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Host–pathogen systems for early drug discovery against tuberculosis

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Tuberculosis (TB) is a global disease causing 1.8 million deaths each year. The appearance of drug-resistant strains raised the demand for new anti-mycobacterial drugs and therapies, because previously discovered antibiotics are shown to be inefficient. Moreover, the number of newly discovered drugs is not increasing in proportion to the emergence of drug resistance, which suggests that more optimized methodology and screening procedures are required including the incorporation of *in vivo* properties of TB infection. A way to improve efficacy of screening approaches is by introducing the use of different host–pathogen systems into primary screenings. These include whole cell-based screenings, zebrafish larvae-based screenings and the impact of artificial granuloma research on the drug discovery process. This review highlights current screening attempts and the identified molecular targets and summarizes findings of alternative, not fully explored host–pathogen systems for the characterization of anti-mycobacterial compounds.

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Tuberculosis is a global health threat

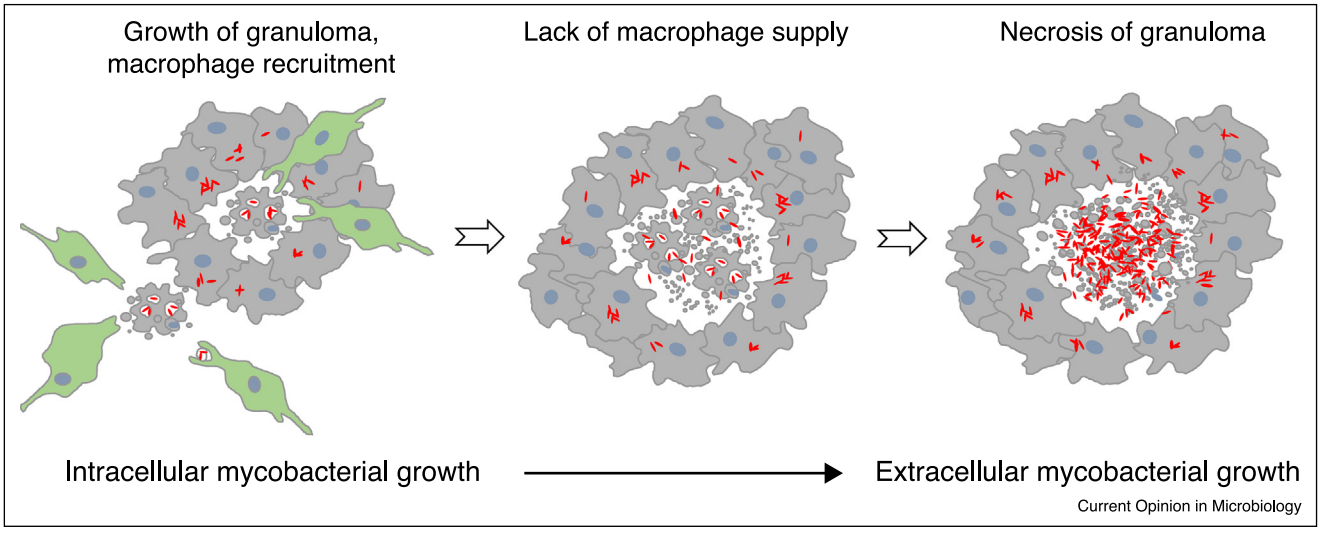
Tuberculosis (TB) is a worldwide threat that causes 1.8 million deaths each year of which 0.4 million individuals are HIV-positive [2]. It also results in more than 10 million new cases of infection each year. *Mycobacterium tuberculosis* (Mtb) is the main causative agent of TB, which mainly targets lung macrophages. The hallmark of TB includes the formation of granulomas, specific multicellular structures, which are formed in response to mycobacterial infection (Figure 1). The therapy of drug-susceptible TB requires an intensive phase of two months treatment with ethambutol, isoniazid, rifampicin and pyrazinamide followed by a four months continuation phase of isoniazid and rifampicin alone. There are several drug candidates in the anti-TB drug pipeline including TBA354, currently in phase I trials, and other drug candidates such as SQ109 [3], imidazopyridine amide Q203 [4], benzothiazinone PBTZ169 [5], with some candidates being shown to act on 'promiscuous' targets [6].

Despite advancements in the treatment of TB, the danger has not been diminished due to the emergence of multi-drug resistance (MDR-TB). MDR-TB manifests itself when strains display resistance to isoniazid and rifampicin, whereas extensively drug-resistant tuberculosis (XDR-TB) implies resistance to second-line drugs, notably against fluoroquinolone, capreomycin, kanamycin and streptomycin. In 2015, MDR-TB affected 3.3% of new cases and 20% of previously treated cases, and accounted for 190 000 deaths [2]. These statistics show clearly a need for more efficient new drugs.

Host–pathogen phenotypic screening as a way to improve early drug discovery approaches

Factors that make the identification of anti-TB drugs a challenging task can be subdivided into several broad categories. These include the intrinsic properties of the pathogen: the generally low metabolic rates of Mtb [7], the highly impermeable hydrophobic cell wall, the intracellular degradation and the activity of molecular excretory pumps of the bacterium [8]. Another category comprises host-associated properties of TB infection: the ability of Mtb to manipulate the environment inside alveolar macrophages by the interference with membrane trafficking and cell signaling [9,10], the modification of autophagy [11,12], and by the induction of programmed cell death [13,14]. Host cells can also decrease the antibacterial effect of compounds *via* the activity of molecular pumps and intracellular degradation. A third category includes complex multicellular interactions involving primary and secondary immune responses, particularly the formation of granulomatous structures that both help to contain and to facilitate dissemination of the infection [15,16]. The list of categories could be expanded even further by encompassing factors of different populations; however, addressing these issues goes beyond the scope of this review.

Figure 1



TB granuloma formation. Intracellular mycobacterial growth is promoted in early TB granulomas by co-opting host macrophages that serve as niche for bacterial growth. Conversely, Pagan *et al.* [1] show that an adequate macrophage supply to the granuloma is required to prevent necrosis of infected macrophages and subsequent extracellular bacterial growth.

The challenges described above explain why many drugs with high bactericidal potential during axenic growth of bacterial cultures show often not significant effects in *in vivo* infection experiments [16]. These specific challenges of TB drug research are the main reason why the probability of a transition from potential hit candidates to an approved TB drug is astonishingly low. Furthermore, it is important to establish a holistic understanding of TB infection that incorporates host–pathogen interactions at different levels. In particular, by systematically neglecting the complex *in vivo* interactions between Mtb and its host, target-based research was often not very successful and initiated a change towards phenotypic screening technologies [17].

Therefore, it is important to improve the efficiency of drug discovery workflows to include several parameters of TB infection simultaneously. One important aspect is to make early drug discovery phenotypically more similar to human TB infection, for example, by introducing a suitable host model into phenotypic screening approaches. The implementation of host models allows the exclusion of chemical entities shown to be suboptimal in *in vivo* conditions, which helps to preserve time, material and human resources. Moreover, the use of host–pathogen screening systems allows targeting of specific host–pathogen interactions [18], such as recognition and uptake of Mtb [19], modulation of phagosomal maturation [20], host cell lipid metabolism [21–23], autophagy [24,25], induction of cell death [14], cytokine therapy [26,27], activity of protein kinase R [28], and granuloma formation [29,15].

Despite new opportunities, it is important to keep in mind that phenotypic screening based on host–pathogen systems raises another set of challenges, for example, the choice of compatible host and pathogen models. None of the existing TB model systems can integrate all the complexity of human TB infection. One have to prioritize some factors and parameters over another, such as costs, reproducibility, ease of manipulations, ethical constraints *etc.* The choice of the model system is largely dependent on the questions that are asked. The parallel use of different screening approaches combined with suitable models could be more beneficial to the overall outcome of the study and to reduce the risks of failure.

Selection of suitable pathogen models for phenotypic screening approaches

The selection of suitable pathogen models for phenotypic screening requires careful attention. The various virulent strains of Mtb, such as H37Rv, are the most straightforward and phenotypically accurate choice. The drawbacks include limitations regarding the handling of biosafety level three pathogens, which reduces the number of available research facilities and demands extensive personal training. In addition, the low growth rates and metabolic turnover of these strains may slow down high-throughput screenings. Among alternative model systems, the most popular ones include *Mycobacterium bovis BCG*, *Mycobacterium smegmatis* and *Mycobacterium marinum* [30]. *M. bovis* BCG belongs to the Mtb complex, yet it is a biosafety level two pathogen, and has similar growth rates compared to Mtb.