Reply to Yew et al., “Vitamin C and *Mycobacterium tuberculosis* Persisters”

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We thank Yew and coauthors (1) for their interest in our article (2) and for highlighting the complexity of the activity of vitamin C on mycobacteria grown *in vitro*. The cited studies reached divergent conclusions whose resolution requires determining whether vitamin C induces a non- or slow-replicating (persister/dormant) phenotype (3, 4) or promotes a constant metabolically active state that prevents or eliminates persister formation in mycobacteria (5).

These conflicting conclusions could arise from differences in experimental procedures (different mycobacterial species, culture conditions, or vitamin C concentrations used) or from the dual property of vitamin C as both an antioxidant and a prooxidant (6–8). This dual property observed both *in vitro* and *in vivo* may depend on the concentration of vitamin C present. In our experiments, vitamin C has a prooxidant phenotype generating reactive oxygen species and sterilizing *Mycobacterium tuberculosis* cultures (9). Vitamin C also increases *M. tuberculosis* oxygen consumption (5), which correlates with persister elimination (10). In view of the potentiation of diverse tuberculosis drugs with vitamin C (2, 11), vitamin C's ability to inhibit both biofilm formation (12) and *M. tuberculosis* in biofilms (13), and vitamin C's inability to control *M. tuberculosis* infection in mice, it seems unlikely that vitamin C induces persister formation in *M. tuberculosis*.

Despite these tantalizing results, developing vitamin C as an adjunct therapy for tuberculosis will face many challenges. Vitamin C by itself did not decrease the *M. tuberculosis* burden in infected mice, but mice synthesize vitamin C, whereas guinea pigs, primates, and humans do not (14, 15). Intriguingly, Svirbely and Szent-Györgyi demonstrated that vitamin C (purified and crystallized from Hungarian paprika!) cured scurvy (16), a disease correlating with susceptibility to infection and which has recently reemerged in the Organisation for Economic Co-operation and Development (OECD) population due to malnutrition. Vitamin C also has many essential functions in humans (17), and with levels of vitamin C in human tissues and plasma dependent upon its mode of delivery (18–20) and regulated absorption (21, 22), it may be difficult to obtain adequate levels of vitamin C to act on *M. tuberculosis*.

The comments offered by Yew et al. highlight the value in examining the redox state of both the host and the pathogen in the context of *M. tuberculosis* infection control. During the infection life cycle of *M. tuberculosis*, the bacteria will have different metabolic needs as it resists the immune response of the human host and establishes an intracellular niche for replication or dormancy. Seen in this context, determining the mechanism of vitamin C efficacy as an adjuvant requires comprehensive examination of the metabolic state of *M. tuberculosis* during infection. This will entail expertise from multiple disciplines, including microbiology, biochemistry, and immunology, to decipher the usefulness of vitamin C as an adjunct therapy for tuberculosis.

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