COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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Mycobacterium tuberculosis virulence: lipids inside and out

Sabine Ehrt & Dirk Schnappinger

During infections, *Mycobacterium tuberculosis* has to acquire nutrients and resist host defense. Lipids are important for both: host-derived lipids provide food, whereas pathogen-derived lipids mediate immune suppression.

Lipids have long been thought to play key roles in the pathogenesis of *Mycobacterium tuberculosis*. In early studies, Segal and Bloch found fatty acids rather than carbohydrates to stimulate respiration of *M. tuberculosis* if the bacteria were isolated from mouse lungs instead of liquid cultures[1]. *In vivo* growth or persistence of *M. tuberculosis* were therefore hypothesized to depend on fatty acids as a major nutrient source. Forty-four years later, McKinney *et al.* described a *M. tuberculosis* mutant in which a gene encoding an isocitrate lyase, now termed *icl1*, had been inactivated[2]. In contrast to wild-type *M. tuberculosis*, this mutant showed a selective survival defect during the chronic phase of infection using a mouse of model of tuberculosis; growth during the acute phase of infection and in liquid media was unaffected.

Isocitrate lyases catalyze an essential reaction of the glyoxylate shunt, an anaplerotic pathway that bypasses the CO_2-generating steps of the tricarboxylic acid cycle and enables bacteria to synthesize carbohydrates and replenish tricarboxylic acid cycle intermediates from fatty acid–derived acetyl–coenzyme A (**Fig. 1**). For many bacteria, this pathway is required for growth on fatty acids as a sole carbon source. So the impaired persistence of the *icl1* mutant implied that *M. tuberculosis* depends on fatty acid metabolism to resist killing by an activated immune system.

Recent work by Muñoz-Elias and McKinney[3] not only confirmed these findings but also expanded previous views on the significance of isocitrate lyases in the virulence of *M. tuberculosis*. The genomes of most *M. tuberculosis* strains encode not one but two functional isocitrate lyases: ICL1 and ICL2. Muñoz-Elias and McKinney investigated the importance of these enzymes for growth on different carbon sources *in vitro*, as well as for growth and persistence in mice, using mutants with deletions in *icl1*, *icl2* or both[3]. All mutants grew like wild-type *M. tuberculosis* in media containing glycerol as carbon source, and little impact was observed on growth with glucose. Mutants containing deletions of either *icl* gene similarly grew well with most short- and long-chain fatty acids. In contrast, the *icl* double mutant (Δ*icl1*Δ*icl2*) failed to grow on fatty acids.

Studies in mice confirmed that deletion of *icl1* caused about a tenfold reduction in bacterial titers in lungs during the chronic phase of infection. Loss of *icl2* alone did not affect growth or persistence of *M. tuberculosis*. Surprisingly, *M. tuberculosis* lacking both *icl1* and *icl2* failed to grow at all in mouse lungs. Even though 1,000 colony-forming units (CFU) were detected in the lungs of mice one day after infection, less than 100 CFU were detected about 10 days later and no CFUs could be recovered after 2 or more weeks. *M. tuberculosis* devoid of all isocitrate lyase activity was therefore not only unable to persist in the face of an activated immune system, but also incapable of growing in immunocompetent and immunodeficient mice.

Although ICL1 and ICL2 of *M. tuberculosis* are also essential for a second biochemical pathway that is required for growth of *M. tuberculosis* on odd-chain fatty acids (the methylcitrate cycle)[4,5], the observed essentiality of isocitrate lyase activity for *in vivo* growth is more likely to be explained by its role in the glyoxylate cycle. This is because inactivation of enzymes of the methylcitrate cycle other than ICL1 and ICL2 do not impair growth or persistence in mice[5].

Standard tuberculosis chemotherapy requires people to take four drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) for two months followed by two drugs (isoniazid and rifampicin) for four months, and it is generally effective. Unfortunately, 'generally effective' does not mean 'always effective'. Although the reasons for treatment failure vary and are always tragic, the spread of drug-resistant *M. tuberculosis* is especially alarming. Strains categorized as multidrug resistant (MDR) are resistant to at least the two first-line tuberculosis drugs (isoniazid and rifampicin), whereas those described as extensively drug resistant (XDR) are additionally resistant to fluoroquinolones and one or more of three injectable second-line drugs (capreomycin, kanamycin and amikacin). XDR tuberculosis cases have been described in at least 17 countries on all continents[6]. Enzymes such as the isocitrate lyases, which might be required for growth and survival of *M. tuberculosis* in all infected individuals, immunocompetent and immunocompromised, but are not part of human metabolism, are therefore attractive

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targets for the development of new tuberculosis drugs. As a first step in the chemical evaluation of isocitrate lyases as drug targets, Muñoz-Elias and McKinney showed that 3-nitropropionate, a prototype ICL inhibitor, blocked growth of *M. tuberculosis* with fatty acids as a sole carbon source and interfered with bacterial replication within mouse and human macrophages in a concentration dependent manner. This mimicked the phenotype of the Δ*icl1*Δ*icl2* mutant, showing that chemical inhibition of both ICL1 and ICL2 can be achieved. The challenge at hand is now to identify lead compounds that inhibit both of the mycobacterial isocitrate lyases and are less toxic than 3-nitroproprionate.

In addition to their role as nutrients, fatty acids, primarily in the form of mycolic acids, constitute a key component of the pathogen's cell envelope. This cell envelope is responsible for many of the unique microbiological characteristics of mycobacteria, and also contains immunomodulatory molecules. Members of the W-Beijing family of *M. tuberculosis* strains have been associated with extensive morbidity and mortality worldwide[7]. Remarkably, infection of mice with the W-Beijing strain HN878 has been shown to accelerate the time to death, even though growth of these strains in mice is not significantly different from several non–W-Beijing isolates[8]. In addition, HN878-infected mice express reduced levels of mRNAs encoding several proinflammatory cytokines, including tumor necrosis factor, interleukin-12 and interferon-γ in their lungs[8,9]. The outcome of infections with different clinical *M. tuberculosis* isolates therefore seems to correlate with their ability to induce a strong T_H1-type T-cell and cytokine response: strains that do not induce a strong inflammatory response are more virulent than strains with robust cytokine-inducing activity. Lipids extracted from HN878 also cause less interleukin-12 production when used to stimulate human monocytes than lipid extracts from non–W-Beijing strains[10]. Thus, differences in the host immune responses to W-Beijing and non–W-Beijing strains are likely to be at least in part attributable to differences in the lipid composition of their cell envelopes.

Reed and colleagues have now provided the first evidence that a particular W-Beijing lipid is capable of suppressing host immune responses (**Fig. 1**). Incubation with [1-^{14}C]propionic acid, which specifically radiolabels lipids possessing methyl branches, and analysis by thin-layer chromatography identified two molecules that were present in HN878 and three other W-Beijing strains but missing in non–W-Beijing strains. Disruption in HN878 of the *pks1-15* locus, which encodes a polyketide synthase, abolished production of the W-Beijing–specific lipids. Genotyping studies further showed that the *pks1-15* locus was intact in all W-Beijing strains tested, but mutated in non–W-Beijing strains[11,12]. Growth of wild-type HN878 and the HN878 *pks1-15* mutant in mouse lungs and spleens was similar, and bacterial titers at times of death of mice were indistinguishable. However, median survival times of mutant-infected mice were 90 days longer than those of mice infected with wild-type HN878. So inactivation of *pks1-15* not only inhibited production of the W-Beijing–specific lipids, but also reduced virulence to that of non–W-Beijing isolates. Previous work had linked the *pks1-15* polyketide synthase to the production of phenolic glycolipids (PGL) in *M. tuberculosis*[12]. Infections of bone marrow–derived macrophages with *M. tuberculosis* strains producing different levels of PGL showed an inverse correlation between PGL levels and the amounts of proinflammatory cytokines and chemokines secreted in response to infection[11]. Finally, addition of purified PGL inhibited cytokine production of bone marrow–derived macrophages stimulated with apolar lipids isolated from the *pks1-15* mutant. Thus, the work by Reed and colleagues establishes PGL as a mycobacterial effector that directly modulates the host innate immune response. This finding is likely to stimulate a number of follow-up studies and facilitate identification of macrophage receptors and signal transduction pathways targeted for immunomodulation by pathogenic mycobacteria.

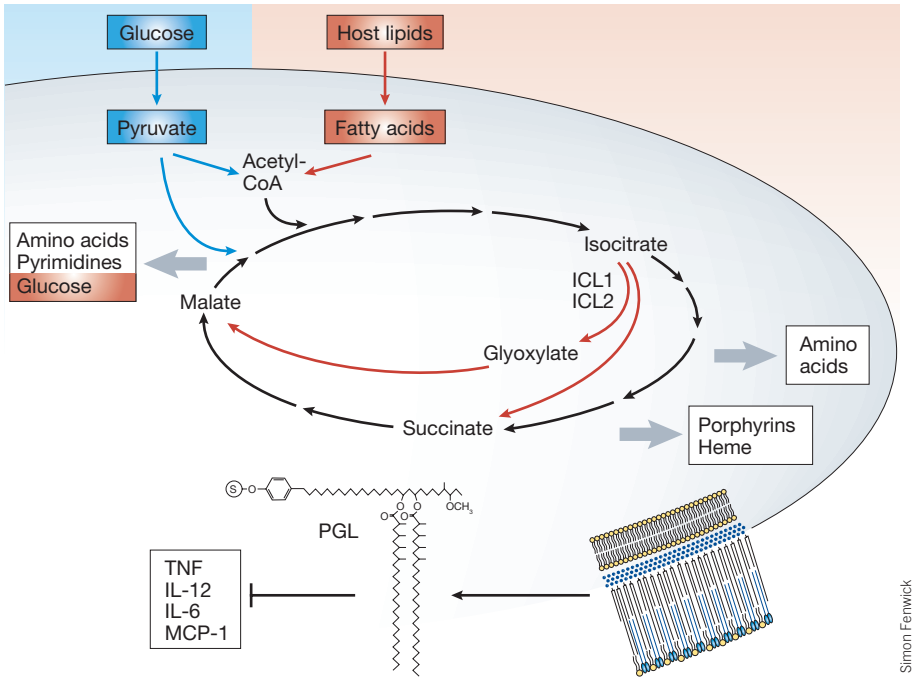

Figure 1 Anaplerotic reactions needed for growth with sugars or lipids and immunomodulation by phenolic glycolipids (PGL). Blue arrows highlight reactions specific for growth with sugars, red arrows highlight reactions specific for growth with lipids or fatty acids, and wide gray arrows indicate biosynthetic pathways that use tricarboxylic acid cycle intermediates. The lower part of the figure shows the mycobacterial cell envelope (cytoplasmic membrane, arabinogalactan-peptidoglycan copolymer and outer membrane) in more detail. It also shows the structure of PGL as described by Reed *et al.*[11] and indicates inhibition of cytokine and chemokine production by PGL. Circled S, variable number of sugar residues; CoA, coenzyme A; TNF, tumor necrosis factor; IL, interleukin; MCP-1, monocyte chemoattractant protein 1.

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