Opinion:

Advancing host-directed therapy for tuberculosis

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Preface

Improved treatments are needed for nearly all forms of *Mycobacterium tuberculosis* infection. Adjunctive host-directed therapies (HDTs) have the potential to shorten tuberculosis treatment duration, prevent resistance and reduce lung injury by promoting autophagy, antimicrobial peptides and other macrophage effector mechanisms, as well as by modifying specific mechanisms that cause lung inflammation and matrix destruction. The spectrum of candidates is broad, including several agents approved for other clinical indications that are ready for evaluation in Phase 2 clinical trials. The promise of new and existing HDTs that could accelerate response and improve tuberculosis treatment outcomes is discussed.

Introduction

For the past 40 years, treatment of tuberculosis has consisted of combinations of antimicrobial drugs that are administered to eradicate active infection while preventing resistance and relapse. However, major unmet needs exist for nearly all forms of *Mycobacterium tuberculosis* infection: these include improved outcomes for drug-resistant disease; shorter treatment durations for both drug-resistant and fully drug-sensitive infections[1-3]; and more effective preventive regimens for individuals with **latent *M. tuberculosis* infection** (LTBI)[4]. There is also growing recognition that following antimicrobial treatment many patients are left with profound, permanent respiratory impairment despite tuberculosis cure[5]. In the pre-chemotherapy era, rest, nutrition and sunlight were frequently successful in arresting pulmonary tuberculosis[6], thus indicating a substantial capacity for self-cure that adjunctive host-directed therapies (HDT) might harness. The discussion in this Opinion article reflects a 2-day workshop sponsored by the US National Institutes of Health that explored the potential of new and existing HDT agents that could accelerate and improve tuberculosis treatment by targeting the host, a partial list of which appears in table 1.

Immune responses to *M. tuberculosis.*

The innate immune response mediated by lung-resident macrophages drives early events in the course of *M. tuberculosis* infection, resulting in **inflammasome** activation, cytokine production, immune receptor expression and initiation of multiple host defence mechanisms, including the production of antimicrobial peptides and reactive oxygen species (ROS)[7] (figure 1). Dendritic cells, epithelial cells, natural killer cells, and other immune cells contribute to this process[8]. Neutrophils also participate through the transfer to macrophages of granules containing antimicrobial agents that traffic to early endosomes containing mycobacteria, and through the production of **S100 proteins**[9,10]. In some individuals who remain tuberculin skin test negative despite prolonged and repeated exposure to *M. tuberculosis*, these innate immune responses may be sufficient to prevent infection[11]. Of note, further genetic studies of these resistant individuals may identify new HDT targets[12].

In most instances, however, these innate immune mechanisms fail to prevent *M. tuberculosis* infection, which must instead be contained by adaptive immune responses. Responding T cells produce interferon-γ (IFNγ) and chemokines that facilitate further T cell recruitment. IFNγ also triggers intracellular antibacterial mechanisms in macrophages by activation of Janus kinase 1 (JAK1), the phosphorylation of signal transducer and activator of transcription 1 (STAT1) and the production ROS[13]. The absolute requirement for IFNγ signaling is evident in individuals with mutations in the IFNγ receptor gene, which confers susceptibility to disseminated infection by mycobacteria and other intracellular pathogens[14].

Ex vivo models of intracellular *M. tuberculosis* infection have been used to study these early immune cell interactions. Clinical interventions that reduce tuberculosis risk, such as vaccination with *M. bovis* bacillus Calmette–Guérin (BCG) or initiation of antiretroviral therapy in individuals with HIV-1 co-infection (box 1), have consistently been shown to inhibit intracellular mycobacterial growth in *ex vivo* models[15,16], but the effects fall short of true bactericidal activity. The persistence of viable mycobacteria *in vivo* drives the formation of necrotic **granulomas**, within which mycobacterial growth can be restricted by lack of oxygen and nutrients[17]. However, mycobacterial survival under these conditions can be facilitated by a dormancy response that limits bacterial cell wall synthesis, cell division and dependence on aerobic respiration[18].

Current tuberculosis drugs have limited capacity to penetrate granulomas, and have reduced effects on dormant bacilli. Tissue damage resulting from sustained inflammation causes permanent pulmonary disability in approximately half of surviving tuberculosis patients, who experience a persistent cough, breathlessness and reduced life expectancy despite tuberculosis cure[5,19,20]. Thus, the human cost of this containment strategy is substantial. Two strategies can be considered through which tuberculosis HDT can shift this stalemate between host and pathogen: augmenting cellular antimicrobial mechanisms, or directly reducing inflammation, thereby preventing lung damage and potentially enhancing the effectiveness of chemotherapy.

Enhancing antimicrobial mechanisms

Autophagy inducers. Inhibition of **phagolysosome** fusion is a key pathogenic mechanism of *M. tuberculosis.* This inhibition occurs due to specific mycobacterial substituents, including mannose-capped lipoarabinomannan (ManLAM), a cell wall component[21]. It can potentially be overcome by the induction of **autophagy**, which is a cellular process that delivers potentially harmful cytosolic macro-

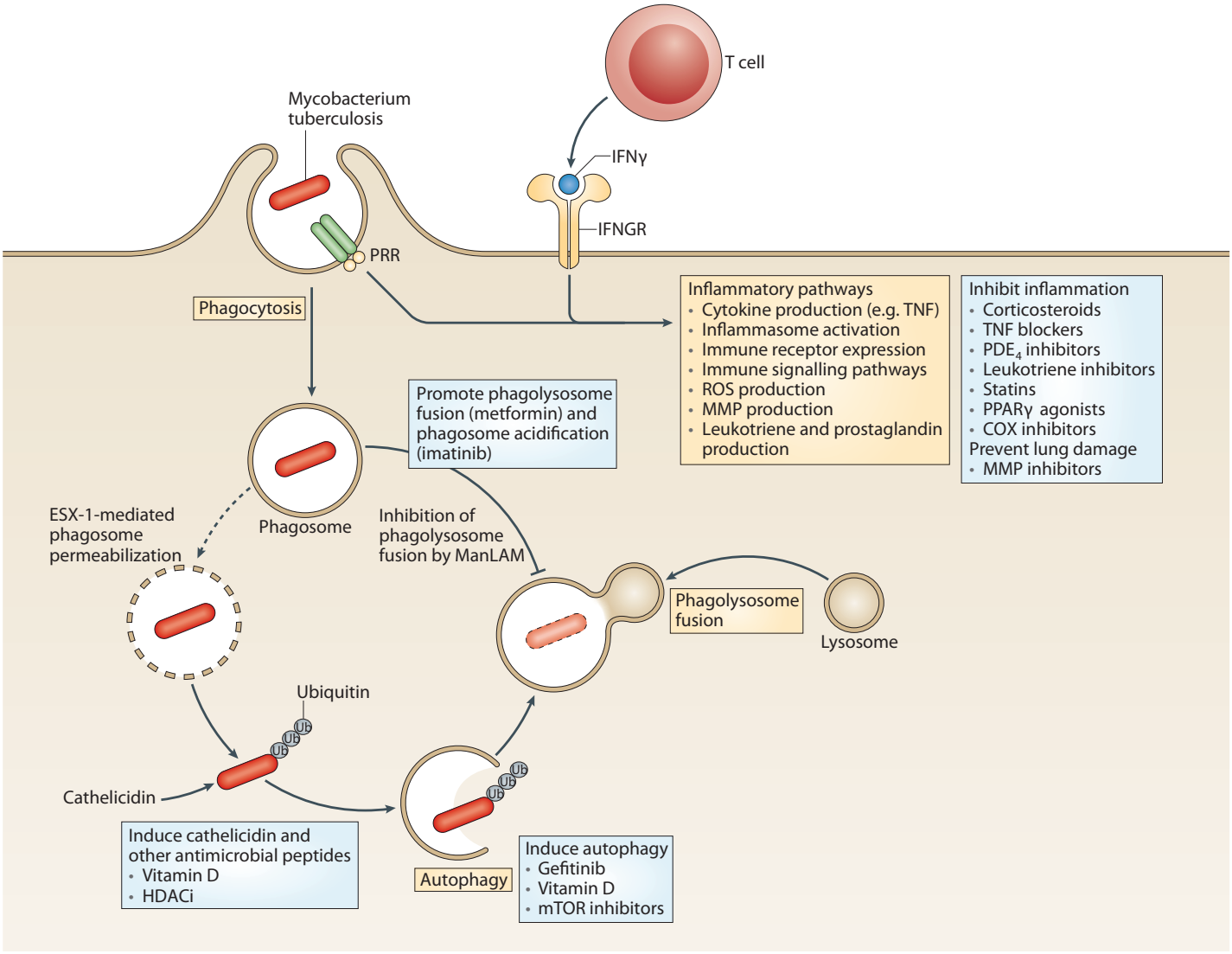

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Figure 1. *Potential targets of host-directed therapy against* Mycobacterium tuberculosis*. Inhibition of phagolysome fusion by mannose-capped lipoarabinomannan (ManLAM) facilitates mycobacterial intracellular survival. This may be overcome by agents promoting phagosome acidification and maturation, or circumvented by agents promoting autophagy and the production of cathelicidin and other antimicrobial peptides. Activation of inflammatory pathways by* M. tuberculosis *results in lung destruction and cavity formation. This may be prevented by anti-inflammatory agents and matrix metalloprotease (MMP) inhibitors. ESX-1=early secreted antigen 6 secretion system 1; HDACi=histone deacetylase inhibitor; PDE_4=phosphodiesterase type 4; PPARγ= peroxisome proliferator-activated receptor γ; PRRs=pattern recognition receptors; TNF=tumor necrosis factor.*

molecules and organelles to lysosomes for degradation[22,23]. Permeabilization of the phagosome by the *M. tuberculosis* ESX-1 secretion system can permit cytosolic components of the ubiquitin-mediated autophagy pathway access to bacilli that are otherwise contained in phagosomes, resulting in reduced *M. tuberculosis* survival (figure 1)[24]. Of note, the use of autophagy inhibitors appears to increase susceptibility to mycobacterial infection in patients with cystic fibrosis[25]. Induction of autophagy can also have secondary effects, such as promoting antigen presentation and reducing inflammation, by sequestering and processing microbial components.

Although autophagy is highly amenable to targeting by small molecules, no currently available inducers of autophagy are entirely suitable for clinical tuberculosis trials. Perhaps the best studied autophagy inducer is rapamycin (also known as sirolimus), an immunosuppressive drug used in organ transplantation. Rapamycin inhibits **mTOR** (mammalian target of rapamycin), which is a strong negative regulator of autophagy[26]. However, the high variability in gastrointestinal absorption of rapamycin has mandated therapeutic drug monitoring in organ transplantation. Ra-

Glossary of terms

Autophagy: A cellular process that delivers potentially harmful cytosolic macromolecules and organelles to lysosomes for degradation. In macro-autophagy, an isolation membrane fuses with itself to enclose the pathogen to form an autophagosome, which can then fuse with lysosomes.

C3HeB/FeJ (Kramnik) mice: A mouse strain that develops granulomatous lesions with central necrosis and hypoxia following *M. tuberculosis* infection. By contrast, lesions in BALB/c mice are non-necrotic and lack hypoxia.

Granuloma: An organized collection of tightly apposed epithelioid macrophages, lymphocytes and fibroblasts, with or without a necrotic center.

Immune reconstitution inflammatory syndrome (IRIS): Paradoxical reactions occurring during combined antimicrobial and antiretroviral treatment in individual with tuberculosis and AIDS.

Inflammasome: A molecular complex of several proteins that upon assembly cleaves pro-IL-1 and pro-IL-18, thereby producing active IL-1 and IL-18.

Latent *Mycobacterium tuberculosis* infection (LTBI): A clinical state in which there is evidence of T cell sensitization to *M. tuberculosis* antigens (by tuberculin skin test or interferon-γ release assay) but no evidence of disease (by chest radiograph and sputum culture). Individuals with LTBI are at risk of developing active tuberculosis if immunosuppressed by medical therapies or other infections.

mTOR: (mammalian target of rapamycin). mTOR is a conserved serine/threonine protein kinase that regulates cell growth and metabolism, as well as cytokine and growth-factor expression, in response to environmental cues. mTOR receives stimulatory signals from RAS and phosphoinositide-3-kinase (PI3K) downstream of growth factors, as well as nutrients, such as amino acids, glucose and oxygen.

Paradoxical reaction: Clinical disease exacerbation (worsened fever, and increased lymph node and lung involvement despite microbiologic improvement (conversion of sputum cultures to negative) occurring after antimicrobial treatment has commenced, attributed to activation of inflammatory mechanisms.

Phagolysosome: A cytoplasmic body formed by the fusion of a phagosome (containing ingested particles at a neutral pH) with a lysosome (containing hydrolytic and other enzymes at an acidic pH). Phagolysosome fusion is inhibited by *M. tuberculosis* as a mechanism for its intracellular survival.

S100 proteins: A family of low molecular weight proteins that participate in the inflammatory response by promoting leukocyte migration.

pamycin can cause interstitial pneumonitis, which can be a concern in patients with pre-existing lung pathology. In addition, rapamycin is metabolized by CYP3A4, a hepatic enzyme that is strongly induced by rifampin, a key TB drug. These factors may limit the clinical role of rapamycin as a TB HDT.

Other potential autophagy inducers include vadimezan (originally DMXAA), which is an antitumor agent that activates the stimulator of IFN genes (STING)-dependent autophagy pathway in mice[27]; however, it appears ineffective in humans[28]. Shoji-Kawata *et al.* reported the induction of autophagy by a Tat–beclin 1 fusion peptide, thereby limiting replication of several pathogens and improving survival of infected mice[29]. The present requirement for daily administration of the fusion peptide by injection may limit its clinical role as a TB HDT, however. Gefitinib (Iressa; AstraZeneca and Teva), an inhibitor of the epidermal growth factor receptor, inhibits *M. tuberculosis* growth in macrophages and reduces mycobacterial replication in the lungs of infected mice, via effects on autophagy[30]. However, there is a current lack of peripheral markers of autophagy. The development of noninvasive methods to assess autophagy induction in involved tissues would accelerate the identification of additional small molecule inducers of autophagy.

Protein kinase inhibitors. Three tyrosine kinase inhibitors — imatinib (Gleevec or Glivec, Novartis), dasatinib (Sprycel, Bristol-Myers Squibb) and nilotinib (Tasigna, Novartis) — are currently licensed for the treatment of chronic myeloid leukaemia (CML). Their target in CML is BCR–ABL (Abelson leukaemia virus protein (ABL) fused with the breakpoint cluster region (BCR)), a fusion protein with tyrosine kinase activity. Inhibition of BCR-ABL in CML arrests myeloid cell proliferation and restores apoptosis. However, two distinct mechanisms pertain to a potential role for tyrosine kinase inhibitors in TB. The first is a direct, pharmacological effect on macrophage function, in which therapeutic concentrations of imatinib promote phagosome acidification and maturation and thereby reduce intracellular *M. tuberculosis* survival *in vitro* and in infected mice[31-33]. The second mechanism is indirect: at sub-therapeutic concentrations, imatinib increases neutrophil and monocyte numbers through effects on myelopoiesis, thereby potentially contributing to the anti-mycobacterial host immune response (D Kalman, Emory Univ., personal communication). Imatinib is generally very well tolerated[34] but studies will be needed to determine its optimal dose for tuberculosis. Imatinib is metabolized by CYP3A4, and is therefore likely incompatible with rifampin. Furthermore, neutrophil induction may exacerbate lung damage. For these reasons, the risks posed by imatinib (and possibly other tyrosine kinase inhibitors) may be more acceptable in individuals with drug-resistant disease.

Protein kinase R (PKR) is a cellular enzyme that participates in host defenses against several viruses and *Toxoplasma gondii* by stimulating autophagy[35]. However, deletion of PKR in mice results in improved control of intracellular *M. tuberculosis* infection, in association with increased macrophage apoptosis, enhanced expression of tumour necrosis factor (TNF) and inducible nitric oxide synthase in response to IFNγ, and reduced production of the immunosuppressive cytokine interleukin-10 (IL-10)[36]. Although there

Table 1. Suitability and clinical readiness of host-directed therapy candidates for tuberculosis.

	Augmenting macrophage effector mechanisms	**Reducing inflammation and/or preventing lung damage**
Suitable for phase 2 clinical trials	Metformin High dose immunoglobulin	TNF inhibitors (including adalimumab) Statins (including rosuvastatin) COX inhibitors (including Ibuprofen) Doxycycline (MMP inhibitor)
Clinical optimization required	Phenylbutyrate plus vitamin D_3 [a] Imatinib [a,b] (tyrosine kinase inhibitor)	CC-10050 [b] (PDE4 inhibitor) Various statins [b] Zileuton (leukotriene inhibitor) [c]
Preclinical R&D required	Gefitinib (autophagy inducer) Various tyrosine kinase inhibitors	Various PDE inhibitors PPARγ agonists (incl. rosiglitazone and telmisartan)
Discovery of new agents required	Protein kinase R inhibitors Autophagy inducers PPARγ antagonists	MMP inhibitors

[a] Dose selection study needed. [b] Pharmacokinetic drug–drug interaction study with rifamycin needed. [c] Identification of appropriate patient subsets needed. PPAR= peroxisome proliferator-activated receptor. TNF=tumor necrosis factor. COX=cyclooxygenase. MMP=matrix metalloproteinase. PDE=phosphodiesterase.

currently are no small molecule PKR inhibitors, this enzyme is a potential TB HDT target.

Cathelicidin inducers. Vitamin D is essential for human anti-mycobacterial host defences[37]. It is required for production of cathelicidin and other antimicrobial peptides following triggering of Toll-like receptors by mycobacterial ligands[38]. This process promotes autophagy and is amplified by IFNγ. Several studies have examined the effects of vitamin D on the early response to tuberculosis therapy. Doses of up to 250 μg of vitamin D_3 per day appear to have no clinical benefit[39,40]. One study of 1250 μg of vitamin D_3 given on days 0 and 28 found no effect on sputum culture status, the 6 minute walk test (a measure of exercise capacity), chest X-ray score or the forced expiratory volume at week 8[41]. Perhaps the most rigorous clinical trial was conducted in a total of 146 patients in England that were randomized to receive 2500 μg of vitamin D_3 or placebo every 2 weeks for 4 doses[42]. Serum vitamin D_3 levels were low at baseline and were augmented by supplementation. Accelerated sputum culture conversion was seen only in the minority of subjects with the *tt* genotype of the vitamin D receptor polymorphism. Vitamin D_3 was generally well tolerated despite the high dose; however, two vitamin D_3-treated patients experienced **paradoxical reactions** (disease worsening despite microbiological improvement) that required therapeutic drainage of deep tissue abscesses. The biological basis of these paradoxical reactions is unclear, as vitamin D_3 administration was shown to dampen the production of pro-inflammatory cytokines in a per-protocol subset of trial participants that excluded the 2 paradoxical reaction cases[43]. Together, these studies indicate that vitamin D supplementation alone is unlikely to have a clinically significant effect on tuberculosis treatment. Supplementation with other micronutrients, such as vitamin A and zinc, may also be of clinical benefit, but has been less well studied[44].

Histone deacetylase inhibitors (HDACi) upregulate cathelicidin expression through epigenetic regulation of transcription. Phenylbutyrate is an HDACi and a non-classical peroxisome proliferator that enhances the action of vitamin D to promote expression of cathelicidin and ROS[45-47]. Phenylbutyrate is approved at total doses of up to 20 g/day for urea cycle disorders. Although generally well tolerated, the relatively high concentrations that are required for cathelicidin induction *in vitro* are only transiently reached *in vivo* after large oral doses. A small study with limited statistical power tested a low range of doses of phenylbutyrate plus vitamin D_3 for combined use as a TB HDT[48]. Based on the results, the combination of 500 mg of phenylbutyrate twice daily plus 5000 IU (125 μg) of vitamin D3 daily is currently under investigation in two clinical trials of 2-month duration (NCT01580007 and NCT01698476). The first of these clinical trials has preliminarily reported finding no effect on sputum culture conversion[49]. Studies of higher doses of phenylbutyrate plus vitamin D_3 will be needed to better evaluate its potential as TB HDT.

Metformin. Metformin is a biguanide that is widely used in the treatment of adult onset diabetes mellitus. Metformin promotes autophagy and expression of AMP-activated protein kinase, which is a sensor of intracellular energy[50]. It inhibits TNF and tissue factor production through inhibition of the extracellular signal-regulated kinase–early growth response 1 (ERK–EGR1) pathway in human monocytes[51]. Recent *in vitro* studies indicate that metformin inhibits *M. tuberculosis* growth by promoting phagolysosome

HIV-1 antiretroviral therapy as host-directed therapy for tuberculosis?

HIV-1 infection profoundly increases TB risk and alters its manifestations[117]. Although recurrent TB is common in HIV-1-infected individuals living in TB-endemic regions, strain typing indicates most recurrences are due to progression of new *M. tuberculosis* infection rather than reactivation (relapse) of persisting infection[118,119]. TB risk is substantially (but not completely) reduced by antiretroviral therapy (ART)[120]. Combined treatment for HIV and TB decreases the risk of subsequent opportunistic infections, reduces the risk of recurrence due to reinfection, and improves long term survival. These have been attributed to ART effects on HIV disease progression, which otherwise is accelerated by TB[121]. ART appears to have little impact on common TB-related outcomes (treatment failure, relapse or death during early TB treatment)[122]. Immune reconstitution in patients treated for both infections may result in clinical exacerbation of disease (IRIS) and may increase the risk of lasting lung injury. Thus restoration of TB-specific immune competence in HIV-1-infected individuals represents a two-edged sword, controlling mycobacterial infection but promoting tissue damage.

fusion and increasing mitochondrial ROS[52]. Testing in mice infected with *M. tuberculosis* revealed that metformin improved pulmonary pathology and reduced bacterial load; however, when combined with the TB drug isoniazid, the magnitude of the added effect of metformin on bacterial load was modest. Metformin is generally well tolerated, rarely causing hypoglycemia in persons without diabetes.

*IFN*γ. Two randomized, controlled clinical trials have examined the therapeutic role of recombinant IFNγ in tuberculosis. The first trial**,** conducted by Intermune in 80 patients with multidrug-resistant tuberculosis using aerosolized recombinant IFNγ was halted by its safety monitoring board due to futility (lack of microbiological or radiographic benefit) plus a trend towards increased mortality (10 deaths in the IFNγ treatment group versus 5 deaths in control group, *P*=0.14). The results of this trial have never been published, but appear in an online protocol supplement to the second trial[53], which compared the administration of recombinant IFNγ by aerosol and subcutaneous injection with standard therapy alone in a total of 77 evaluable drug-susceptible tuberculosis patients. Neither route of IFNγ administration accelerated sputum culture conversion or resolution of lung lesions as determined by chest computed X-ray tomography (CT). Similarly, a study of adjunctive IL-2 therapy during the first month of treatment in 110 tuberculosis patients found no clinical or microbiological benefit[54]. Thus, the finding that a cytokine is essential for host defenses against *M. tuberculosis* does not guarantee that its therapeutic administration will provide clinical benefits.

High dose immunoglobulin. The role of antibody in defence against *M. tuberculosis* is uncertain[55]. Treatment with high doses of human immunoglobulin results in large reductions in organ bacterial burden in both acute and chronic *M. tuberculosis* infection models in mice[56]. The potential of high dose immunoglobulin as HDT could be readily tested in a small trial in patients with extensively drug-resistant TB.

Thus, diverse TB HDT candidates with the potential to induce antimicrobial activity in macrophages are available for clinical testing.

Reducing inflammation

Corticosteroids. Recognition that lung damage in tuberculosis is mainly due to a prolonged host inflammatory response led in the 1960s to multiple controlled clinical trials of adjunctive corticosteroids during early tuberculosis chemotherapy[57]. These studies found that for the most part steroids hastened the resolution of constitutional, radiographic and pulmonary function abnormalities, although they did not affect long-term outcomes. A formal meta-analysis indicated a trend toward a survival advantage favoring steroid administration in pulmonary tuberculosis that fell short of statistical significance[58]. Greater benefit from adjunctive corticosteroid treatment has been observed in patients with tuberculosis meningitis who have a specific leukotriene A_4 hydrolase genotype[59]. A recent meta-regression analysis examined the relationship between corticosteroid dose and sputum culture conversion[60]. The main finding was that corticosteroids accelerated sputum culture conversion, but that high doses (134 mg/day of the corticosteroid prednisolone or its equivalent) would be required to reduce the proportion of patients with positive of sputum cultures after 2 months of treatment using solid culture medium from 15% to 1–2%, the proposed target for new 4-month TB regimens[61]. The mechanism of this effect is uncertain, but corticosteroids likely improve drug penetration to lung lesions, and enhance antimicrobial drug action by promoting bacillary aerobic metabolic activity. Some degree of caution is warranted in using a predictive model developed from studies of tuberculosis chemotherapy for dose selection of adjunctive immunotherapy. However, 3 studies of corticosteroid HDT assessed effects on relapse and found them to be consistent with effects observed on sputum cultures at 2 months[62-64].

Clinical experience with corticosteroids in tuberculosis at a dose sufficient to reach the target for a 4-month regimen is limited to a single trial (NCT00057421) in which 187 HIV-1-infected tuberculosis patients were randomly assigned to receive 2.75 mg/kg/d (approximately 150 mg/day) prednisolone or placebo for the first 4 weeks of standard treatment[63]. The dose had been selected based on a pilot study that indicated it would reduce TNF production by more than half. At this dose, prednisolone induced a striking reduction in the proportion of patients whose sputum culture was positive at 1 month (38% *vs*. 63%, P<0.001). However, its adverse event profile, which included hyperglycemia, hypertension, fluid retention and 1 steroid-relat-

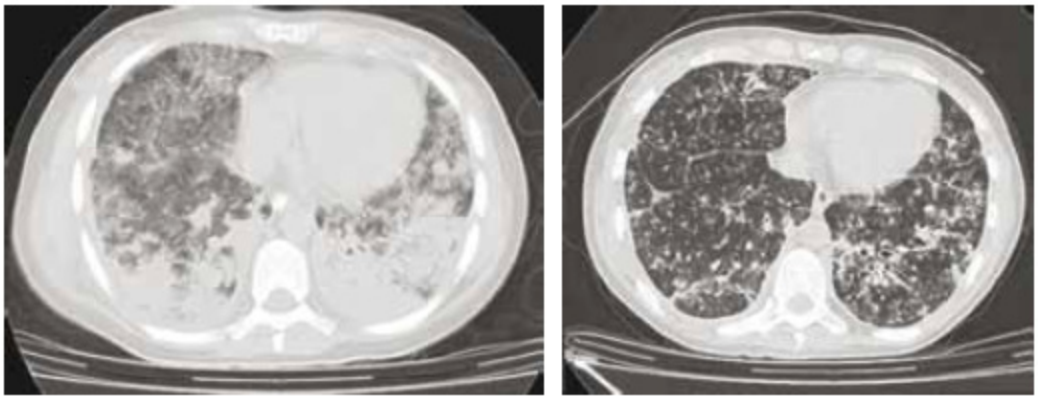

Figure 2. *Chest computed X-ray tomography of a patient with life-threatening pulmonary tuberculosis, on hospital day 13 (left) and day 63 (right). Adjunctive treatment with the anti-TNF monoclonal antibody adalimumab, initiated on day 17, was followed by a rapid therapeutic response. Modified with permission from reference [78].*

ed fatality, is unlikely to be considered acceptable from a benefit-risk perspective.

TNF blockers. TNF has a central role in the pathogenesis of inflammation and is required for host defenses against mycobacterial infection[65]. Anti-TNF therapies can alter the course of chronic inflammatory conditions (for example, preventing progression of joint damage in patients with rheumatoid arthritis, and promoting healing of the mucosal lesions in Crohn's Disease that otherwise lead to fistula formation and fibrosis[66,67]). Treatment with corticosteroids does not accomplish these objectives. One trial of adjunctive etanercept (soluble TNF receptor), in which patients with tuberculosis were given 25 mg of the drug twice weekly for 1 month, found etanercept to be safe and well tolerated, and observed a small benefit on sputum culture conversion and a trend toward improved chest radiography[68]. It is now recognized that etanercept is ineffective for other chronic granulomatous inflammatory conditions such as Crohn's disease and sarcoidosis[69,70], and that it is correspondingly inefficient in reactivating LTBI (doing so at a rate estimated at estimated at 1.7% per month)[71]. These observations make it unlikely that a clinically meaningful benefit of etanercept would occur in larger tuberculosis trials.

In contrast, anti-TNF monoclonal antibodies (mAbs) are highly active in the treatment of granulomatous inflammation, and are as efficient as high dose corticosteroids in reactivating LTBI (at a rate estimated at 21% per month)[72,73]. Concurrent administration of antimycobacterial chemotherapy mitigates this risk[74,75]. There have been no randomized clinical trials to date of adjunctive anti-TNF mAbs in tuberculosis. However, there have been several case reports of rapid therapeutic responses to the anti-TNF mAbs adalimumab and infliximab in cases of central nervous system (CNS) tuberculosis that are refractory to corticosteroids[76,77] and in life-threatening pulmonary tuberculosis (figure 2)[78]. These cases indicate that, despite the essential role for TNF in antimycobacterial host defence, TNF blockade may be of therapeutic benefit if administered concurrently with antimicrobial chemotherapy. Although the effects of TNF mAbs are lost as their plasma concentrations decline[79], these agents persist substantially longer than current antimicrobial chemotherapy. Mitigation strategies will be required to ensure that patients do not prematurely discontinue antimicrobial chemotherapy shortly after receiving a dose of an anti-TNF mAb.

JAK inhibitors. Tofacitinib (Xeljanz and Jakvinus, Pfizer) is a JAK inhibitor licensed in the US for treatment of rheumatoid arthritis at a dose of 5 mg/day. Higher doses (equivalent to 10 mg/day) interfere with containment of *M. tuberculosis* infection in mice[80], and have been associated with reactivation of LTBI in clinical trials[81]. However, this dose was not approved due to safety concerns. Tofacitinib is metabolized by CYP3A4, and is likely incompatible with rifampin, limiting its role as a TB HDT. However, JAK inhibitors with different enzyme specificities and pharmacologic profiles may be potential HDT agents.

Thalidomide analogues. Thalidomide was approved in the 1950s in Europe as a sedative and anti-emetic. It was withdrawn from the market in the 1960s when it was found to cause phocomelia (congenital limb defects) if administered during pregnancy. In 1965 a small study found thalidomide to be beneficial in erythema nodosum leprosum, an inflammatory complication of leprosy[82]. These therapeutic effects have been attributed primarily to the inhibition of

Links:

Participants and agenda, Advancing Host Directed Therapy for Tuberculosis" Workshop, April 15 - 16, 2014, Rockville, Maryland. http://www.newtbdrugs.org/meetings/hdt-2014.php

Report of the expert consultation on immunotherapeutic interventions for tuberculosis. World Health Organization, 2007. Geneva, Switzerland. http://apps.who.int/tdr/publications/tdr-research-publications/immunotherapeutic-interventions-tb/pdf/interventions-tb.pdf

TNF production[83]. Several case reports describe favorable responses to thalidomide in patients with CNS tuberculosis and refractory paradoxical inflammatory reactions[84,85]. However, the sole randomized controlled trial of adjunctive thalidomide in pediatric tuberculosis meningitis was halted by its safety monitoring board due to excess deaths in the thalidomide arm of the trial that appeared allergic in etiology[86].

Recent studies indicate thalidomide initiates its teratogenic effects by binding to cereblon and modulating a specific ubiquitin ligase[87], and that this activity is dissociable from its immunomodulatory effects[88]. Thalidomide analogs have since been developed that reduce expression of TNF and certain other pro-inflammatory cytokines by inhibiting type 4 phosphodiesterase (PDE_4) activity, thereby increasing levels of cyclic adenosine monophosphate (cAMP). These PDE_4 inhibitors do not bind cereblon, and have not been found to have thalidomide-like teratogenicity[89]. The analogs have been studied as adjuncts to isoniazid monotherapy in the mouse and rabbit models of tuberculosis, in which they reduce necrosis, fibrosis, granuloma number and size, and mycobacterial burden[90,91]. One such analog, CC-11050, has been well tolerated in phase 1 clinical trials, and is currently being studied as an adjunct to chemotherapy in cynomolgus macaque and rabbit models of tuberculosis. A pharmacokinetic drug–drug interaction study with CC-11050 and the TB chemotherapy drugs rifampin and rifabutin will be required, as CC-11050 is metabolized by CYP3A4.

Other PDE inhibitors. Phosphodiesterases (PDEs) differ in their amino acid sequence, substrate preference, organ distribution and extent of inhibition by various small molecules. Several other PDE inhibitors have been studied as TB HDT candidates. Rolipram (Schering AC), a PDE_4 inhibitor not used clinically due to poor gastrointestinal tolerability, has been reported to interfere with standard TB treatment in mice[92]. Cilostazol (Pletal, Otsuka Pharmaceutical) and sildenafil (Viagra, Pfizer) are PDE_3 and PDE_5 inhibitors approved, respectively, for treatment of intermittent claudication and erectile dysfunction. One study in mice found cilostazol plus sildenafil accelerated the clearance of bacilli from the lung during standard TB treatment[93]; however, a second study showed they afforded no protection against relapse when treatment was shortened[92]. Roflumilast (Daxas and Daliresp, Takeda UK) and apremilast (Otezla, Celgene) are PDE_4 inhibitors approved, respectively, for prevention of chronic obstructive pulmonary disease (COPD) exacerbation, and treatment of psoriasis and psoriatic arthritis. The effects of these drugs in tuberculosis are not known. Pentoxifylline (Trental, Sanofi), a nonspecific PDE and TNF inhibitor, is licensed in the US for the treatment of intermittent claudication. In mice, in the absence of antimicrobial chemotherapy, pentoxifylline destabilizes granulomas and exacerbates disease[94]. One randomized controlled clinical trial of adjunctive pentoxifylline using a sustained release formulation found it to be safe in tuberculosis, but found no benefit on sputum culture conversion or rate of relapse[95,96].

Leukotriene inhibition. Zileuton (Zyflo, Cornerstone Therapeutics), a 5-lipoxygenase inhibitor, is licensed for prophylaxis and treatment of asthma. In a mouse model in which polyinosinic–polycytidylic acid (polyI:C)-driven production of type I IFN suppresses IL-1-mediated control of mycobacterial growth, oral administration of zileuton or intranasal administration of prostaglandin E_2 (PGE_2) prevented weight loss and improved survival by reducing production of type I IFN, IL-10, and IL-1Ra, and restoring that of IL-1 and PGE_2[97]. Administration of both drugs together induced the greatest benefit. Available evidence indicates IL-1 production is increased, rather than reduced, in human tuberculosis[98-100]. Studies to identify subsets of patients with high levels of type 1 interferons and a decreased ratio of PGE_2/leukotriene A_4 could help advance evaluation of this therapeutic approach.

Cyclooxygenase inhibitors. Ibuprofen, a cyclooxygenase (COX) inhibitor and analgesic, lacks direct antimycobacterial activity at standard therapeutic concentrations, and has no effect when administered alone to *M. tuberculosis*-infected BALB/c mice[101]. However, in **C3HeB/FeJ (Kramnik) mice**, which develop large necrotic lesions following *M. tuberculosis* infection, ibuprofen reduces pathology and prolongs survival in the absence of antimicrobial chemotherapy, apparently by reducing lung damage by neutrophils[102]. Ibuprofen appears to be of benefit in paradoxical **immune reconstitution inflammatory syndrome** (IRIS) in patients with tuberculosis and AIDS, although it has not been formally studied. The effects of COX inhibition in human tuberculosis are otherwise not known, but could be readily studied. COX inhibitors decrease macrophage production of PGE_2, increase that of TNF[103], lack disease-modifying activity in arthritis, and are ineffective for treatment of chronic granulomatous inflammatory conditions.

Statins and PPARγ agonists. Inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase (statins) have both lipid-lowering and anti-inflammatory effects, the latter mediated in part through effects on transforming growth factor-β (TGFβ) and peroxisome proliferator-activated receptor-γ (PPARγ)[104]. Statins reduce mycobacterial lipid droplet formation and reduce *M.tuberculosis* survival in macrophages by induction of autophagy and phagosome maturation[105]. 3-methyladenine, an autophagy inhibitor, reversed these effects. Treatment with the statin simvastatin (25 mg/kg) was shown to accelerate bacillary clearance in the lungs of mice when administered in combination with standard therapy[106]. However, a large retrospective analysis using a national medical claims database found statins afforded no protection against tuberculosis in individuals with diabetes mellitus[107]. Some statins, such as rosuvastatin, are not metabolized by CYP3A4 and are therefore unlikely to require dose adjustment due to rifampin. Additional research

may be required to identify the optimal agent and dosing schedule for clinical trials.

PPARγ is highly expressed in alveolar macrophages. It is further upregulated in *M. tuberculosis*-infected cells, in which it negatively regulates several pro-inflammatory pathways through inhibition of nuclear factor-κB (NF-κB) and other nuclear transcription factors[108]. Approved PPARγ agonists include rosiglitazone (Avandia, GlaxoSmithKline, for the treatment of diabetes mellitus), and telmisartan (Micardis, Boehringer Ingelheim, for the treatment of hypertension). It is hypothesized that PPARγ agonists used together with antimicrobial chemotherapy may reduce inflammation and prevent lung damage during active tuberculosis. PPARγ antagonists, such as GW9662, remain in preclinical research and development. PPARγ antagonists may augment host defense mechanisms, particularly during early stages of mycobacterial infection.

Metalloproteinase inhibition. Matrix metalloproteinases (MMPs) contribute to tissue damage in inflammatory conditions through the loss of collagen and other structural proteins. This process can be decreased by corticosteroids, other anti-inflammatory agents and MMP inhibitors[109]. In periodontal disease, sub-antimicrobial doses of the MMP inhibitor doxycycline prevent collagen degradation and reduce loss of supporting dental structures by non-specific MMP inhibition[110]. An adjunctive role for doxycycline in tuberculosis has been proposed based on MMP inhibition in the lung[111]. Agents that prevent cavity formation might reduce infectiousness.

Lastly, mesenchymal stromal cell infusion has been proposed as an adjunct therapy to reduce inflammation and improve outcomes in MDR TB[112]. Thus, diverse TB HDT candidates with the potential to prevent lung damage are available for clinical evaluation. Several of these agents may also accelerate eradication of infection.

HDT development strategies

Many of the agents under consideration here have been approved for other clinical indications and therefore have well-established safety profiles. The question arises as to the value or need to conduct experiments with these agents in preclinical models of tuberculosis, particularly if the models are poor representations of human disease. Prostaglandins, for example, can be beneficial, inactive, or deleterious, depending on the specific mouse model of tuberculosis that is used[97,101,102]. Parallel experimental modeling and early clinical studies that mutually inform each other may be of benefit. In addition, HDT agents with effects on multiple host targets may require studies to establish the appropriate dose for its specific molecular target in tuberculosis. Biomarkers indicating proximate effects on these targets *in vivo* will accelerate development of HDT agents.

Tuberculosis biomarkers have to date been considered interchangeable whether they measured microbial or host characteristics. However, by directly modulating the host response, TB HDT agents can alter the relationship between infection and inflammation, thereby altering the prognostic significance of host-specific biomarkers. As a result, the development of TB HDT agents will require separate assessments of inflammation and tissue damage, protective immunity, and overall antimicrobial effectiveness. Combined imaging with ^{18}F-fluorodeoxyglucose positron emission tomography (PET) plus CT is emerging as an important research tool to assess lung inflammation and lung structure integrity in tuberculosis[113,114]. Whole blood bactericidal activity is a candidate biomarker of protective antimycobacterial immunity[16] and successful chemotherapy[115] with the potential to assess the combined effects of host-directed and antimicrobial therapies. Sputum culture status at 2 months may help assess the treatment-shortening potential of HDT candidates prior to phase 3 clinical trials by predicting effects on relapse[61].

HDT agents that reduce inflammation and thereby prevent lung damage may be of most benefit for patients with far advanced pulmonary disease (defined by radiographic criteria)[116], whereas agents that enhance macrophage antimicrobial activity may be of particular benefit to patients with drug-resistant tuberculosis. Experience with the adjunctive use of corticosteroids indicates that anti-inflammatory HDTs are best avoided in patients with extensive drug resistance[64]. By contrast, inducers of autophagy may have a unique role in drug-resistant tuberculosis by enhancing host defenses while also reducing inflammation. The study of HDT agents in drug-resistant tuberculosis also avoids potential pharmacokinetic interactions with rifampin. Tuberculosis patients with advanced AIDS are at increased risk of acquired drug resistance, IRIS and death. Inasmuch as these risks are immunologically based, they will likely benefit from host-directed interventions. Specific biomarkers are needed to guide the selection, testing, and use of HDT agents in this population. Sequential treatment with anti-inflammatory and then antimicrobial HDTs over a period of months may be optimal with respect to preventing lung injury, enhancing chemotherapeutic effects, and preventing recurrence while minimizing the risk of IRIS-like events.

Summary and conclusions

The spectrum of potential tuberculosis HDT research and development is broad. Increased translational research capacity, with advanced capabilities in human immunology, microbiology, and imaging, is needed now to study these agents. Peripheral markers of HDT effects on host and microbe in the lung must be developed and validated. Assessment of HDT agents must include evaluations of pulmonary function to both detect possible benefit mediated by reduction of tissue destruction and possibly increased inflammatory lung damage caused by an HDR

agent. Closer interactions are needed among basic, translational, and clinical researchers, including those in the pharmaceutical industry, to capitalize on advances in other areas of medicine that may be adapted for use in tuberculosis. Better methods are needed to identify candidate molecular therapies in the laboratory and to reliably deliver them to the site of disease in tuberculosis patients. Resource allocation will be challenging as specific HDT research priorities are developed across this wide spectrum of objectives. For many of the proposed agents, the main risk is that HDT effects on total mycobacterial burden will be small compared to those of antimicrobial chemotherapy. Agents that prevent lung injury and preserve lung function therefore deserve special consideration, as these appear to be unique HDT objectives that are unlikely to be met by antimicrobial treatment alone.

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