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Mycobacterium tuberculosis: A Pathogen That Can Hold Its Breath a Long Time

In this issue of the *Journal*, Bucşan and colleagues (pp. 94–104) present an extensive comparative analysis of two widely used, virulent strains of *Mycobacterium tuberculosis* (Mtb) in the nonhuman primate (NHP) model (1). They found that the Erdman strain was considerably more virulent than the CDC1551 strain by multiple parameters: larger areas of necrotic granulomas, poorer survival, increased bacterial lung burden, increased lung pathology associated with greater systemic inflammation, and a high and early inflammatory myeloid cell influx to the lung with evidence of greater macrophage and T-cell activity in the lung. Comparison of gene expression signatures in the lungs of infected animals by RNA sequencing revealed that pathways associated with the hypoxia response and lung tissue remodeling were induced to higher degrees in Erdman-infected animals.

Combining the observation of hypoxia response genes being elevated in the Erdman-infected animals with the fact that the Erdman-induced necrotic granulomas demonstrated impressively larger volumes and higher bacterial burdens than those from CDC1551, the authors hypothesized that the Erdman strain might be better able to survive and proliferate under hypoxic conditions. To evaluate this, bacterial RNA sequencing was used to study the responses of the two strains to hypoxia *in vitro*. The authors found that transcription of the DosR (dormancy survival regulator) regulon was significantly elevated in the Erdman strain compared with CDC1551. Even when *in vitro* hypoxia was terminated and the bacteria were grown in reaerated conditions, the DosR regulon gene members remained highly expressed in the Erdman strain, whereas expression of these genes returned to basal levels in CDC1551.

DosR is part of a bacterial two-component gene regulatory system that controls the expression of a 48-gene regulon first identified by Sherman and colleagues as a key regulator of hypoxia in Mtb (2). Indeed, it is already known that Mtb DosR regulon mutants fail to persist or cause disease in the NHP model (3). However, before this study, the impact of DosR expression magnitude and its response to reaeration had not been studied in detail in the NHP model. Hence, the importance of this study is that it underscores a key

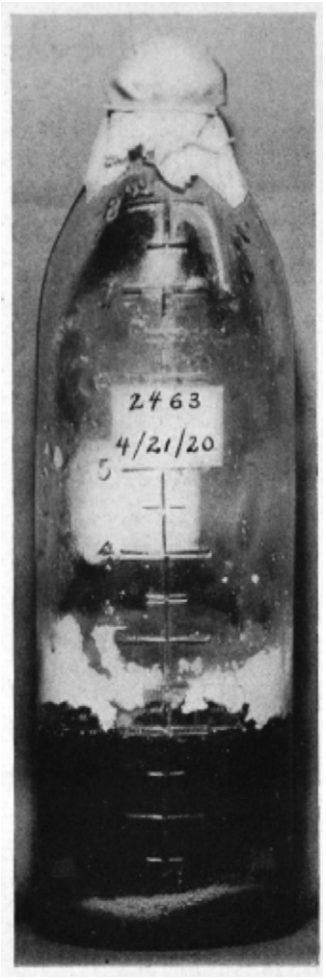

Figure 1. A sealed culture of *Mycobacterium tuberculosis* inoculated on April 4, 1920, and held at 37°C until the spring of 1932. Cultures from the sediments of this bottle grew viable *M. tuberculosis*, which was shown to be virulent in guinea pigs. Reprinted from Reference 7.

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bacterial mechanism that may explain why certain strains may lead to early progressive tuberculosis (TB) disease.

The necrotic granuloma, the pathological hallmark structure of mycobacterial infection (4), is known to be acidic and hypoxic (5, 6), conditions that would be lethal for most pathogens. Yet despite the old dogma that Mtb prefers the more aerated upper lung lobes, Mtb has long been known to survive under hypoxia. *In vitro* studies from more than a century ago demonstrated the unusual ability of Mtb to survive hypoxia. For example, Corper and Cohn showed that sealed Mtb cultures held at 37°C for 12–30 years yielded viable bacilli in their anaerobic sediments (Figure 1) (7, 8). Later, pioneering studies by Lawrence Wayne showed that gradual depletion of oxygen *in vitro* triggers Mtb to enter a nonreplicating but viable state (9), and it was this classic "Wayne model" that enabled Sherman and colleagues' discovery of the role of the DosR regulon in the pathogen's hypoxia response (2).

Although hypoxic conditions are clearly present in the necrotic granulomas of active TB disease, hypoxia in microscopic granulomatous lesions has also been proposed as a trigger for Mtb to enter a latent (nonreplicating or intermittently replicating) state in which the microbe may then survive for decades during latent TB infection (LTBI).

The study by Bucşan and colleagues may offer clues to the divergent roles of hypoxia in active TB disease versus LTBI. Interestingly, the Erdman strain studied by Bucşan and colleagues is from a patient with progressive active TB at the Mayo Clinic in 1946 (10), while the CDC1551 strain, which became a standard laboratory strain for its ability to *1*) be highly transmissible and *2*) cause LTBI, was isolated from an active case of TB in a factory worker on the Tennessee-Kentucky border that spawned 18 subsequent cases of LTBI (11). Although further study is needed, it is possible that Erdman's pattern of elevated *dosR* transcription may predispose it (and related strains) to progressive active TB, whereas lower *dosR* transcription under hypoxia may correlate with a propensity for LTBI.

The study by Bucşan and colleagues raises a number of questions for future study. First is the question of whether the high degree of *dosR* expression observed in Erdman under hypoxic conditions is *causal* in the production of large, necrotic granulomas or whether this *dosR* transcription pattern is *secondary* or *correlative* with another virulence trait of Erdman. A study by Mehra and colleagues showing that four distinct DosR regulon mutants (Δ*dosR*, Δ*dosS*, Δ*dosT*, and Δ*dosST*) failed to persist and to cause disease in NHPs clearly defines the necessity of having an intact *dosR* regulon for Mtb virulence (3), and it certainly suggests that degrees of *dosR* expression in the host might have a causal role in disease progression. However, CDC1551 has a fully intact DosR regulon but nevertheless generates smaller granulomas. Studies with isogenic Mtb strains that express differential degrees of *dosR* may help clarify this causality question.

A second question raised by this study surrounds the genetic basis of the elevated *dosR* transcription phenotype observed in Erdman versus CDC1551. Although the two strains are closely related, lineage 4 Mtb strains (12), it is not yet known what genetic alterations drive the differential *dosR* expression patterns between the two strains. Interestingly, strains from the Mtb Beijing lineage (lineage 2) constitutively express the DosR regulon by virtue of a C601T SNP in the *dosR* promoter (13, 14). However, this Beijing-associated SNP is not present in either Erdman or CDC1551. Hence, novel genetic changes must be present in Erdman that remain to be identified.

In summary, the paper by Bucşan and colleagues adds yet another chapter to the ongoing tale of how Mtb craves oxygen yet can generate large hypoxic, granulomatous lesions and survive in and around them. In light of the centrality of necrotic granuloma formation in the pathogenesis of tuberculous lung damage, cavitation, and disease transmission (4), we need to learn more about the intimate relationship between this pathogen and hypoxia. ■

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New Tricks for an Old Drug: Prostacyclins and Right Ventricular Contractility in Pulmonary Arterial Hypertension

Prostacyclin analogs are the cornerstone medical therapy for pulmonary arterial hypertension (PAH), a disease characterized by progressive pulmonary endothelial dysfunction and remodeling of distal pulmonary arterioles, which can lead to right ventricular (RV) failure (1). Prostacyclins improve cardiac output (CO), functional status, and survival in PAH—effects classically attributed to vasodilatory, antithrombotic, and antiproliferative properties at diseased pulmonary arterioles (2–5). In this model, prostacyclins target PAH vasculopathy to reduce RV afterload and restore ventriculoarterial coupling, a metric of mechanical efficiency. Conversely, approved therapies for RV contractile dysfunction in PAH, a key determinant of survival, do not exist currently. In this issue of the *Journal*, Tello and colleagues (pp. 111–114) challenge this contemporary paradigm of prostacyclin pharmacology in PAH. Through high-fidelity hemodynamic analysis, they conclude that inhaled iloprost enhances CO not only through effects on the pulmonary circulation but also through enhanced RV contractility (6).

To disentangle the relative contributions of afterload and contractility to RV performance, effective arterial elastance (Ea) and end-systolic ventricular elastance (Ees), respectively, can be quantified through analysis of RV pressure waveforms (single-beat method) or pressure–volume loops (multibeat method). When the Ees/Ea ratio is approximately 1.5–2, RV contractility is matched to afterload, and blood is ejected from the RV into the pulmonary circulation with optimal efficiency (7, 8). It follows that decreases in afterload (Ea), mediated by pharmacologic (or surgical) intervention, may lead to a decrease in contractility (Ees) to spare the RV from inefficient energy consumption. Indeed, in patients with PAH initiated on continuous intravenous treprostinil, it has been shown that Ees declined together with Ea after days to months of pulmonary vasodilator therapy (9). This observation would appear to validate the classical model that prostacyclins improve CO in PAH through effects on the pulmonary circulation and RV afterload (9). Importantly, this prior work did not profile the real-time Ees/Ea response to prostacyclin administration, opening a path forward to clarify the acute RV response to prostacyclin therapy.

To understand the acute effects of prostacyclin on RV function, Tello and colleagues administered inhaled iloprost to 32 patients with PAH undergoing high-fidelity conductance catheterization. This approach enabled real-time assessment of RV pressure–volume loops and, thus, direct Ees/Ea measurement through the multibeat method. As a comparator to iloprost, inhaled nitric oxide (NO) or oral riociguat, which stimulates the NO effector, soluble guanylyl cyclase, was administered to a comparator group. Full methodologic details, critically important to the interpretation of sophisticated hemodynamic analysis, are provided in an online supplement. The authors observed that iloprost improved CO and RV ejection fraction versus the pretreatment baseline, but through an unexpected mechanism: lower Ea and increased Ees, leading to a net increase in Ees/Ea from 0.93 ± 0.44 to 1.46 ± 0.70. Conversely, patients treated with inhaled NO/riociguat demonstrated decreased Ea, decreased Ees, and unchanged Ees/Ea. Collectively, these findings suggest that the acute benefits of inhaled iloprost are mediated by afterload reduction and positive inotropy, in contrast to the purely vasodilatory effects of NO/riociguat. The investigators went on to validate the effect of intravenous iloprost using a rodent pulmonary arterial banding model, a system used to study RV performance under conditions of fixed afterload. They observed directionally similar findings for increased Ees and CO with iloprost administration, adding additional plausibility to the concept that iloprost improves RV contractility.

How did the present study detect inotropic effects which have not been demonstrated in patients with PAH before? First, although the efficacy of prostacyclin analogs is generally regarded to be similar, it is conceivable that iloprost may have unique inotropic properties relative to previously studied agents such as treprostinil (10). This study did not compare the effects of iloprost to other prostacyclin formulations. Second, the study by Tello and colleagues was the first to use multibeat determination of Ees and Ea during the acute initiation of prostacyclin therapy. As the multibeat approach may predict PAH outcomes better than the single-beat method, it is possible that multibeat analysis is a more sensitive measure of load-independent RV function (11). Last, the study by Tello and colleagues was designed to test only the short-term hemodynamic effects of iloprost; taken together with prior data collected over a longer period, prostacyclin enhancement of RV contractility may wane during

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