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Advanced cellular systems to study tuberculosis treatment

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Mycobacterium tuberculosis (Mtb) kills more humans than any other infection and drug resistant strains are progressively emerging. Whilst the successful development of new agents for multi-drug resistant Mtb represents a major step forward, this progress must be balanced against recent disappointments in treatment-shortening trials. Consequently, there is a pressing need to strengthen the pipeline of drugs to treat tuberculosis (TB) and develop innovative therapeutic regimes. Approaches that bridge diverse disciplines are likely to be required to provide systems that address the limitations of current experimental models. Mtb is an obligate human pathogen that has undergone extensive co-evolution, resulting in a complex interplay between the host and pathogen. This chronic interaction involves multiple micro-environments, which may underlie some of the challenges in developing new drugs. The authors propose that advanced cell culture models of TB are likely to be an important addition to the experimental armamentarium in developing new approaches to TB, and here we review recent progress in this area and discuss the principal challenges.

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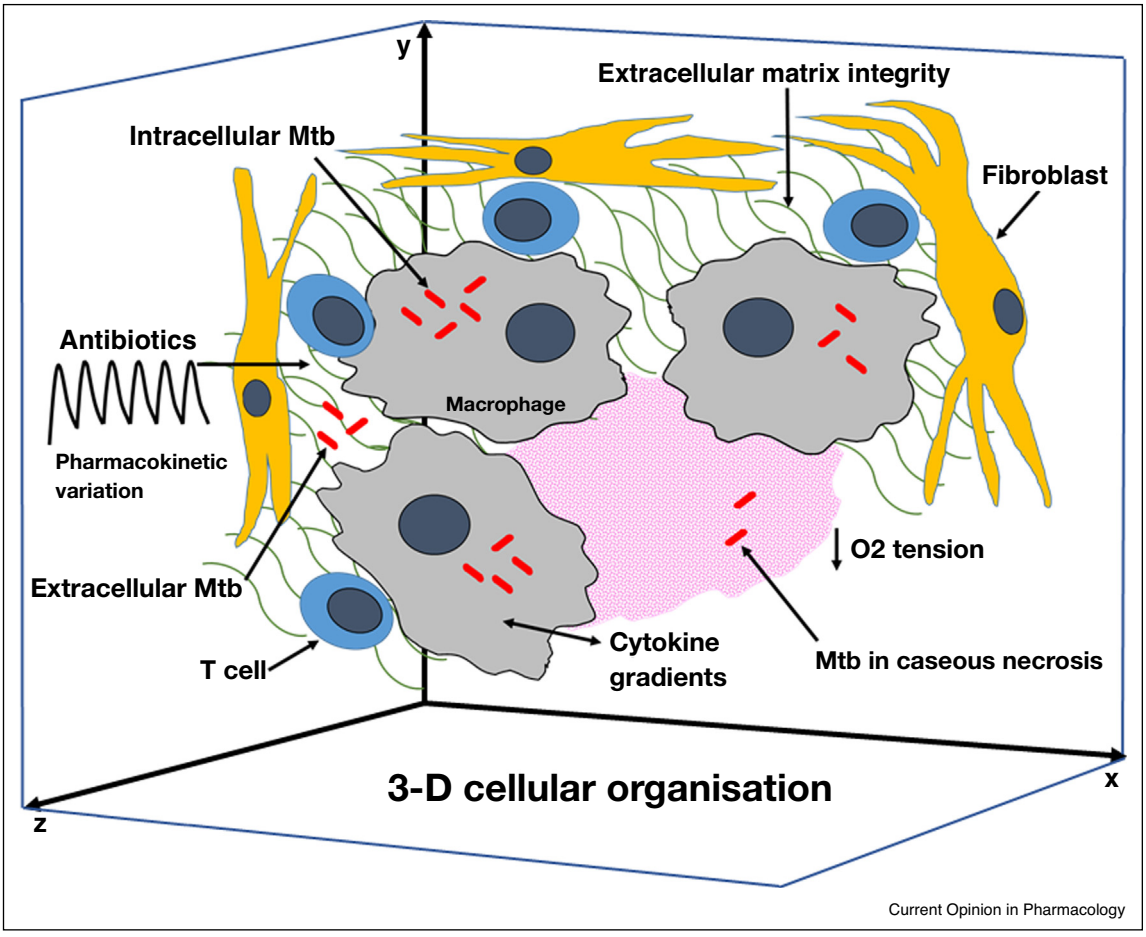
Introduction

Tuberculosis (TB) is a major global pandemic, killing more people than any other infectious disease [1]. TB treatment is complicated by prolonged duration of treatment, ranging from six months for drug sensitive disease to 24 months for drug resistant disease, which is increasing in incidence. Therefore, it is widely accepted that a much stronger pipeline of new anti-tuberculous drugs is required. The current standard system of developing new antibiotics relies on the '3M's': Minimal inhibitory concentration (MIC), Mouse and Man [2]. This system has successfully identified new agents now clinically used for treating multidrug resistant TB, but the failure of recently studied treatment shortening regimes indicates limitations in this approach [3]. Each *in vivo* experimental model has benefits, but also limitations. For example, the mouse is widely used and has the benefit of being genetically tractable and relatively inexpensive, but the histology of wild type mouse granulomas differs from man and lacks hypoxia [4]. Novel models, such as the 'Kramnik' C3HeB/FeJ mouse, develop hypoxic caseating granulomas, although with much higher mycobacterial loads than human granulomas [5]. The guinea pig and rabbit TB models are well characterised and form hypoxic granulomas, but are relatively limited by cost of housing and lack of immunological reagents [6]. The zebrafish model has the potential of high throughput [7], but uses *Mycobacterium marinum* as opposed to Mtb and zebrafish larvae lack T cells. The non-human primate model is limited by cost, throughput and ethical concerns [6,8]. Furthermore, it has been recently reported that extreme drug tolerance of *Mycobacterium tuberculosis* may occur in caseum [9], suggesting that it is important to use models that represent the diverse micro-environments encountered during human TB (Figure 1) [10].

Pyrazinamide is one of the most important front-line agents in the treatment of human TB, and was discovered relatively fortuitously. Significantly, it would have not have been discovered by current approaches used to develop new TB treatments [2]. Due to its structural similarity to nicotinamide, which showed some activity against mycobacteria in animal models, pyrazinamide was directly tested *in vivo* and found to be effective, bypassing nutrient rich selection where it would have been ineffective [11]. This indicates the need to develop and investigate novel systems that replicate the complex physiology of the host-pathogen interaction that occurs in human patients. Human granulomas are multicellular structures containing both inflammatory and stromal cells organised in 3-dimensions, with the matrix regulating the host-pathogen interaction [12] and often with central caseous necrosis. In addition, Mtb can be cultured from macroscopically normal lung tissue. Consequently, it seems likely that testing drug efficacy at the single cell level will not reflect the complexity of micro-environments within humans. Considering the nature of clinical TB, we propose that the key attributes of such a system should include primary human cells, fully

Figure 1



The complexity of human TB granulomas. Mtb resides in different micro-environments with the granuloma, a multicellular structure organised in 3 dimensions with different extracellular matrix composition. Modelling antibiotic killing of Mtb *in vitro* may need to reflect all these micro-environments.

virulent *Mycobacterium tuberculosis*, multiple host cell types, three-dimensional organisation, prolonged duration of infection, high throughput and with the potential for dynamic environmental modelling and study of different micro-environments. In recent years, there has been significant progress in developing such model systems to study novel treatment approaches and we review these and then discuss future directions.

Emerging advanced cellular models

Formation of multicellular organised granulomas is a hallmark of TB infection. *Mycobacterium tuberculosis* is capable of residing within granulomas for a prolonged time asymptomatically during latent infection. Several researchers have developed *in vitro* 2-dimensional models of human mycobacterial granulomas [[17••],13]. Progressive recruitment of macrophages around live bacteria have been observed in these models, which reflects initial steps in host granulomatous response enabling cellular and molecular analysis of this event [14–16]. The complexity of interactions that take place inside human TB granulomas have been elegantly presented by Guirado and colleagues. They developed an *in vitro* granuloma model using human primary blood cells from individuals with and without latent TB infection and demonstrated that the granulomatous response was significantly different between the two groups [17••]. Crouser subsequently demonstrated that mRNA expression patterns of granulomatous response from latent TB patients significantly differs from the molecular profiles of individuals with sarcoidosis [18•].

Emerging concepts within TB granulomas are the importance of 3-dimensional (3-D) organisation and the regulatory role of the extracellular matrix [12,19], and 3-D models of *M. tuberculosis* granuloma have been developed. In these systems, infected primary human cells are co-cultured with collagen matrix gels, agarose beads or agarose-coated plates. Kapoor and colleagues created