ACS central science

FIRST REACTIONS

Major Global Killer Tamed by Hydrogen

Clifton E. Barry III

National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, Bethesda, Maryland, United States, and Institute for Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, Western Cape, South Africa

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB) has only one host—us. Specimens of our earliest known upright ancestors, *Homo erectus*, from more than 500,000 years ago show pathology consistent with TB disease, and whole genome sequences of TB strains have been constructed from the remains of Peruvian mummies that were over 1000 years old.[1] Despite our long and intimate relationship with the bacterium, we still know remarkably little about how we have adapted to provide its living quarters and conversely what adaptations the pathogen has made to respond to our defense systems.

Since bedaquiline, the first new antituberculosis drug in decades, was approved for the treatment of TB in 2012, all eyes have been on energy metabolism. That's because bedaquiline inhibits the proton-pumping ATP synthase that fuels the mycobacterial cell.[2]

Despite our long and intimate relationship with the bacterium, we still know remarkably little about how we have adapted to provide its living quarters and conversely what adaptations the pathogen has made to respond to our defense systems.

And yet, three years after the initial clinical trials that led to its approval it is still unclear what impact inhibiting ATP synthesis has on our ability to cure patients with TB. This has led to the realization that we do not know half as much about respiration and energy metabolism in this organism as we thought we did. The surprising results reported in the last issue of this journal serve to further thicken the plot and reveal an unexpectedly subtle connection between the structure of the bacterial electron carrier and life outside, and inside, the macrophage (Figure 1).[3]

Aerobically, the process of moving electrons during respiration results in protons being pumped from the cytoplasm to the periplasm, generating a pH gradient that drives the action of the ATP synthase. Mycobacteria rely on menaquinone (MK) for shuttling electrons between oxidoreductases within respiratory complexes. The business end of these electron carriers is the napthoquinone that can be reversibly reduced to napthoquinol allowing the smooth flow of electrons from reduced cofactors to molecular oxygen (under aerobic conditions) or alternative electron acceptors (under microaerophilic or anaerobic conditions). MK also contains a lipid tail region composed of about nine repeating isoprene units that presumably serves to anchor them to the membrane where the respiratory complexes assemble. Different bacteria show distinct patterns within this tail (Figure 2).

Clifton Barry III explains the significance of the saturation in menaquinone for tuberculosis proliferation in macrophage reported by Upadhyay et al.

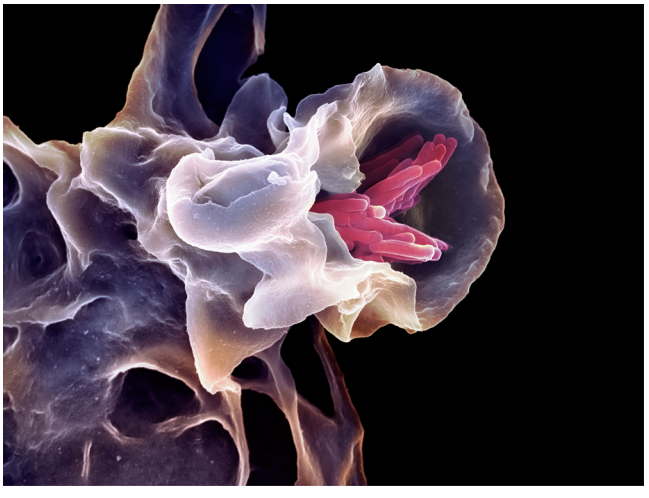

Figure 1. *Mycobacterium tuberculosis* normally resides in macrophage, as shown above.

Published: September 3, 2015

DOI: 10.1021/acscentsci.5b00293
ACS Cent. Sci. 2015, 1, 286−288

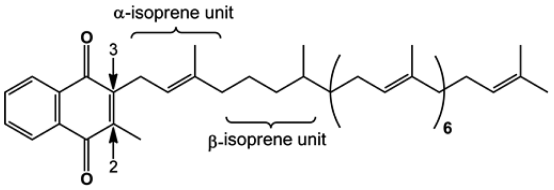

Figure 2. Menaquinone (MK) contains a napthoquinone and lipid tail.

In mycobacteria, for example, all but one of these isoprene units are unsaturated. The odd-ball saturated unit is localized to the second position distal to the napthoquinone. On the basis of similarity to other structural lipids with isoprene side-chains Upadhyay et al. reasoned that the reduced isoprene was likely formed enzymatically through the action of a hydrogenase. One candidate was a hydrogenase from Archaea involved in phospholipid biosynthesis that had some similarity to a mycobacterial protein of unknown function. *Escherichia coli* produces primarily ubiquinone under aerobic conditions but shifts to MK when grown anaerobically. When the authors cloned and expressed this putative hydrogenase from mycobacteria in *E. coli* the usually fully unsaturated MK suddenly shifted 2 mass units up, and tandem mass spectrometry showed that this shift was not in the napthoquinone nucleus.

Genetic deletion of the gene in *M. tuberculosis* also showed a consistent result, knocking out the putative hydrogenase resulted in a MK shifted down by 2 mass units. Replacing the gene in that strain restored the normal partially saturated structure leading them to conclude that this gene was, in fact, the hydrogenase they were seeking. Using this gene as a template they find that this MK reductase appears to be widespread throughout Gram-positive bacteria with reduced isoprenoid side chains. Thus, the authors have therefore added a new enzyme to the list of MK biosynthetic enzymes.

The most surprising result in the paper though is that when the authors infected macrophages with this mutant *in vitro*, the mycobacteria were entirely incompetent to replicate, and even appeared to die off over a matter of a few days.

As one might expect for an unsaturated system located far from the site of intrinsic redox chemistry, the phenotype of the resulting mutant at first appears subtle. There was no difference in growth rate of the mutant compared to wild type under either normal aerobic conditions or those with reduced oxygen tension where alternative respiratory complexes would be expected to be required. A subtle difference in how fast hypoxic cultures depleted the available oxygen led the group to explore the respiratory competence of the mutant more closely. ATP levels were maintained at wild type level, but the rate of electron transport through the respiratory complex was reduced 3-fold. Looking at the total levels of MK revealed that the cells had preserved respiratory competence by doubling the overall concentration of MK.

The most surprising result in the paper though is that when the authors infected macrophages with this mutant *in vitro*, the mycobacteria were entirely incompetent to replicate, and even appeared to die off over a matter of a few days. This striking result shows that the respiratory complexes allowing growth *in vitro* under aerobic conditions are inadequate to support respiration *in vivo*, but not in a fashion that is dependent on anoxia since macrophage cultures are at atmospheric levels of oxygen. It has been previously shown that TB produces both the β-saturated and β-unsaturated MK during aerobic growth, but only the monosaturated MK could be detected in anaerobic culture.[4]

Although it has previously been suggested that MK may perform a signaling function related to changes induced by anoxia, the present work makes it clear that monosaturated MK plays a direct functional role. The essentiality of this molecule during macrophage infection, but not during aerobic or anaerobic growth, implies that *M. tuberculosis* utilizes unique assemblies of oxidoreductases *in vivo* or that the assemblies used *in vitro* experience more demand during infection and are therefore less tolerant to the lower efficiency produced by the fully unsaturated MK. It is worth bearing in mind that in human TB infections, the bacteria occur within a wide range of different environments of which the macrophage reflects only one. Understanding the breadth of potential terminal electron acceptors and reduced intracellular substrates across all of the environments during infection would allow a much more refined approach to targeting specific bacterial subpopulations. The ultimate utility of bedaquiline, and the role of inhibitors of respiration in general, in the treatment of TB demand a more detailed understanding of these systems. This study makes an important contribution to exploring the structural determinants important for MK function and shows that in the long coevolution of humans and TB even very small changes can make a big difference.

Author Information
E-mail: cbarry@niaid.nih.gov.

REFERENCES

(1) Bos, K. I.; Harkins, K. M.; Herbig, A.; Coscolla, M.; Weber, N.; Comas, I.; Forrest, S. A.; Bryant, J. M.; Harris, S. R.; Schuenemann, V. J.; Campbell, T. J.; Majander, K.; Wilbur, A. K.; Guichon, R. A.; Wolfe Steadman, D. L.; Cook, D. C.; Niemann, S.; Behr, M. A.; Zumarraga, M.; Bastida, R.; Huson, D.; Nieselt, K.; Young, D.; Parkhill, J.; Buikstra, J. E.; Gagneux, S.; Stone, A. C.; Krause, J. Pre-Columbian

DOI: 10.1021/acscentsci.5b00293
ACS Cent. Sci. 2015, 1, 286−288

mycobacterial genomes reveal seals as a source of New World human tuberculosis. *Nature* **2014**, *514*, 494−497.
(2) Andries, K.; Verhasselt, P.; Guillemont, J.; Göhlmann, H. W.; Neefs, J. M.; Winkler, H.; Van Gestel, J.; Timmerman, P.; Zhu, M.; Lee, E.; Williams, P.; de Chaffoy, D.; Huitric, E.; Hoffner, S.; Cambau, E.; Truffot-Pernot, C.; Lounis, N.; Jarlier, V. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* **2005**, *307*, 223−227.
(3) Upadhyay, A.; Fontes, F. L.; Gonzalez-Juarrero, M.; McNeil, M. R.; Crans, D. C.; Jackson, M.; Crick, D. C. Partial Saturation of Menaquinone in *Mycobacterium tuberculosis*: Function and Essentiality of a Novel Reductase, MenJ. *ACS Cent. Sci.* **2015**, DOI: 10.1021/acscentsci.5b00212.
(4) Honaker, R. W.; Dhiman, R. K.; Narayanasamy, P.; Crick, D. C.; Voskuil, M. I. DosS responds to a reduced electron transport system to induce the Mycobacterium tuberculosis DosR regulon. *J. Bacteriol.* **2010**, *192*, 6447−55.

DOI: 10.1021/acscentsci.5b00293
ACS Cent. Sci. 2015, 1, 286−288