Host Directed Therapies for Tuberculosis: Futures Strategies for an Ancient Disease

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Tuberculosis · Antibiotic resistance · Host-directed therapies · Personalized medicine

Abstract

The emergence and spread of drug-resistant strains of *Mycobacterium tuberculosis* is worsening the global threat of tuberculosis (TB). There is a need and urgency for the development of new treatments for TB, for the management of drug resistant TB (MDR-TB) and for improved regimens against drug-susceptible TB, with the goal of reducing toxicity and length of therapy that will boost patient compliance. The paucity of new drugs is a major obstacle to design new regimens while host-directed therapies (HDTs) are emerging as a promising area of research and are opening new avenues to fight TB. In this review, we discuss examples of potentially promising strategies aimed at improving the host response to *M. tuberculosis*, and argue how a better understanding of TB pathogenesis, with the fine characterization of the immunological mediators involved, may pave the way for the design of new therapies, the identification of new drugs or the

repurposing of some already in use for other diseases. We emphasize that any HDTs shall be included as adjunct therapy to the drug-combination regimens already in use for TB, with the goal to reduce tissue damage and immunopathology and enhance bacterial clearance. We anticipate that the benefits of HDTs against TB will be highest against MDR-TB, where the activity of current regimens is poor and the cost high.

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The Challenge of Treating Tuberculosis

Despite the availability of effective drugs against *Mycobacterium tuberculosis* (*Mtb*) since more than 50 years, tuberculosis (TB) remains a major infectious disease at global level [1]. The most recent report by WHO estimated about 10.4 million as the new TB cases and 1.7 million deaths in 2016 [1]. Most of the TB cases occur in sub-Saharan Africa, South-East Asia and Eastern Europe, although the recent migrations of people from these geographic areas have seen a re-emergence of the

disease in Western Europe. In recent years, a main cause of concern has been the emergence of *Mtb* strains showing resistance to 2 of the most commonly used effective drugs: isoniazid and rifampicin. These multi-drug resistant *Mtb* strains (MDR-TB) were responsible for 0.49 million cases of TB, mostly in India, China and the Russian Federation [1]. Moreover, approximately 6.2% of MDR-TB strains showed a pattern of extensively drug resistance (XDR-TB), with additional non-susceptibility to fluoroquinolones and an injectable drug [1]. Currently, WHO recommends that patients with MDR-TB should be treated with a combination of at least 4 active anti-TB drugs for a minimum of 20 months [2]. This is an exceptionally long duration of therapy for a bacterial disease, which causes several adverse and toxic side effects that undermine patient compliance to treatment. The prolonged and complex anti-TB chemotherapy for MDR- and XDR-TB cases includes antibiotics that are very expensive. For instance, combining direct and indirect costs, the average cost per-TB case in the original EU states was estimated at 10,282 EUR for drug susceptible TB, 57,213 EUR for MDR-TB and 170,744 EUR for XDR-TB, with a total cost for TB treatment in Europe surpassing the 0.5 billion EUR in 2011 [3]. Hence, the emergence of drug resistance is posing a major threat to control TB, with important health, social and economic consequences [2].

Many efforts have been made in the last 2 decades to develop new drugs against TB, though only recently and after nearly half a century since the approval of the last anti-tuberculous medication, 2 new drugs, delamanid and bedaquiline, have been included in regimens to treat MDR- or XDR-TB patients [2]. Moreover, the repurposing of other drugs as linezolid and carbapenems/clavulanate offer another option under specific conditions, though the anti-tuberculous activity of these drugs remain much lower compared to that of first-line drugs [4].

These limitations emphasize the need and urgency for the development of new treatments primarily for the management of drug-resistant TB, although improved regimens against drug-susceptible TB aimed at reducing toxicity and duration of the current combination therapy are also needed to reduce the risk for the emergence of drug-resistance [5]. In the last few years, thanks to a better understanding of TB pathogenesis and the characterization of the immunological mediators involved, innovative and potentially highly rewarding approaches have been proposed as host-directed therapies (HDTs) that can be used as adjunct to antibiotic-based anti-TB treatment.

Potential of HDTs for TB

The term HDTs is used to define treatment options with small molecules or biologics that can provide an antimicrobial or beneficial effect by (a) interfering with host mechanisms exploited by the pathogen to persist or replicate in host tissues; (b) boosting host immune defences against the pathogen; (c) targeting pathways that may contribute to disease or immunopathology, as classically highlighted by hyper-inflammation; (d) modulating host factors at local level that are associated with pathogenetic responses [6]. HDTs can be used to target specific pathways that play a causal role in the pathogenesis of a given infectious disease or to ease the symptoms, as when targeting hyperinflammation. For all these reasons, several HDTs are emerging as valid opportunities to treat TB, also because they can be administered in combination with antibiotic regimens against drug-resistant *Mtb* strains and they raise little concerns for the emergence of therapy resistance, since they directly target highly conserved host pathways. The goals of HDTs against TB may be several and may include shortening the course of treatment; reducing the number of agents required in combination drug therapy, particularly when toxicity is a concern; improving the efficacy of regimens against drug-resistant TB, which are known to be usually lower than the standard one; preserving lung function in a context of extensive tissue damage (Fig. 1). The complex immunological events unfolding during *Mtb* infection and TB pathogenesis offer many opportunities for HDTs and recent findings on the cellular and molecular players involved are further widening the list of molecules and biologics that can serve the purpose.

The Host Immune Response during *Mtb* Infection

The most common outcome following *Mtb* infection in an immunocompetent host is latent TB infection, with no signs or symptoms of active disease [7]. This underscores the critical role of host innate and adaptive immune responses in the control of *Mtb* infection and the intrinsic ability of host responses to contain *Mtb* replication while preventing the development of the typical tissue damage, which forms the hallmark of active TB [8]. Remarkably, latent TB is characterized by the persistence and a certain degree of replication of *Mtb* in host tissues that remain in a dynamic equilibrium with the host, which in most cases lasts for lifetime [9, 10]. Experimen-

Fig. 1. HDTs are aimed at supporting the host immune response to contain bacterial replication and tissue damage. Infection with *Mtb* results in a dynamic equilibrium between the bacilli and the host that may lead to a spectrum of conditions spanning from LTBI to active TB. Different HDTs can support the host in controlling *Mtb* replication and/or reducing tissue damage or the pathological response and their use as adjunct therapy in combination with anti-TB drugs may significantly improve treatment outcomes, particularly in the case of MDR-TB. HDT, host directed therapies; TB, tuberculosis; *Mtb*, *Mycobacterium tuberculosis*.

tal evidences obtained in the non-human primate model of TB indicate that animals latently infected with *Mtb* have a significant number of replicating bacilli in lung lesions and that animals with active TB can sterilize certain granulomas but not others due to the heterogeneity in the innate and adaptive responses in individual lesions, which dictate the clinical outcome of TB infection [11]. The factors (chemokines, cytokines, type and phenotype of immune cells involved, etc.) responsible for the consequences at local level remains to be determined, but these findings highlight the critical role of the host immune response in the control of *Mtb* replication, or emergence of active disease instead. Notably, most lung lesions appear to be funded by a single bacterium and reach a similar maximum bacterial burden [11], further underscoring the clinical implications of the events occurring at the cellular level in each lesion.

The Intracellular Outcomes of *Mtb* Infection

The fate of intracellular *Mtb* within the phagocytes is determined by various cellular processes, including autophagy, activation of, or evasion from, 1 or more host defence pathways and by the type of cell death occurring in infected cells (apoptosis, necrosis, pyroptosis, efferocytosis) [12]. *Mtb* is rapidly phagocytosed by macrophages through a number of mechanisms and once inside the cell, the tubercle bacillus actively secretes proteins and virulence factors that by inhibiting the phagolysosomal fusion prevent the acidification of the *Mtb*-containing vacuole and the full activation of the macrophage antimicrobial response [13, 14]. Inside these non-acidified phagosomes *Mtb* flourishes, causes vacuole membrane permeabilization that promotes translocation in the cytoplasm of *Mtb* proteins, lipids and other mole-

cules that can directly interfere with several intracellular pathways including autophagy [15, 16]. Under these circumstances, *Mtb* can also translocate into the cytoplasm where it can reside and multiply protected from the classical antibacterial host defences [17]. At the same time, phagosome permeabilization mediated by the bacterial ESX-1 secretion system allows cytosolic components of the ubiquitin-mediated autophagy pathway to access mycobacteria-containing phagosomes, further promoting microbial killing and phagosome delivery to the lysosome [15, 18].

Autophagy is an evolutionary-conserved cellular process that mediates the degradation of bulk cytoplasm and entire organelles, primarily regulated by the mammalian target of rapamycin (mTOR) complex 1 [19, 20] and the adenosine monophosphate-activated protein kinase (AMPK) [20]. Seminal studies have indicated that activation of the autophagic flux by starvation or rapamycin treatment restricts *Mtb* intracellular growth [21] and that virulent *Mtb* can inhibit autophagy in an ESX-1-dependent mechanism to resist killing [22, 23]. Other reports have highlighted a more complex relationship, with *Mtb* capable of selectively modulating autophagy as a strategy to survive intracellularly and preventing infected cells from undergoing apoptosis [24, 25]. In line with these findings, *Mtb* DNA reaching the cytoplasm activates the host DNA-sensing pathway, targeting bacteria to autophagy in a process involving cGAS and the STING/TBK1/IRF3 pathway [26, 27]. This pathway has been shown to activate the Type I IFN that, by promoting inflammation, may be linked to TB pathogenesis [27], which plays a critical role in dictating the clinical outcome of *Mtb* infection [12, 28]. Indeed, mice where ATG5 has been geneti-

cally inactivated are more susceptible to *Mtb* infection, though depletion of the PMNs using a specific antibody was able to protect these mice from the emergence of extensive tissue damage and inflammatory response in the lung [12], pointing to a key link between autophagy and inflammation.

The Consequences of Mtb Infection

When the immune response is partially successful, activated macrophages and other host cells (T cells, B cells, and fibroblasts) surround the *Mtb*-infected cells in an organized display, a granuloma, creating hypoxic, acidic, nutrient-poor conditions that are less permissive for *Mtb* replication. However, these lesions are not always sterilized, as *Mtb* employs a number of strategies to ensure its survival, co-opting the trafficking of cells within the granuloma to expand the number of infected cells, and inhibiting macrophage apoptosis to preserve its host niche [29].

The failure to contain bacterial replication may lead to the release of *Mtb* in the extracellular environment where it can actively replicate, release toxic lipids and proteins and interact with host cells. The interaction between phagocyte pattern recognition receptors and *Mtb* antigens triggers the production of various pro-inflammatory cytokines, including tumour necrosis factor- α (TNF- α) and interleukin-12 (IL-12) as well as chemokines that recruit and activate other innate and adaptive immune cells from the circulation to the site of infection [30]. Macrophages activation, supported by the emergence of the adaptive immune response with the key role of T cells, leads to phagosome-lysosome fusion, secretion of cytokines such as TNF- α , IFN- α , IFN- β , IL-6, IL-12, and IL-1 β that while potentially enhancing the antimicrobial response, can also cause overt lung inflammation and tissue damage, which is an hallmark of TB disease [31, 32]. It is well known that acquired or primary immunodeficiencies are associated with an increased risk of developing TB disease following *Mtb* infection due to the host's failure to efficiently control bacterial replication. However, it remains still unclear how, in the immunocompetent host, the dynamic equilibrium between the immune system and *Mtb* can permit the control of bacterial replication in the absence of any substantial inflammation (latent TB), or conversely lead to tissue damage and overt disease (active TB) [33]. In the latter case, it is the exuberant immune response, with the plethora of inflammatory cytokines promoting tissue damage that supports the emergence of active disease.

Targeting the Host to Treat TB

The contribution of the host immune response to the emergence of active TB indicates the possibility to target the host, in 1 or more of the numerous biological and immunological mechanisms involved, with HDTs aimed for instance at lessening the inflammatory response and associated host tissue damage. In the last few years, several drugs have been tested or repurposed for the treatment of TB. Table 1 summarizes the main features of some of the most relevant HDTs that have been tested in pre-clinical experiments or in clinical trials, listed based on the biological process that they target.

HDTs that Target the Autophagic Pathway

Autophagy plays a critical role in restraining *Mtb* replication within the macrophage and contributes to efficient antigen presentation and modulation of cell death [21]. One of the best-studied molecules for inducing autophagy is rapamycin, which inhibits mTOR kinase activity. Rapamycin has been used for over 30 years to counter transplant rejection due to its global immunosuppressive effects [34]. Blockade of mTOR by rapamycin delays dendritic cell (DC) maturation and inhibits antigen uptake and presentation by DCs [35], enhancing the generation and function of regulatory T cells, which may account for its immunosuppressive effects during transplantation [35]. Interestingly, in contrast to what was expected, when administered at lower concentrations, rapamycin has been shown to induce immunostimulatory effects [36]. For example, rapamycin administration to BCG-vaccinated mice was shown to elicit better vaccination efficacy against *Mtb* infection, and it was associated with induced autophagy, increased antigen presentation on DCs and elevated Th1-type immune response [21, 37]. In short, several drugs or natural products that are known activators of autophagy, or which have been identified in the context of other diseases and are now recognized as modulators of autophagy, may be effective against TB. Recent studies suggest that lithium, valproic acid, prochlorperazine, and haloperidol all induce autophagy [38]. Specifically, lithium has been shown to act via inhibition of TBK-1 to inhibit growth of *Mycobacterium kansasii*, promote host resistance to *Pseudomonas aeruginosa* keratitis, and attenuate IFN- β production and antiviral responses to Sendai virus [39]. Two anticonvulsants, carbamazepine and valproic acid, were shown to stimulate autophagy through an mTOR-independent pathway and enhance intracellular killing of *Mtb* in primary human macrophages at concentrations achievable in humans

Table 1. HDTs that target host pathways and regulate *Mtb* pathogenesis

HDTs compound	Host target	Mechanism of action	Effect	Reference(s)
<i>Autophagy</i> Lithium, prochlorperazine, valproic acid	Autophagy	Reduction phosphorylation of mTOR and PI3-kinase	Inhibition TBK-1, reduction TNF-mediated tissue damage	Wang et al. [39], 2013
Rapamycin	Autophagy	Inhibition of mTOR	Promotion of autophagy	Stepkowski and Kahan [34], 1991
Vitamin D ₃	Autophagy via cathelicidin	Stimulation of VDR (vitamin D receptor) to induce cathelicidin expression; upregulation the expression of Atg5 and Beclin-1	Immunomodulation and direct antimicrobial activity	Yuk et al. [42], 2009
Metformin	Autophagy	Reduction phosphorylation of mTOR and p70S6k	Induction of mitochondrial ROS, phagolysosome fusion and increase MTB-infected cell apoptosis	Singhal et al. [41], 2014
<i>Immunometabolism</i> Statin	Cholesterol	Inhibition of HMG-CoA reductase	Promotion phagosomal maturation, phagolysosome fusion, and autophagy	Skerry et al. [58], 2012 Parihar et al. [47], 2014
Aspirin	Eicosanoids	Enhance the LXA4 production	Inhibition of COX and activation of vitamin-D antimicrobial activities	Tobin et al. [55], 2012
Diclofenac	Eicosanoids	Unselective COX-inhibitor	Inhibition of bacterial DNA synthesis	Dutta et al. [52], 2007
<i>Granuloma structure</i> Enbrel	Granuloma	TNF- α neutralization	Disruption of granuloma and reduction of lung pathology	Bourigault et al. [59], 2013
Bevacizumab	Granuloma	Neutralization of VEGF	Normalization of vascular structure, decreases the hypoxia, and improvement the entry of drug in the granuloma	Datta et al. [61], 2015

[40]. Interestingly, mice infected with a virulent MDR-TB strain when treated with carbamazepine, showed a robust reduction in tissue bacterial burden, improved lung pathology and an enhanced adaptive immunity, highlighting the potential anti-*Mtb* activity of these drugs in an in vivo model of TB [40].

Patients with diabetes mellitus are at higher risk of *Mtb* infection and of developing active TB disease, which may present with increased severity. Metformin, a drug commonly administered to diabetes mellitus patients, was shown to reduce *Mtb* survival in infected macrophages by inducing maturation of phagolysosomes and production

of reactive oxygen radicals [41]. The anti-*Mtb* activity of metformin was also demonstrated in vivo where it enhanced host immune responses and reduced chronic inflammation and lung pathology in infected mice. Indeed, in human diabetic patients with TB, metformin treatment was associated with improved control of *Mtb* and decreased disease severity, indicating that metformin could be used as adjuvant therapy to treat TB [41].

Vitamin D deficiency has been associated with increased risk of TB disease in adults and children. Vitamin D₃ (1,25-dihydroxycholecalciferol) was shown to induce autophagy in *Mtb*-infected macrophages via cathelicidin

(hCAP18) at physiological concentrations [42, 43]. An increased level of hCAP18 were observed when THP-1 cells were treated with anti-TB drugs in the presence of the active form of vitamin D (1N1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃]), but not when THP-1 cells were treated with anti-TB drugs alone, which correlated with an enhanced anti-*Mtb* activity, supporting the potential benefits associated with the assumption of vitamin D during standard anti-TB therapy [44].

miRNAs, small, non-coding RNAs, are a novel class of drugs with potential applications also in the treatment of infectious diseases [45]. In TB disease, some miRNAs have been implicated in the modulation of autophagy and apoptosis, such as miRNA-155 that inhibits ATG3 expression, essential for the correct formation of autophagosomes during *Mtb* infection [46]. Notably, silencing of miRNA-155 rescued autophagosomes formation during *Mtb* infection [46].

Immunometabolism

Host cells and *Mtb* both rely extensively on lipid and carbohydrate metabolic pathways for homeostatic functions. Drugs targeting these metabolic pathways offer promise because they can target pathways in both the host and the pathogen. Particularly, the dysregulation of host lipid synthesis and sequestration plays a critical role in the formation of foamy macrophages, which can sustain persistent bacteria and contribute to the tissue pathology that leads to the cavitation and release of infectious bacilli in patients who have progressed to active disease. It has been hypothesized that cholesterol inhibition by statins could potentially alter protective immunity to *Mtb* infection, with the statin-mediated reduction in cholesterol levels within phagosomal membranes promoting host-induced autophagy that improves host protection against TB [47].

A hallmark of active TB is the aggressive immune response that by inducing inflammation promotes host tissue damage leading to necrosis and cavitation [48]. Attenuating excessive host inflammatory responses in active TB might thus be beneficial during treatment and may improve disease outcome [49]. There is a wide range of HDTs, including non-steroidal anti-inflammatory drugs (NSAIDs) that act on host immune effectors to achieve decrease in host-destructive pathology, potentially leading to clinical improvement, decreased morbidity and mortality. NSAIDs, based on their anti-inflammatory effect, could act on attenuating excessive (neutrophil-mediated) inflammation in active TB disease [48]. Byrne et al. [50] treated *Mtb*-infected mice with either the first-line anti-TB drugs pyrazinamide or isoniazid, or with the

NSAIDs ibuprofen or aspirin, alone or in combination with isoniazid [50], demonstrating that ibuprofen and aspirin alone did not have any significant effect on bacterial counts compared to controls but enhanced the isoniazid-dependent clearance of *Mtb* in tissues. The authors suggest the use of ibuprofen rather than aspirin as adjunctive analgesic in TB-treatment [50].

Eicosanoids are signalling molecules derived from fatty acids that serve as precursors to prostaglandins, thromboxanes and leukotrienes [51]. This class of small molecules exerts significant control over the immune response to TB via various immune mechanisms that include the cytokines TNF, IL-6, and IL-1 β and cell death pathways in macrophages and T cells. Studies conducted in mice and zebrafish have shown that mycobacterial virulence factors can modulate macrophage necrosis by generating specific eicosanoids from arachidonic acid [51]. The virulent *Mtb* H37Rv strain was shown to induce the production of lipoxin A4 (LXA4) by 5- and 15-lipoxygenases to promote necrosis, while the *Mtb* H37Ra attenuated strain induced the synthesis of the pro-apoptotic prostaglandin E2 (PGE2) by cyclooxygenase 1 and 2 and prostaglandin synthases [51]. In line with these findings, diclofenac (2-[2, 6-dichloranilino] phenylacetic acid), an unselective cyclooxygenase-inhibitor, was shown to restrict *Mtb* replication in spleen and liver when administered alone, and a further synergistic activity was observed when administered in combination with streptomycin [52].

The reduction of PGE2 is considered one mechanistic explanation underlying the anti-inflammatory benefit of NSAIDs in the context of *Mtb* infection [48], as PGE2 inhibits phagocytosis and bacterial killing at late stage of *Mtb*-infection.

Granuloma Structure

A prominent pathological feature of TB is the formation of granulomas, driven by both bacterial and host factors in the lungs of infected patients [53]. A granuloma is an organized and compact immunological structure rich with immune cells, such as macrophages, monocytes, DCs, neutrophils, epithelioid cells, foamy macrophages, and multi-nucleated giant cells [54]. Upon induction of the adaptive immunity, the granuloma develops a peripheral lymphocytic cuff mainly characterized by B and T cells, reaching a balanced solid state in which many granulomas persist, restricting bacilli replication. However, as the disease progresses, increased necrotic breakdown of granuloma cells leads to caseous necrosis, which may result in cavitation. Ultimately, as the granuloma cavitates

and collapses into the lung, *Mtb* bacilli are released into the airway. Hence, the events unfolding within granulomas are responsible for the outcome of *Mtb* infection. Among the key players involved is the chemokine TNF- α that is required to induce macrophage-killing activities, though excessive- or low-TNF- α production is detrimental to the host by reducing antimicrobial activity or causing augmented tissue pathology respectively [55, 56]. Inhibition of TNF- α activity has been successfully used to treat inflammatory diseases, like rheumatoid arthritis, Crohn's disease, and psoriasis [57]. Hence, blocking the activity of TNF- α has been explored as a potential host-directed therapy for TB. Treatment of Kramnik mice with a TNF- α inhibitor etanercept or enbrel in conjunction with standard TB treatment reduced pulmonary bacterial burden and ameliorated lung pathology [58, 59]. The key role of TNF- α in TB pathogenesis is highlighted by the increased risk of TB reactivation in patients receiving TNF- α inhibitors [7, 58, 59]. However, a clinical trial in HIV patients with pulmonary TB where TNF- α was administered in combination with anti-TB drugs, reported an improved response to TB treatment without major side effects [59].

Granulomas are morphologically similar to solid cancerous tumours in that they contain hypoxic microenvironments and can be highly fibrotic. Datta et al. [61] shown that TB-infected rabbits have impaired small molecule distribution into these disease sites due to a functionally abnormal vasculature, with a low-molecular-weight tracer accumulating only in peripheral regions of granulomatous lesions. Granuloma-associated vessels are morphologically and spatially heterogeneous, with poor vessel pericyte coverage in both human and experimental rabbit TB granulomas. Moreover, they found enhanced VEGF expression in both species. In tumours, antiangiogenic, specifically anti-VEGF, treatments can “normalize” their vasculature [60], reducing hypoxia and creating a window of opportunity for concurrent chemotherapy; thus they investigated vessel normalization in rabbit TB granulomas. Treatment of TB-infected rabbits with the anti-VEGF antibody bevacizumab significantly decreased the total number of vessels while normalizing those vessels that remained [61]. As a result, hypoxic fractions of these granulomas were reduced and small molecule tracer delivery was increased. These findings demonstrate that bevacizumab treatment promotes vascular normalization, improves small molecule delivery and decreases hypoxia in TB granulomas, thereby providing a potential avenue to improve delivery and efficacy of current treatment regimens [61].

Concluding Remarks

TB still remains a major public health problem further complicated by the emergence of antimicrobial resistance against the most effective drugs. The current standard first-line treatment regimen consisting of isoniazid, rifampicin, ethambutol, and pyrazinamide requires long duration of treatment (at least 6 months for first-line TB therapy and 18–20 months for MDR-TB therapy) to achieve cure. Moreover, the toxic side effects of drugs may lead to non-compliance to treatment, thus, opening a window for development of drug resistance. Hence, novel treatment strategies are required to shorten the duration of treatment and improve treatment outcomes particularly in drug-resistant patients.

Given their features, HDTs may be considered valid and potentially effective tools in the treatment of TB when administered as adjunct therapy to the commonly used anti-TB drug regimens. Compared to antibiotics that target bacteria, HDTs present some features that could be of great relevance in a context of emerging antimicrobial resistance. First, HDTs make it more difficult for the pathogen to evolve drug-resistant variants, as the bacteria would have to develop an entirely new suite of interactions with host factors while still under immune selection. Second, since HDTs have a similar activity against drug-susceptible and drug-resistant strains, the combination of HDTs with the less effective “second-line” antibiotics, commonly used in the treatment of MDR-TB or when issues of toxicity emerge in the patient, could result in a significant improvement in treatment efficacy. Hence, combined use of HDTs in anti-TB drug regimens may promote a faster clearance of *Mtb* and improve the general clinical conditions of the patients that may potentially lead to a shortening of the anti-TB therapy. This is a major goal also for regimens against drug-susceptible strains, though the lack of reliable biomarkers to monitor efficacy of anti-TB activity makes this goal challenging.

Nevertheless, the success and opportunities for HDTs against TB will depend upon the combined effort of basic and clinical sciences to tackle key issues. Among the most relevant are certainly the need to improve our understanding of TB pathogenesis, with the identification of the events at molecular level, to identify potentially relevant host targets and molecules that by interfering in these processes will serve as a HDTs. The recent studies on the role of miRNA in TB pathogenesis may lead to the identification of new targets for HDTs, and to the identification of the TB patients for which a given HDT would be

more appropriate [45, 62]. Moreover, it will also be of paramount importance to develop relevant *in vitro* and *in vivo* experimental models to test the anti-TB activity of these HDTs when administered alone or in combination. The animal models currently available for TB do not mimic TB infection and disease in humans. The use of *ex vivo* model like for instance the granuloma-like model obtained from human peripheral blood monocyte may provide a useful tool to assess HDTs in experimental settings involving different cell type. While most of the evi-

dences currently available are from preclinical studies, the ongoing clinical trials or those that are starting will help assess safety and efficacy, paving the way for the introduction of HDTs as adjunct therapies against TB.

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References

- World Health Organization: Global Tuberculosis Report 2017, 2017.
- Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, Furin J, Nardell EA, London L, Lessem E, Theron G, van HP, Niemann S, Merker M, Dowdy D, Van RA, Siu GK, Pasipanodya JG, Rodrigues C, Clark TG, Sirgel FA, Esmail A, Lin HH, Atré SR, Schaaf HS, Chang KC, Lange C, Nahid P, Udwadia ZF, Horsburgh CR Jr, Churchyard GJ, Menzies D, Hesselning AC, Nuermberger E, McIlleron H, Fennelly KP, Goemaere E, Jaramillo E, Low M, Jara CM, Padayatchi N, Warren RM: The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. *Lancet Respir Med* 2017;pii:S2213-2600(17)30079-6.
- Greco E, Quintiliani G, Santucci MB, Serafino A, Ciccaglione AR, Marcantonio C, Papi M, Maulucci G, Delogu G, Martino A, Goletti D, Sarmati L, Andreoni M, Altieri A, Alma M, Caccamo N, Di Liberto D, De Spirito M, Savage ND, Nisini R, Dieli F, Ottenhoff TH, Fraziano M: Janus-faced liposomes enhance antimicrobial innate immune response in *Mycobacterium tuberculosis* infection. *Proc Natl Acad Sci U S A* 2012;109:E1360-E1368.
- Caminero JA, Scardigli A: Classification of antituberculosis drugs: a new proposal based on the most recent evidence. *Eur Respir J* 2015;46:887-893.
- Gomez GB, Dowdy DW, Bastos ML, Zwerling A, Sweeney S, Foster N, Trajman A, Islam MA, Kapiga S, Sinanovic E, Knight GM, White RG, Wells WA, Cobelens FG, Vassall A: Cost and cost-effectiveness of tuberculosis treatment shortening: a model-based analysis. *BMC Infect Dis* 2016;16:726.
- Kaufmann SHE, Dorhoi A, Hotchkiss RS, Bartschlag R: Host-directed therapies for bacterial and viral infections. *Nat Rev Drug Discov* 2018;17:35-56.
- Delogu G, Goletti D: The spectrum of tuberculosis infection: new perspectives in the era of biologics. *J Rheumatol Suppl* 2014;91:11-16.
- O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP: The immune response in tuberculosis. *Annu Rev Immunol* 2013;31:475-527.
- Gengenbacher M, Kaufmann SH: *Mycobacterium tuberculosis*: success through dormancy. *FEMS Microbiol Rev* 2012;36:514-532.
- Chao MC, Rubin EJ: Letting sleeping dogs lie: does dormancy play a role in tuberculosis? *Annu Rev Microbiol* 2010;64:293-311.
- Lin PL, Ford CB, Coleman MT, Myers AJ, Gawande R, Ioerger T, Sacchettini J, Fortune SM, Flynn JL: Sterilization of granulomas is common in active and latent tuberculosis despite within-host variability in bacterial killing. *Nat Med* 2014;20:75-79.
- Kimmey JM, Huynh JP, Weiss LA, Park S, Kambal A, Debnath J, Virgin HW, Stallings CL: Unique role for ATG5 in neutrophil-mediated immunopathology during *M. tuberculosis* infection. *Nature* 2015;528:565-569.
- Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, Allen RD, Gluck SL, Heuser J, Russell DG: Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* 1994;263:678-681.
- Schorey JS, Schlesinger LS: Innate immune responses to tuberculosis. *Microbiol Spectr* 2016;4.
- Houben D, Demangel C, van Ingen J, Perez J, Baldeon L, Abdallah AM, Caleechurn L, Bot-tai D, van ZM, de PK, van der Laan T, Kant A, Bossers-de VR, Willemsen P, Bitter W, van SD, Brashers R, van der Wel N, Peters PJ: ESX-1-mediated translocation to the cytosol controls virulence of mycobacteria. *Cell Microbiol* 2012;14:1287-1298.
- Dey B, Dey RJ, Cheung LS, Pokkali S, Guo H, Lee JH, Bishai WR: A bacterial cyclic dinucleotide activates the cytosolic surveillance pathway and mediates innate resistance to tuberculosis. *Nat Med* 2015;21:401-406.
- van der WN, Hava D, Houben D, Fluittsma D, van ZM, Pierson J, Brenner M, Peters PJ: *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. *Cell* 2007;129:1287-1298.
- Alonso S, Pethe K, Russell DG, Purdy GE: Lysosomal killing of *Mycobacterium* mediated by ubiquitin-derived peptides is enhanced by autophagy. *Proc Natl Acad Sci USA* 2007;104:6031-6036.
- Mizushima N, Levine B, Cuervo AM, Klionsky DJ: Autophagy fights disease through cellular self-digestion. *Nature* 2008;451:1069-1075.
- Kim J, Guan KL: Amino acid signaling in TOR activation. *Annu Rev Biochem* 2011;80:1001-1032.
- Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V: Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell* 2004;119:753-766.
- Romagnoli A, Etna MP, Giacomini E, Pardini M, Remoli ME, Corazzari M, Falasca L, Goletti D, Gafa V, Simeone R, Delogu G, Piacentini M, Brosch R, Fimia GM, Coccia EM: ESX-1 dependent impairment of autophagic flux by *Mycobacterium tuberculosis* in human dendritic cells. *Autophagy* 2012;8:1357-1370.
- Petrucchioli E, Romagnoli A, Corazzari M, Coccia EM, Butera O, Delogu G, Piacentini M, Girardi E, Fimia GM, Goletti D: Specific T cells restore the autophagic flux inhibited by *Mycobacterium tuberculosis* in human primary macrophages. *J Infect Dis* 2012;205:1425-1435.
- Chandra P, Ghanwat S, Matta SK, Yadav SS, Mehta M, Siddiqui Z, Singh A, Kumar D: *Mycobacterium tuberculosis* inhibits RAB7 recruitment to selectively modulate autophagy flux in macrophages. *Sci Rep* 2015;5:16320.
- Chandra P, Kumar D: Selective autophagy gets more selective: uncoupling of autophagy flux and xenophagy flux in *Mycobacterium tuberculosis*-infected macrophages. *Autophagy* 2016;12:608-609.
- Watson RO, Manzanillo PS, Cox JS: Extracellular *M. tuberculosis* DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. *Cell* 2012;150:803-815.
- Watson RO, Bell SL, MacDuff DA, Kimmey JM, Diner EJ, Olivas J, Vance RE, Stallings CL, Virgin HW, Cox JS: The cytosolic sensor cGAS detects *Mycobacterium tuberculosis* DNA to induce type I interferons and activate autophagy. *Cell Host Microbe* 2015;17:811-819.

- 28 Groschel MI, Sayes F, Simeone R, Majlessi L, Brosch R: ESX secretion systems: mycobacterial evolution to counter host immunity. *Nat Rev Microbiol* 2016;14:677–691.
- 29 Ernst JD: The immunological life cycle of tuberculosis. *Nat Rev Immunol* 2012;12:581–591.
- 30 Algood HM, Chan J, Flynn JL: Chemokines and tuberculosis. *Cytokine Growth Factor Rev* 2003;14:467–477.
- 31 Zumla A, Maeurer M, Chakaya J, Hoelscher M, Ntoumi F, Rustumjee R, Vilaplana C, Yeboah-Manu D, Rasolof V, Munderi P, Singh N, Aklillu E, Padayatchi N, Macete E, Kapata N, Mulenga M, Kibiki G, Mfinanga S, Nyirenda T, Maboko L, Garcia-Basteiro A, Rakotosamimanana N, Bates M, Mwaba P, Reither K, Gagneux S, Edwards S, Mfinanga E, Abdulla S, Cardona PJ, Russell JB, Gant V, Noursadeghi M, Elkington P, Bonnet M, Menendez C, Dieye TN, Diarra B, Maiga A, Aseffa A, Parida S, Wejse C, Petersen E, Kaleebu P, Oliver M, Craig G, Corrah T, Tientcheu L, Antonio M, Rao M, McHugh TD, Sheikh A, Ippolito G, Ramjee G, Kaufmann SH, Churchyard G, Steyn A, Grobusch M, Sanne I, Martinson N, Madansein R, Wilkinson RJ, Mayosi B, Schito M, Wallis RS: Towards host-directed therapies for tuberculosis. *Nat Rev Drug Discov* 2015;14:511–512.
- 32 Tobin DM, May RC, Wheeler RT: Zebrafish: a see-through host and a fluorescent toolbox to probe host-pathogen interaction. *PLoS Pathog* 2012;8:e1002349.
- 33 Barry CE, III, Boshoff HI, Dartois V, Dick T, Ehrt S, Flynn J, Schnappinger D, Wilkinson RJ, Young D: The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Microbiol* 2009;7:845–855.
- 34 Stepkowski SM, Kahan BD: Rapamycin and cyclosporine synergistically prolong heart and kidney allograft survival. *Transplant Proc* 1991;23:3262–3264.
- 35 Turnquist HR, Fischer RT, Thomson AW: Pharmacological modification of dendritic cells to promote their tolerogenicity in transplantation. *Methods Mol Biol* 2010;595:135–148.
- 36 Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, Bachmann MF, Larsen CP, Ahmed R: mTOR regulates memory CD8 T-cell differentiation. *Nature* 2009;460:108–112.
- 37 Jagannath C, Lindsey DR, Dhandayuthapani S, Xu Y, Hunter RL Jr, Eissa NT: Autophagy enhances the efficacy of BCG vaccine by increasing peptide presentation in mouse dendritic cells. *Nat Med* 2009;15:267–276.
- 38 Wu J, Liu W, He L, Huang F, Chen J, Cui P, Shen Y, Zhao J, Wang W, Zhang Y, Zhu M, Zhang W, Zhang Y: Sputum microbiota associated with new, recurrent and treatment failure tuberculosis. *PLoS One* 2013;8:e83445.
- 39 Wang L, Zhang L, Zhao X, Zhang M, Zhao W, Gao C: Lithium attenuates IFN- β production and antiviral response via inhibition of TANK-binding kinase 1 kinase activity. *J Immunol* 2013;191:4392–4398.
- 40 Schiebler M, Brown K, Hegyi K, Newton SM, Renna M, Hepburn L, Klapholz C, Coulter S, Obregon-Henao A, Henao TM, Basaraba R, Kampmann B, Henry KM, Burgon J, Renshaw SA, Fleming A, Kay RR, Anderson KE, Hawkins PT, Ordway DJ, Rubinstein DC, Floto RA: Functional drug screening reveals anticonvulsants as enhancers of mTOR-independent autophagic killing of *Mycobacterium tuberculosis* through inositol depletion. *EMBO Mol Med* 2015;7:127–139.
- 41 Singhal A, Jie L, Kumar P, Hong GS, Leow MK, Paleja B, Tsenova L, Kurepina N, Chen J, Zolezzi F, Kreiswirth B, Poidinger M, Chee C, Kaplan G, Wang YT, De LG: Metformin as adjunct antituberculosis therapy. *Sci Transl Med* 2014;6:263ra159.
- 42 Yuk JM, Shin DM, Lee HM, Yang CS, Jin HS, Kim KK, Lee ZW, Lee SH, Kim JM, Jo EK: Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. *Cell Host Microbe* 2009;6:231–243.
- 43 Luong K, Nguyen LT: Impact of vitamin D in the treatment of tuberculosis. *Am J Med Sci* 2011;341:493–498.
- 44 Chesdachai S, Zughaier SM, Hao L, Kempker RR, Blumberg HM, Ziegler TR, Tangpricha V: The Effects of First-Line Anti-Tuberculosis Drugs on the Actions of Vitamin D in Human Macrophages. *J Clin Transl Endocrinol* 2016;6:23–29.
- 45 Harapan H, Fitra F, Ichsan I, Mulyadi M, Miotto P, Hasan NA, Calado M, Cirillo DM: The roles of microRNAs on tuberculosis infection: meaning or myth? *Tuberculosis (Edinb)* 2013;93:596–605.
- 46 Etna MP, Sinigaglia A, Grassi A, Giacomini E, Romagnoli A, Pardini M, Severa M, Cruciani M, Rizzo F, Anastasiadou E, Di CB, Barzon L, Fimia GM, Manganello R, Coccia EM: *Mycobacterium tuberculosis*-induced miR-155 subverts autophagy by targeting ATG3 in human dendritic cells. *PLoS Pathog* 2018;14:e1006790.
- 47 Parihar SP, Guler R, Khutlang R, Lang DM, Hurdal R, Mhlana MM, Suzuki H, Marais AD, Brombacher F: Statin therapy reduces the *Mycobacterium tuberculosis* burden in human macrophages and in mice by enhancing autophagy and phagosome maturation. *J Infect Dis* 2014;209:754–763.
- 48 Kiran D, Podell BK, Chambers M, Basaraba RJ: Host-directed therapy targeting the *Mycobacterium tuberculosis* granuloma: a review. *Semin Immunopathol* 2016;38:167–183.
- 49 Hawn TR, Matheson AI, Maley SN, Vandal O: Host-directed therapeutics for tuberculosis: can we harness the host? *Microbiol Mol Biol Rev* 2013;77:608–627.
- 50 Byrne ST, Denkin SM, Zhang Y: Aspirin and ibuprofen enhance pyrazinamide treatment of murine tuberculosis. *J Antimicrob Chemother* 2007;59:313–316.
- 51 Chen M, Divangahi M, Gan H, Shin DS, Hong S, Lee DM, Serhan CN, Behar SM, Remold HG: Lipid mediators in innate immunity against tuberculosis: opposing roles of PGE2 and LXA4 in the induction of macrophage death. *J Exp Med* 2008;205:2791–2801.
- 52 Dutta NK, Mazumdar K, Dastidar SG, Park JH: Activity of diclofenac used alone and in combination with streptomycin against *Mycobacterium tuberculosis* in mice. *Int J Antimicrob Agents* 2007;30:336–340.
- 53 Maeurer M, Rao M, Zumla A: Host-directed therapies for antimicrobial resistant respiratory tract infections. *Curr Opin Pulm Med* 2016;22:203–211.
- 54 Cosma CL, Sherman DR, Ramakrishnan L: The secret lives of the pathogenic mycobacteria. *Annu Rev Microbiol* 2003;57:641–676.
- 55 Tobin DM, Roca FJ, Oh SF, McFarland R, Vickery TW, Ray JP, Ko DC, Zou Y, Bang ND, Chau TT, Vary JC, Hawn TR, Dunstan SJ, Farrar JJ, Thwaites GE, King MC, Serhan CN, Ramakrishnan L: Host genotype-specific therapies can optimize the inflammatory response to mycobacterial infections. *Cell* 2012;148:434–446.
- 56 Tobin DM, Vary JC Jr, Ray JP, Walsh GS, Dunstan SJ, Bang ND, Hagge DA, Khadge S, King MC, Hawn TR, Moens CB, Ramakrishnan L: The *Ita4h* locus modulates susceptibility to mycobacterial infection in zebrafish and humans. *Cell* 2010;140:717–730.
- 57 Wallis RS, Maeurer M, Mwaba P, Chakaya J, Rustumjee R, Migliori GB, Marais B, Schito M, Churchyard G, Swaminathan S, Hoelscher M, Zumla A: Tuberculosis – advances in development of new drugs, treatment regimens, host-directed therapies, and biomarkers. *Lancet Infect Dis* 2016;16:e34–e46.
- 58 Skerry C, Harper J, Klunk M, Bishai WR, Jain SK: Adjunctive TNF inhibition with standard treatment enhances bacterial clearance in a murine model of necrotic TB granulomas. *PLoS One* 2012;7:e39680.
- 59 Bourigault ML, Vacher R, Rose S, Olleros ML, Janssens JP, Quesniaux VF, Garcia I: Tumor necrosis factor neutralization combined with chemotherapy enhances *Mycobacterium tuberculosis* clearance and reduces lung pathology. *Am J Clin Exp Immunol* 2013;2:124–134.
- 60 Carmeliet P, Jain RK: Angiogenesis in cancer and other diseases. *Nature* 2000;407:249–257.
- 61 Datta M, Via LE, Kamoun WS, Liu C, Chen W, Seano G, Weiner DM, Schimel D, England K, Martin JD, Gao X, Xu L, Barry CE, III, Jain RK: Anti-vascular endothelial growth factor treatment normalizes tuberculosis granuloma vasculature and improves small molecule delivery. *Proc Natl Acad Sci USA* 2015;112:1827–1832.
- 62 Miotto P, Mwangoka G, Valente IC, Norbis L, Sotgiu G, Bosu R, Ambrosi A, Codecasa LR, Goletti D, Matteelli A, Ntinginya EN, Aloï F, Heinrich N, Reither K, Cirillo DM: miRNA signatures in sera of patients with active pulmonary tuberculosis. *PLoS One* 2013;8:e80149.