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New Drugs for an Old Foe: *Mycobacterium tuberculosis* Meets PSC-Derived Macrophages

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Infections with *Mycobacterium tuberculosis* (*Mtb*) are still among the top 10 causes of death worldwide, highlighting the utmost need for new forms of medical treatments. In this issue of *Stem Cell Reports*, Han et al. (2019) describe a technique to screen therapeutically active compounds targeting *Mtb* using pluripotent stem cell-derived macrophages.

According to the World Health Organization (WHO), infections with *Mycobacterium tuberculosis* (*Mtb*) still remain a global health burden with 10 million infected individuals and more than 1 million deaths worldwide in 2018. To counteract this threat, one of the most common forms of anti-tuberculosis treatment today is the prolonged administration of antibiotics, e.g., isoniazid, rifampicin, pyrazinamide, and/or ethambutol, which interfere with the life cycle of *Mtb* at various stages. While antibiotic treatment is the current backbone of anti-*Mtb* therapy, multi-drug resistant (MDR) strains of *Mtb* have been described, highlighting the need for new drugs and/or molecules that interfere with *Mtb*. To develop such new compounds, improved understanding of the host-pathogen-interaction as well as the mode of action of described anti-*Mtb* antibiotics would be beneficial. While the latter can potentially be addressed by recent technological advances (Greenwood et al., 2019), host-pathogen interaction and thereby the identification of new therapeutically active compounds requires ambitious cell culture systems.

It is worth mentioning that alveolar macrophages in the lung act as the first line of host defense, protecting the host from invading mycobacteria. Alveolar macrophages belong to the class of tissue resident macrophages and are important cellular components in (1) the maintenance of lung homeostasis and (2) pulmonary host defense machinery. Given their important function as phagocytes, alveolar macrophages phagocytose the entering mycobacteria bacilli following inhalation. While most of the mycobacteria are successfully degraded within the macrophages by (phago)lysosomal degradation, some mycobacteria have the ability to evade this process and survive within the alveolar macrophages (Pieters, 2008). Given the essential role of macrophages in the context of mycobacterial infections, new drugs, especially new candidates that interfere with the life cycle of *Mtb* within macrophages, are of high therapeutic interest.

In this issue of *Stem Cell Reports*, Han and colleagues demonstrate the use of human pluripotent embryonic stem cell (ESC)-derived macrophages (referred to as induced macrophages; iMACs) for the identification of new drugs that interfere with the life cycle of *Mtb* (Han et al., 2019). In addition, the authors not only present an advanced protocol to derive macrophages from pluripotent stem cells (PSCs), but they also use this technique to establish a screening platform for new compounds that interfere with *Mtb* within macrophages.

In fact, the use of PSCs as an inexhaustible cell type to derive multiple different mature cells of a human organism and their subsequent use in drug screenings has been proposed and shown previously. While induced pluripotent stem cells (iPSCs) have also entered the stage in 2006, the authors have decided to use human ESCs as well as iPSCs as starting material to derive defined iMAC populations (Figure 1). As of yet, current studies and experimental efforts that aim to elucidate macrophage and *Mtb* interaction rely either on the use of primary monocyte-derived macrophages or on macrophage-like cell lines (e.g., THP1, MonoMac6, and others). While primary monocyte-derived macrophages can be isolated from human blood tissue samples, clearly the cost intensive isolation/generation, donor to donor variability, and limitations with respect to up-scaling limit the use of these cells. In contrast, human macrophage-like cell lines can in principle be up-scaled; however, limitations with respect to continuous cultivation, and the respective genetic background, hamper the reliability of this system. To circumvent these problems, the authors used defined ESCs and further improved a previously published macrophage generation protocol (Yanagimachi et al., 2013). Using this new technique, the authors were able to generate approximately $5x10^8$ macrophages from only 20 colonies. This number of macrophages is clearly a step forward in the generation of both ESC and iPSC-derived macrophages. While current classical adherent based-protocols are sufficient to generate macrophages in a magnitude of 10^5–10^7, scalable suspension-based differentiation techniques performed in stirred tank

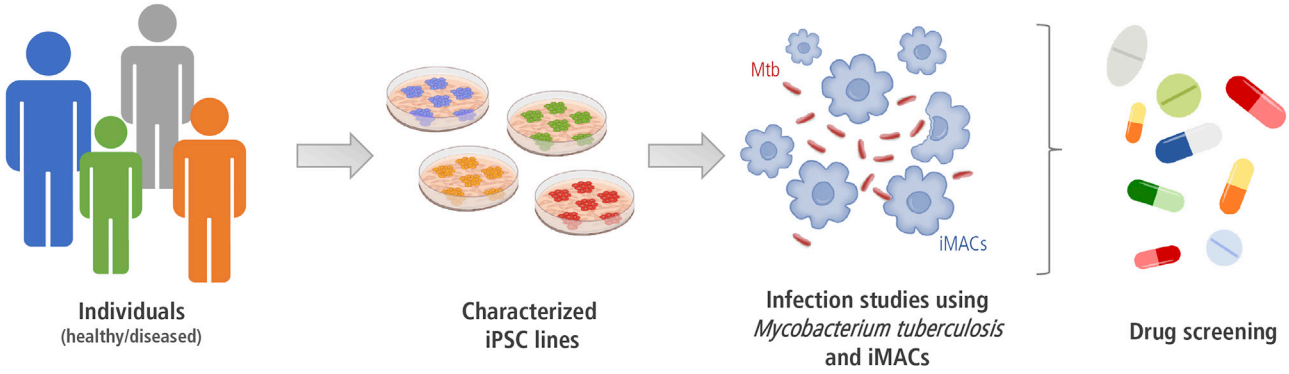

Figure 1. Generation of iMACs for Drug Screening

Defined and fully characterized induced pluripotent stem cells (iPSCs) can be generated from healthy or diseased individuals and subjected to the generation of induced macrophages (iMACs). These iMACs can subsequently be used in infection experiments using *Mycobacterium tuberculosis* (*Mtb*) or other pathogens in order to identify new drug candidates.

bioreactors are able to generate cumulative macrophage populations in the magnitude of 10^8 (Ackermann et al., 2018).

In fact, the iMACs generated by the authors represent a very homogeneous population of macrophages that are positive for classical monocyte/macrophage markers such as CD45/CD11b and CD86. Furthermore, the authors show high expression of HLA-DR on iMACs, which is an MCH class II molecule primarily involved in foreign antigen presentation that gets upregulated upon stimulation with IFNγ or infection. This observation is surprising, since many protocols for the generation of iMACs show no or low expression of HLA-DR in unstimulated cells (Hale et al., 2015; van Wilgenburg et al., 2013), which is in contrast to peripheral blood derived monocytes and alveolar macrophages. The high expression might be an advantage in faithfully recapitulating the host-pathogen interaction between *Mtb* and macrophages. It is worth mentioning that the authors can also produce iMACs in a continuous fashion and cells can be harvested at various time points. To also ensure continuous quality of the cells with each harvest, the authors compared iMACS from an early (day 20) with a late (day 60) time point and found that the cells were highly similar. They clustered together in multidimensional scaling analysis and showed a similar transcriptomic profile. In fact, previous studies employing macrophages from various steps of differentiation could also see similar surface marker expression (Ackermann et al., 2018), further highlighting the continuous quality of produced iMACs.

Besides the phenotypic characterization of produced iMACs, the transcriptomic fingerprint of generated iMACs is of great interest, especially considering the stage of differentiation or developmental origin of iMACs. Here, the authors compared the iMACs to human primary macrophages derived from $CD14^+$ peripheral blood cells (hMDMs) as well as a human macrophage cell line (THP-1) and ESCs as a negative control. The iMACs from both the early and late harvest points showed a high degree of similarity to the hMDMs and both were distinct from the THP-1 cells. However, while iMACs and hMDMs were mostly similar, there were still differences in their transcriptomic profile and the authors found that in this case they were mainly related to cell adhesion. Others have noted as well that iMACs share a core transcriptomic profile with hMDMs (Karlsson et al., 2008) but subtle differences still exist. Although the authors showed similarities between hMDMs and iMACs, it has to be noted that recent studies could also show a more primitive fingerprint of iPSC-derived macrophages, putting them in close proximity to yolk sac or fetal liver macrophages (Buchrieser et al., 2017). The differentiation of iPSC-derived macrophages seems to exhibit common transcription factor requirements that are in line with primitive yolk sac hematopoiesis, which is MYB independent and RUNX1 dependent. The lung harbors two distinct macrophage populations—interstitial and alveolar macrophages—which are thought to be of distinct origins (monocyte-derived and of primitive origin, respectively).

To test iMACs' functionality, Han et al. challenged the cells with the influenza virus H3N2 first. Cells were able to internalize the viral particles and showed typical response in regard to reactive oxygen production and cytokine secretion. As a next step, they challenged the cells with *Mtb* (Figure 1). iMACs were permissive for *Mtb* at various MOIs and exhibited a strong response measured by cytokine release and upregulation of genes connected to innate immune response. Other groups have also used iPSC-derived

macrophages to study infection with a wide variety of pathogens such as *Salmonella* spp. (Hale et al., 2015), HIV (Higaki et al., 2018), and *Pseudomonas aeruginosa* (Ackermann et al., 2018) and were able to show similar results.

Given this promising data, the authors used the iMACs to establish a screening platform based on microscopic scoring of the cells infected with a GFP-fluorescent *Mtb* strain. Using this assay, they screened a drug library and were able to identify a novel compound called 10-DEBC. 10-DEBC's mode of action is via inhibition of AKT1 signaling, a known pathway involved in regulating *Mtb* growth. This compound was also tested in primary macrophages by the authors and showed similar results here, again highlighting the suitability of using iPSC-derived macrophages for drug discovery. To the best of our knowledge, this is the first drug screening platform for *Mtb* using iPSC-derived macrophages. Macrophages play an important role in many different diseases and iPSC-derived macrophages have been shown to be useful disease modeling tools. In this line, the drug screening platform could also be expanded to encompass the screening of small molecules for other diseases and indeed other pathogens.

In summary, Han et al. introduce a protocol for producing iMACs in large quantities and utilize these cells to build a drug screening platform for *Mtb*, which could be expanded to include other pathogens and diseases.

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