomal environment, *J Exp Med,* 198(5) (2003) 693.

11 Brzostek A, Dziadek B, Rumijowska-Galewicz A, Pawelczyk J & Dziadek J, Cholesterol oxidase is required for virulence of *Mycobacterium tuberculosis*, *FEMS Microbiol Lett*, 275(1) (2007) 106.

12 McKinney J D, Honer zu Bentrup K, Munoz-Elias E J, Miczak A, Chen B, Chan W T, Swenson D, Sacchettini J C, Jacobs W R, Jr & Russell D G, Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase, *Nature,* 406(6797) (2000)735.

13 Perez-Guzman C, Vargas M H, Quinonez F, Bazavilvazo N & Aguilar A, A cholesterol-rich diet accelerates bacteriologic sterilization in pulmonary tuberculosis, *Chest*, 127(2) (2005) 643.

14 Deniz O, Gumus S, Yaman H, Ciftci F, Ors F, Cakir E, Tozkoparan E, Bilgic H & Ekiz K, Serum total cholesterol, HDL-C and LDL-C concentrations significantly correlate with the radiological extent of disease and the degree of smear positivity in patients with pulmonary tuberculosis*, Clin Biochem*, 40(3-4) (2007) 162.

15 Gatfield J & Pieters J, Essential role for cholesterol in entry of mycobacteria into macrophages*, Science*, 288(5471) (2000) 1647.

16 Nguyen L & Pieters J, The Trojan horse: Survival tactics of pathogenic mycobacteria in macrophages*, Trends Cell Biol*, 15(5) (2005) 269.

17 Joshi S M, Pandey A K, Capite N, Fortune S M, Rubin E J & Sassetti C M, Characterization of mycobacterial virulence genes through genetic interaction mapping, *Proc Natl Acad Sci USA*, 103(31) (2006) 11760.

18 Pandey A K & Sassetti C M, Mycobacterial persistence requires the utilization of host cholesterol*, Proc Natl Acad Sci USA,* 105(11) (2008) 4376.

19 Rengarajan J B, Bloom B R & Rubin E J, Genome-wide requirements for *Mycobacterium tuberculosis* adaptation and survival in macrophages, *Proc Natl Acad Sci USA*, 102(23) (2005) 8237.

20 Sassetti C M & Rubin E J, Genetic requirements for mycobacterial survival during infection, *Proc Natl Acad Sci USA,* 100(22) (2003) 12989.

21 Van der Geize R, Yam K, Heuser T, Wilbrink M H, Hara H, Anderton M C, Sim E, Dijkhuizen L, Davies J E, Mohn W W, & Eltis L D, A gene cluster encoding cholesterol catabolism in a soil actinomycete provides insight into *Mycobacterium tuberculosis* survival in macrophages*, Proc Natl Acad Sci USA*, 104(6) (2007) 1947.