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EDITORIAL COMMENTARY

Sputum Microscopy and *Mycobacterium tuberculosis* Infectiousness

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(See the major article by Datta et al, on pages 514–24.)

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Tubercle bacilli with reduced metabolic activity are present in clinical samples, including sputum, in large numbers. These slowly replicating or nonreplicating bacilli are more able to tolerate antibiotics and resistant to multiple stresses, but their role, if any, in the transmission of tuberculosis is less well established.

In this issue of *The Journal of Infectious Diseases*, Datta et al [1] report mycobacterial counts, after staining with auramine or fluorescein diacetate, in pooled sputum specimens collected overnight at the time of diagnosis from 35 patients with tuberculosis who tested highly positive for acid-fast bacilli. Close household contacts of these patients were monitored for 6 years. Strikingly, the close contacts of patients with a higher proportion of fluorescein diacetate microscopy–negative cells were significantly more likely to develop tuberculosis. This result might be considered unexpected, as fluorescein diacetate microscopy has been successfully used to follow mycobacterial killing during treatment by Salim et al [2], as well as by Datta et al [3]. Dead mycobacterium cells are auramine positive and fluorescein diacetate negative. These results may be less striking to those more familiar with tuberculosis, who will probably immediately think of latent/dormant/fat lazy mycobacteria [4]. Thus, this careful microscopy-based study with detailed follow up, performed over years, provides clinical data that suggest the variously named phenotype of *Mycobacterium tuberculosis* with reduced metabolic activity is fluorescein diacetate negative and may indeed be the most infectious form. Further studies, such as those investigating any correlations between the proportion of acid-fast bacilli–positive and FOA-negative cells in cases and the probability of tuberculin skin test or IGRA positivity among their close contacts, as suggested by Datta et al, would be a logical follow up to this work.

It is widely accepted that a dormant phenotype is critical in the life cycle of *M. tuberculosis.* Latent mycobacteria are the likely reason for long periods of subclinical infection in many contacts and also why such long treatment is required to achieve lasting cure. The cells present in a normal *M. tuberculosis* laboratory culture are indeed visibly different from those seen in patient samples [4], and in an attempt to study this phenotype in vitro, the Wayne model [5] and related culture methods have been widely applied. The elegant study by Datta et al suggests that that the fluorescein diacetate–negative cells are also more suited to establishing new infections, presumably because they survive longer in the environment and/or more efficiently resist the initial assault by the host's immune system.

In the study of Datta et al, a median of 2069 bacteria/μL stained positive by auramine, but only a median of 119 bacteria/μL were FOA positive; thus, the vast majority of mycobacterial cells stained by auramine in this study were FOA negative. Quantitative cultures recovered an even smaller proportion of the auramine-positive cells (median, 40 colony-forming units/μL vs 2069 bacteria/μL). The impact of sputum decontamination, which is required to prevent the overgrowth of other species of bacteria, is difficult to quantify, but the proportions of FOA-negative uncultivable bacteria are impressive. Others have also shown that around 90% of the bacilli in sputum are persisters that are difficult to recover in culture [6], adding credibility to these numbers.

Variations in the proportion of these FAO-negative cells are presumably influenced by the host disease status and the infecting mycobacterial strain. It is tempting to speculate that the differing propensities to enter a latent state observed in certain mycobacterial lineages [7, 8] may be one of the factors explaining their success. Attacking *M. tuberculosis* bacilli by disrupting the apparently critical balance between an active and a latent phenotype is also being considered [9].

In conclusion, the striking findings by Datta et al, as well as a method to identify

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the most infectious patients and target their contacts for preventive therapy, should also stimulate efforts to develop a more complete understanding of the bacteriological details of fat lazy, latent, fluorescein diacetate–negative bacteria.

Note

Potential conflicts of interest. R. M. A. reports widely promoting the use of auramine microscopy for tuberculosis diagnostics over the past 15 years. The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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