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Dormancy and persistence in chronic infection: role of the general stress response in resistance to chemotherapy

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Sir,

In a recent correspondence, Dick[1] usefully refers to phenotypic antibiotic resistance in *Mycobacterium tuberculosis* related to dormancy, the possibility of anti-dormancy drug targets and problems of susceptibility assays. In this context it would be helpful to draw attention to the wider implications of dormancy in persistence and chronic infection in general. Such infections are commonly associated with biofilm/biomass or intracellular growth. We have proposed that growth in these situations leads to expression of the stationary phase general stress response (GSR) relatively early compared with that in conventional laboratory planktonic culture, which contributes to resistance to chemotherapy and persistence.[2] Hengge-Aronis has described the GSR in the natural environment during which key bacterial structures are co-ordinately protected under the control of alternative sigma factors such as RpoS in Gram-negative bacteria.[3] The cells become non-replicating, dormant and resistant to multiple physical and chemical stresses. There are numerous papers showing a general tendency for nutrient depletion and slow or no growth to be associated with antibiotic and biocide resistance.[2] In retrospect, it seems probable that, in addition to the consequences of adaptation to the specific nutrient starvation and to the enforced reduction in growth rate, a major role in resistance is played by the GSR. In at least some cases, density-dependent regulation (quorum sensing) of virulence determinants, biofilm formation and RpoS expression are closely integrated.[4,5]

A quorum-sensing density has typically been synonymous with cessation of exponential growth and entry into stationary phase in planktonic batch culture. By definition, cell density will be high in a compact, adherent biofilm population. As a consequence, relatively small biofilm populations may demonstrate signal-driven, stationary phase survival responses, which equivalent numbers of free-growing planktonic counterparts would not. This could partially explain the general high resistance of biofilm organisms to exogenous stress. We have demonstrated expression of *rpoS* mRNA in sputum from cystic fibrosis (CF) patients.[6] Quorum-sensing signals associated with *Pseudomonas aeruginosa* biofilms have also been identified in CF sputum.[4] These data suggest that, at least in this chronic infection, quorum-sensing signal-driven and *rpoS*-dependent events occur.

Similarly, intracellular or intra-vacuole growth is also likely to lead to an early quorum-sensing cell density and could give rise to an early expression of density-dependent phenomena. Clearly, signal diffusion will be influenced by the nature of the intracellular boundary, or indeed the biofilm substratum. Thus, an impermeable substratum would concentrate any signal, while the degree of hydrophobicity of a vacuole boundary could influence entrapment or diffusion, depending on the chemistry of the signal. A hydrophobic signal could be trapped as aggregates/micelles within biofilm exopolymer and maintain an equilibrium concentration of monomer close to the cell, while hydrophilic molecules could diffuse away.

For many bacteria, including *M. tuberculosis*, co-evolution with lower-order environmental eukaryotic organisms such as protozoa, involving intra-vacuole growth, could have selected the strains now seen in the clinic even before higher animals evolved.[2] This has equipped them not only for enhanced environmental survival, but also for invasion of and survival/replication in cells and tissues of higher animals. Thus, intra-amoebal and intra-macrophage growth of pathogens (involving *rpoS* in at least some cases) has resulted in enhanced invasiveness for macrophages and non-professional phagocytic cells, as well as increased resistance.[2,7] Although circumstantial, it is interesting to note that environmental and clinical strains of a pathogen proved to be equally virulent, showing that the environmental strains already possessed the necessary virulence mechanisms.[8] Thus it may prove helpful to use biofilm and protozoal models to reveal targets for new anti-dormancy drugs.

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