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Mycobacterium tuberculosis: new tricks for an old bug

'...there is potentially much to be learned from examining whether mechanisms of drug resistance, or perhaps better referred to as tolerance, in tuberculosis may be similar to those found in other organisms or diseases that are quite distinct.'

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One of the most challenging aspects of tuberculosis (TB) treatment is the need for prolonged therapy to ensure eradication of the disease in a high percentage of patients. Even with the advent of rifampicin (Rifadin®, Aventis Pharma) and pyrazinamide, the standard treatment of drug-susceptible TB still requires 6 months of chemotherapy. Considering the parts of the world in which TB is endemic and the difficulty of delivering long-term therapy in such socioeconomically depressed and medically underserved areas, the goal of shortening the duration of therapy remains one of the highest priorities in the search for newer, more effective regimens.

The most commonly accepted construct explaining the need for prolonged therapy hypothesizes the existence of a subgroup of microorganisms called persisters that are phenotypically resistant to, or tolerant of, the commonly used antibiotics, even though there is no evidence of the classical forms of genetically mediated resistance. The factors responsible for microbial persistence remain largely unknown. As a consequence, there is no general agreement on which experimental system best mimics the persister state. However, there is general agreement that the study of the nonpersister population can be readily accomplished in a variety of laboratory settings or models. The conceptualization of these persister and nonpersister subgroups of mycobacteria has not only contributed to the formulation of current research paradigms, but also to the currently accepted and pursued treatment regimens. TB drugs are viewed as having either bactericidal activity – an ability to kill rapidly growing bacteria (the nonpersisters) – and/or sterilizing activity – an ability to kill persistent bacilli. Current therapy for TB consists of an initial intensive phase of therapy (four drugs given daily for 2 months) usually followed by 4 months of a less intensive phase of treatment (two drugs given from seven-times weekly down to twice weekly). In many ways, these distinct phases of therapy focus on the different subsets of mycobacteria.

While this view of TB mycobacteriology is consistent with some of the empiric observations of the organism's behavior in the human host, the use of a fairly unique set of concepts and terms has perhaps deflected attention away from other conceptual frameworks that would view the behavior of the TB bacillus as being similar, not only to other bacterial microorganisms, but even to eukaryotic cells. The implications of such a shift in thinking would include a consideration that the underlying mechanisms of drug resistance, in other words, those reasons why prolonged therapy is necessary in TB, may be similar to the mechanisms that have evolved in a host of other living cells or organisms. Such an approach could create alternative hypotheses in the search for more effective drugs.

As noted by Young, multiple bacterial organisms respond to stress by actively reducing their metabolic activity and transforming their growth characteristics [1]. For example, *Escherichia coli* synthesizes new proteins under

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selective pressure through changes in transcriptional patterns associated with specific RNA polymerase-σ subunits [2]. This observed reduction in metabolic activity, which may be caused by a variety of stimuli, is phenomenologically very similar to that observed in *Mycobacterium tuberculosis*.

In a recent review of therapeutic opportunities in the treatment of biofilm-associated infections, Ma and colleagues describe multiple characteristics of those infections [3]. Bacterial biofilms may be defined as bacterial communities adherent to an inert or living surface that can tolerate what would otherwise be lethal antibiotic treatment [4]. Biofilms are not only found in device-associated infections such as on prosthetic surfaces, but have also been linked to infections such as endocarditis, osteomyelitis and otitis media. Biofilm-associated infections usually require prolonged antibiotic therapy. A similarity between biofilm-associated infections and TB is that even though conventional antibiotics will often have substantial efficacy against the bacteria that are embedded into biofilms, the biofilms themselves will be much more tolerant of the same antibiotics. Experimentally, when biofilms are disrupted and bacteria resuspended in planktonic culture, they typically regain their original drug susceptibility. Thus, as hypothesized for persisters in TB, bacterial organisms found in the biofilm setting are not drug resistant in the classical sense, rather in a temporary, environmentally dependent manner. It is of interest that in an experimental *Staphylococcus aureus* model, only rifampicin, the most effective drug against persister *M. tuberculosis* cells, retained an equally potent minimal bactericidal concentration in the biofilm model [5].

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Another phenomenon that one could hypothesize plays a role in both biofilm formation and TB persistence is quorum sensing. Bacterial cells produce small molecules that are excreted from the cell for the purpose of signaling and regulating the behavior of other bacterial cells. In some bacterial biofilm systems, disruption of the quorum sensing signals will influence biofilm behavior and thereby antibiotic sensitivity [6].

Environmentally dependent crosstalk between microorganisms that influences parameters such as growth rate and metabolic activity is, however, probably not limited to the prokaryotic world. In the field of antineoplastic therapy, the enigma also exists that killing off a residual small number of malignant cells in an *in vivo* setting appears to be much more difficult than would be predicted based on the known inherent drug sensitivities of the cells. Norton and Simon hypothesized that this was due to an underlying biological phenomenon that the kinetics of tumor growth and tumor regression were best described by Gompertzian kinetics [7]. In this model, tumors are hypothesized to grow at a relatively slow rate during the early stages of tumor growth (perhaps similar to the latent stage in the setting of TB) and also regress slowly when the tumor burden has been considerably decreased (perhaps similar to the phenomenon of persistency in TB).

As one example of the potential commonalities between TB and a seemingly unrelated disease, if one looks empirically at the therapy of Hodgkin's disease, there are potentially striking similarities to the treatment of TB. The fact that the tandard therapy of Hodgkin's Disease also consists of a four-drug regimen is interesting. However, of much greater potential importance is the observation that Hodgkin's Disease is typically treated for 6 months, but it is during the first weeks up until 2 months of therapy that the vast majority of tumor cells are killed. Treatment is still given for a total of 6 months to ensure total eradication of the small residual tumor burden. Many other examples exist in the area of neoplastic growth of eukaryotic cells *in vivo,* whereby it is much more difficult to eradicate a small number of residual cells than would be anticipated based on the killing effects of the same drugs when the body burden of tumor cells is much greater. It is tempting to hypothesize that a generalized phenomenon of quorum sensing is an underlying mechanism that controls changes in drug susceptibility not only in the bacterial world, but also in the area of neoplastic growth of eukaryotic cells.

Based on these phenomenological similarities, there is potentially much to be learned from examining whether mechanisms of drug resistance, or perhaps better referred to as tolerance, in TB may be similar to those found in other organisms or diseases that are quite distinct. Many of the underlying observations that have led to the use of terms such as dormancy, persistence, latency and sterilizing versus bactericidal activity, are fundamentally not dissimilar from observations that have been made in a variety of other systems. The information that has already been gained from the study of these other cells and organisms may provide extremely important leads in furthering the understanding of the operant mechanisms in TB, an understanding which could lead to newer, significantly more effective treatments for TB.

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