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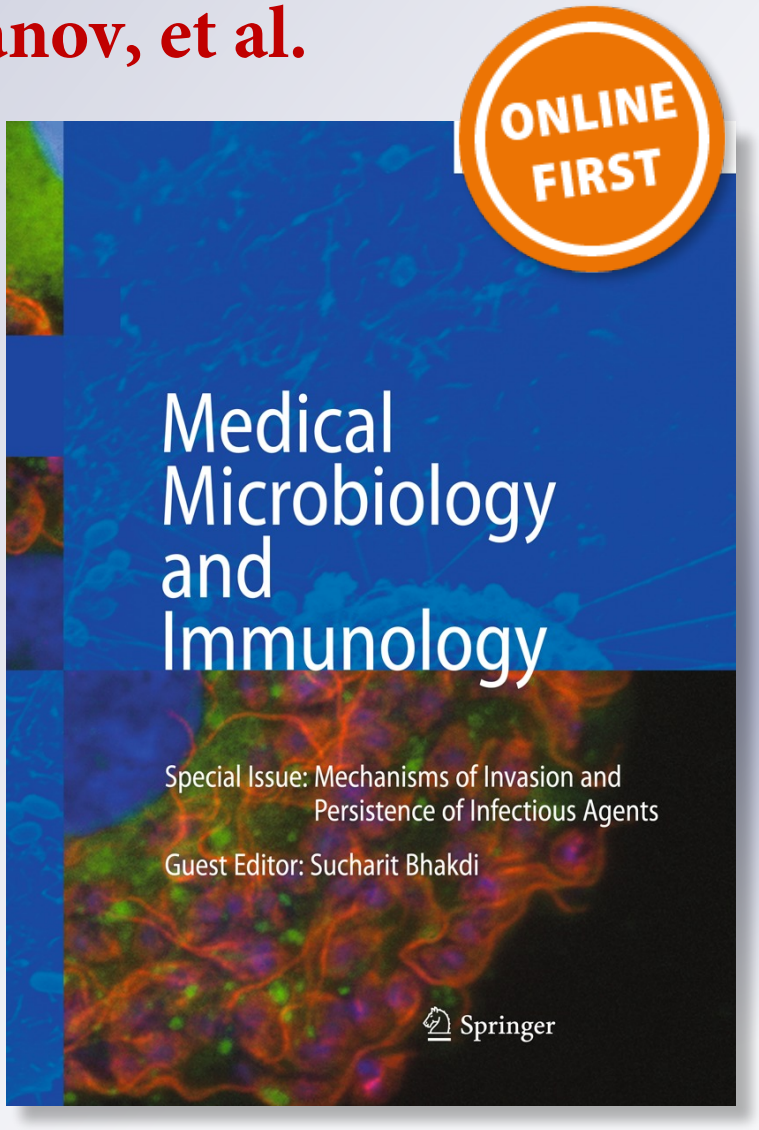
Medical Microbiology and Immunology

ISSN 0300-8584

Med Microbiol Immunol
DOI 10.1007/s00430-012-0278-6



Springer

Med Microbiol Immunol
DOI 10.1007/s00430-012-0278-6

REVIEW

Targeting multidrug-resistant tuberculosis (MDR-TB) by therapeutic vaccines

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Received: 14 October 2012 / Accepted: 25 October 2012

Abstract Tuberculosis (TB) has scourged humankind for millennia, and latent infection affects nearly one-third of today's world population. The emergence of multidrug-resistant (MDR)-TB is a major global threat and reflects treatment failure of drug-sensitive disease. MDR-TB management is a burden for patients and society; success rates are unacceptably low with prolonged treatment duration. *Mycobacterium tuberculosis* (*Mtb*) possesses the ability to transform into a dormant state in which it can persist in the face of antimicrobial treatment and host defense. This sub-population of persisters is largely responsible for lengthy and difficult treatment. Targeting persistent bacilli could eventually improve the treatment success rate (currently 50–65 %) and shorten duration of treatment. A subset of therapies in the pipeline, termed therapeutic vaccines, use the host immune response to attack *Mtb*. The historical occurrence of an exacerbated host response has resulted in a negative perception of therapeutic vaccines. Thus, a renewed concept of immunotherapy is needed. We review current perspectives of immunotherapy in MDR-TB based on the knowledge of

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Published online: 10 November 2012

TB immunology and briefly discuss the profiles of several therapeutic vaccine products.

Keywords Tuberculosis · Immunology · Drug-resistant · Persistent bacteria · Therapeutic vaccine · Immunotherapy · Review

Tuberculosis (TB) has been a scourge to humankind for thousands of years. With the advent of effective chemotherapy and declining incidence rates in affluent societies, interest in the disease waned [1]. In 1994, however, drug-resistant TB emerged [2, 3], and the World Health Organization (WHO) declared TB a Global Emergency [4]. Reports from New York show that drug-resistant TB was readily transmitted in the early 1990s among hospitalized AIDS patients. In 2010 alone, nearly 9.2 million new cases of TB occurred and an estimated 1.4 million deaths were caused by it [5]. The spread of HIV/AIDS accelerated the TB epidemic in large parts of the world, as the risk of developing the disease is markedly increased in HIV-infected persons [6].

MDR-TB and XDR-TB

When susceptibility to the two most powerful anti-tuberculosis drugs, isoniazid (H) and rifampicin (R), is lost, short-course treatment is no longer an option. HR resistance is referred to as multidrug-resistant (MDR)-TB. When resistance developes to not only HR, but also to two major second-line TB drug classes, the condition is referred to as extensively drug-resistant (XDR)-TB. By 2010, the WHO estimated the global prevalence of MDR-TB to be around 650,000 among the world's 12.0 million cases of TB and 58 countries around the world have reported at least one case of XDR-TB [7, 8]. Countries of the Former Soviet Union have been among the most severely affected by the MDR-TB epidemic. A representative survey conducted in 2010 in Minsk, Belarus, showed alarming levels of drug resistance with nearly one out of two tuberculous patients being affected by MDR-TB [9]. Surveillance data from South Africa indicate a high prevalence of drug-resistant TB in the region [10], with a hall-mark paper reporting an alarmingly high mortality rate in XDR-TB patients co-infected with HIV [11]. Recent data from China have raised even greater concern. In a nation-wide survey on drug-resistant TB, 5.7 % of culture-positive pulmonary treatment-naïve cases appeared to have MDR-TB [12]. An independent survey in China revealed that 12 % of TB cases in one particular province were MDR-TB [13]. China has the second highest TB incidence in the world with India having the highest [7]. The case of China underlines the fact that MDR-TB has become an important global health threat. Increasing mobility and cross-border travel demands radical changes in the approach to combat TB [14, 15]. Currently available treatment for MDR- and XDR-TB requires administration of longer treatment with less effective, more costly, and more toxic drugs in comparison with the standard 6-month short-course chemotherapy for drug-susceptible TB: Indeed, the current treatment for MDR-TB is a burden for patients as well as for society [16, 17]. Nevertheless, treatment success with MDR-TB is low compared with that of drug-sensitive TB [18–20]. The emergence of a type of TB resistant to all currently available first- and second-line drugs—totally drug-resistent (TDR)-TB or very extensively drug-resistent (XXDR)-TB—has been reported in the last few years [21–23]. In summary, the emergence of ever-increasing resistance represents a major challenge for TB management in the future.

Risk factors for MDR-TB

Inadvertent monotherapy in multi-bacillary TB has been considered the driving mechanism in the bacillary repopulation of lesions by drug-resistant mutants, leading to monodrug resistance. When this process is repeated with another drug, MDR-TB results [24]. Previously defaulted or retreated cases, prolonged treatment time (>180 days), delayed initiation of chemotherapy, and misconducted treatment have all been associated with the development of MDR-TB [13, 25]. All of these factors have been alluded to as mistakes made by patients, doctors, and pharmacists. An alternative explanation was proposed by the group of Tawanda Gumbo, suggesting the role of interindividual pharmacokinetic (PK) variability of a single drug in the regimen—particularly H or R—as a major contributing factor in the development of MDR-TB rather than inappropriate treatment [26]. This would imply that drug resistance cannot be prevented even by well-designed standardized directly observed treatment, and that in one way or another, PK variability should be taken into account as an essential component in treatment and control programs.

Pathogenesis and immunology of TB

Several reviews on pathogenesis and immunology of TB have recently been published [27–31]. For the purpose of this review, we have summarized the main findings. Following aerogenic transmission from an individual with active disease, infectious droplets of *Mycobacterium tuberculosis* (*Mtb*)—the bacilli causing TB—reach the

alveolar spaces. During its residency in lung parenchyma, *Mtb* can be phagocytosed either by alveolar macrophages (AM), dendritic cells (DC), epithelial cells, or neutrophils. These phagocytes are able to recognize the molecular components of *Mtb*—referred to as pathogen-associated molecular patterns—through pattern recognition receptors (PRR) on their cell surfaces. Much work has been done to reveal the PRR involved in the recognition process, which includes several receptor families such as toll-like receptors, nucleotide oligomerization domain-like receptors, C-type lectins, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (also known as CD209 or DC-SIGN), and dectin-1. Among the phagocytes expressing PRR, AM play a major role. AM possess the capability to produce essential cytokines and also act as a reservoir for *Mtb* bacilli. Once phagocytosed by these AM, *Mtb* may resist bactericidal actions by inhibiting phagolysosome function. Typically, macrophages acidify their phagosomes to pH 5.2 and kill the bacteria by the production of antimicrobial peptides (i.e., cathelicidin), activating a vitamin-D-dependent pathway [32]. The release of nitric oxide (NO) also constitutes an important defense mechanism in mouse models, but evidence in human studies has been conflicting [33, 34]. Several different mechanisms have been hypothesized and identified in halting phagosome maturation. Reduced recruitment of vacuolar H-ATPases hampers the acidification process and thus enables *Mtb* to persist inside the phagosomal vacuole [35, 36]. While lysosome fusion is altered, the vacuoles still fuse with other early endosomal vesicles while acquiring some lysosomal molecules by the conventional synthetic pathway [37]. GTPases of the Rab protein family are involved in lysosome fusion. Normally, Rab 5 is replaced by Rab 7, but Rab 5 is retained in phagosomes that contain *Mtb* [37]. Lipid bodies (LB)—dynamic and functionally active lipid-rich organelles—are the sites for these GTPases, and they play a role in transporting Rab into and from the phagosome in order to procure phagosome maturation [38]. LB also supply neutral lipids to the phagosome which can be utilized as an energy source by *Mtb* during its residency inside AM [39, 40]. Calcium-binding calmodulin is involved in early endosome fusion and calmodulin inhibitors inhibit fusion [41]. Suppression of the actin-binding coronin I (TACO in humans), which associates with early phagosomes, results in defective phagosome fusion [42]. In summary, *Mtb* inhibits phagosome maturation by several different mechanisms, and the pathogen persists intracellularly while avoiding macrophage apoptosis. During its residency in macrophages, *Mtb* can either multiply and eventually kill the host cell or alternatively once the infected macrophage is activated, *Mtb* can be destroyed. A third possibility, a unique feature of *Mtb* and leprosy bacilli, is that they fall into a stage of dormancy with low to absent metabolic and replicative activity.

If macrophages become necrotic, the bacilli enter the extracellular space where they are phagocytosed by other macrophages. This cycle ends only after the cell-mediated immune response is elicited with interferon-gamma (IFN-γ) released by $CD4^+$ Th1 lymphocytes [43]. Upon recognition of major histocompatibility (MHC) class II molecules expressed by the infected macrophages, $CD4^+$ T lymphocytes are able to polarize into distinct types of effector cells [44]. Th1 and Th17 T-lymphocytes play an important role in immunity to *Mtb*. Th1 lymphocytes produce several cytokines, including IFN-γ which is strongly associated with protective immunity. IFN-γ promotes macrophage activation by reviving phagosomal maturation, inducing NO-dependent apoptosis, and modulating autophagy thus enhancing *Mtb* clearance [45, 46]. IFN-γ as an immunogenic marker has been recommended by WHO as an assessment tool for new TB vaccine trials although it does not suffice as a marker of protection [47]. Th17 lymphocytes are involved in the early phase of host defense as they produce cytokines essential for neutrophil and monocyte recruitment to the site of infection. Th2 lymphocytes produce interleukin (IL)-4 that downregulates the Th1 response, thereby contributing to the development of progressive disease [48]. Treg lymphocytes produce transforming growth factor-beta (TGF-β) and IL-10, which inhibit all other $CD4^+$ T cells. Apart from these $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, which are MHC I-restricted, also contribute to protective immunity by directly lysing *Mtb* and producing Th1 type cytokines. Several innate immune cells, such as natural killer (NK) cells and gamma-delta (γδ) T cells, also play a role in the immune response against *Mtb*. As disease progresses, surface density of MHC class II molecules is decreased [49]. Costimulatory molecules are downregulated reducing cellular immune responses even further. *Mtb* also alters the cytokine profile around the macrophage. IL-6 attracts B-lymphocytes but reduces T-lymphocyte proliferation [49]. Hence, *Mtb* not only reduces the initiation of an effective host immune response, it also minimizes its effects.

The dormancy and persister stage of *Mtb*

Mtb and possibly leprosy bacilli have the unique capability to turn off their cellular metabolism, halt replication, and transform into a dormant stage under stress conditions (see Fig. 1) [50, 51]. While dormancy is typically induced by stress imposed by the host on *Mtb*, persistence refers to the survival of *Mtb* under harsh conditions, be they caused by drug treatment or host immunity. This makes it difficult for antituberculosis drugs to eliminate these organisms, which are therefore called "phenotypic persisters" or "phenotypic

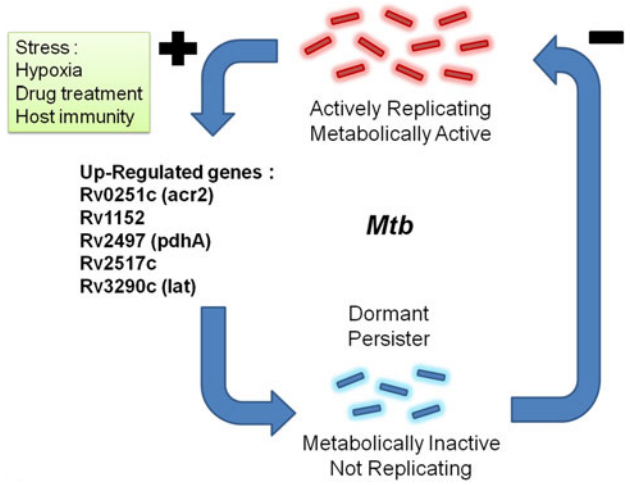

Fig. 1 Mtb changes phenotype by changing the genetic program during stress

resistance" [52]. The sub-population of persistent *Mtb* organisms—though genetically identical to susceptible, fast-replicating, metabolically active bacteria—resist antimicrobial treatment and host immunity. At the same time, these dormant *Mtb* may not cause major harm to the host. This is the very reason why treatment is so lengthy and difficult [53]. *Mtb* enter the persister state expressing a range of different genes challenged by cellular immunity [54]. Through dormancy, the bacillus escapes the host immune response by many mechanisms [29, 53]. Dormant *Mtb* can persist in healthy individuals in a stage termed latent TB infection (LTBI).

Granulomas act as a suitable microenvironment where persisters of *Mtb* reside and persist [55], although earlier reports failed to show the presence of TB bacilli in lung granulomatous lesions [56]. Several other possible locations of these persistent *Mtb*, including adipose tissue, normal lung parenchyma, and several other organs, have been tentatively identified [57–59]. Tumor necrosis factor-alpha (TNF-α) plays a major role in granuloma formation and preservation [29], possibly with the involvement of other factors [60–62]. Granulomatous inflammation presents a complex cellular interplay to protect the host against invasion of organisms by walling *Mtb* off and attracting specific lymphocytes at the site of infection [63, 64]. An alternative hypothesis toward this assumption has however been proposed [65]. The *Mtb* region of difference-1 (RD-1) encoded ESX-1 secretion system enhances host expression of matrix metalloproteinase 9 (MMP-9), which is essential in initiating macrophage recruitment to the granuloma [66]. An increase in RD-1 expression by *Mtb* has been associated with an increased bacterial load [60]. If this is true, granuloma formation in its early stage—prior to T cells' arrival—should be considered a pathogen-initiated response favoring the bacilli rather than a host defense mechanism.

The granuloma environment imposes several stress factors—such as low pH, NO, hypoxia, and limited nutrients—driving *Mtb* into dormancy. During this *Mtb* persister stage, stress-related genes are upregulated, while several central metabolism genes are downregulated [52]. The dormancy survival regulator (DosR)—transcription factors of *Mtb* responsible for increased expression of dormancy genes—upregulates the expression of several proteins crucial for *Mtb* survival under stress conditions. Some of these DosR regulon-encoded *Mtb* proteins evoke immunogenic responses when administered in a latently TB-infected individual [67]. Subsequent to phagocytosis, *Mtb* expresses DosR-encoded genes in the phagosome [68]. Transcriptome analysis from human smear-positive sputum samples revealed that *Mtb* persisters express signals of the DosR regulon [69]. IFN-γ production upon stimulation with some of these DosR regulon-encoded antigens may differentiate active and latent TB [70, 71]. Of the different stress factors, hypoxia is probably more critical than low pH, or increased NO and carbon monoxide production, in inducing activation of the DosR regulon [72, 73]. Disruption of DosR genes only slightly impairs the survival of *Mtb* in several animal models [52], suggesting that it might not be the only factor involved in the switch into dormancy. DosR is important for the initial hypoxic adaptation but not for the survival of *Mtb* during chemotherapy [74]. Genes encoded by sigma E transcription regulator are also involved in the stress response, and *Mtb* persistence inside macrophages [75].

Across a range of different stressor models studied, a set of five genes appear consistently upregulated. These genes are Rv0251c (acr2), encoding an α-crystallin heat shock protein (Hsp); Rv1152, a transcriptional regulator of the gntR family; Rv2497c (pdhA), encoding a possible pyruvate dehydrogenase component; Rv2517c, encoding a hypothetical protein; and Rv3290c (lat), encoding an L-lysine-epsilon-aminotransferase [74]. In a mouse model of *Mtb* infection, six other genes (fadE5, sigE, Rv2030c, Rv2660c, sigB, and ppsD) were consistently expressed—both during early and late stages of infection. One of these genes, Rv2660c—the most upregulated gene in an in vitro starvation model—encodes a hypothetical protein essential for adaptation to lack of nutrition and hypoxia [75]. A new multistage vaccine based on the Rv2660c antigen combined with two other early secreted antigens (Ag85B and early secreted antigenic target-6, or ESAT-6) appeared to prevent TB disease in a post-exposure TB infection model [76]. To what extent these persister genes are important in humans remains to be further elucidated, but their potential

role in the switch of *Mtb* into dormancy may be important as a novel target for therapy.

Targeting persisters by drugs: old and new

Targeting the persister stage of *Mtb* may eventually lead to shorter drug regimens resulting in enhanced treatment success. Several old and new TB drugs are currently being tested for their activity against persistent bacteria, with the hope of shortening current regimens [53, 77]. Only a few new powerful drugs are in the pipeline [78], such as delamanid [79], bedaquiline [80], and the linezolid derivative sutezolid [81].

Animal models have been developed to assess elimination of persisters by using sterilization as an endpoint [82] but pharmacokinetics/pharmacodynamics (PK/PD) as well as the immune response in these animals is quite different from that of humans. Apart from novel therapeutic agents and novel therapeutic schedules, an important goal is to improve and optimize available second-line drugs by therapeutic drug monitoring and modeling [83–87]. Interindividual differences in PK/PD may be a more important driving force in the development of drug resistance than mistakes in medications [26, 88]. The strategy to optimize the use of current and novel drug therapy may however ultimately fail.

TB vaccines

Bacille Calmette–Guérin (BCG)—the only currently available TB vaccine—is a live attenuated strain of *Mycobacterium bovis*. It has been an important part of the Expanded Program on Immunization since the 1970s and has since been given more than 4 billion times. Its safety record is astonishing as few serious adverse events have been reported [89]. Yet, this 90-year-old vaccine does not protect against pulmonary TB in adults, its efficacy is minimal (0–80 %) in adults, and it provides only limited protection in children [90]. BCG remains controversial, and different policies and practices can be seen across nations [91]. Moreover, the current HIV epidemic places immune-compromised patients, who are being BCG vaccinated, at risk of developing BCG disease [92]. Promising progress can be seen in new TB vaccine development as many candidates have entered first clinical safety and immunogenicity evaluation. Eleven candidates have passed phase I clinical trials [93, 94]. Even more products are in the pipeline of preclinical development. Although these candidate vaccines represent potential improvements compared with BCG [95–97], they have not been shown to provide sterilizing immunity in a mouse model [98], and their potential value as therapeutic vaccines has not been assessed [99].

Therapeutic TB vaccine

Early initiation of anti-retroviral therapy provides benefit in preventing TB in HIV-infected individuals, highlighting the importance of enhanced cellular immunity to control LTBI [100]. Few vaccines use the host immune response to target active TB; these are termed therapeutic vaccines, though this is a misnomer. Two inactivated or fragmented mycobacterial formulations are considered for TB immunotherapy. Heat-killed *Mycobacterium vaccae* is an inactivated environmental mycobacterium. In phase III clinical trials, it was shown to be safe in HIV patients who had previously received BCG [92, 101]. *M. vaccae* immunotherapy enhances host defense against *Mtb* by promoting Th1 and suppressing Th2 response. When added to chemotherapy, *M. vaccae* improves sputum conversion and chest radiographic resolution [102, 103]. RUTI is composed of detoxified fragments representing a whole range of inactivated latency-associated antigens. In phase I clinical trials, its safety and immunogenicity has been demonstrated [104]. A phase II study for RUTI was recently completed, and a phase III trial is envisaged. Both are by far the most advanced therapeutic vaccines today. *M. smegmatis* and *M. indicus pranii* (MIP) are other saprophytic non-TB mycobacteria (NTM) that also share antigens with *Mtb*, similar with *M. vaccae* [105]. V-5 immunitor (V5)—an oral therapeutic vaccine initially developed for management of chronic hepatitis—has been shown in several studies to be beneficial when administered to TB patients [106, 107]. The authors suggest it could contain latency-associated *Mtb* antigen, resembling RUTI.

Current concept of immunotherapy

The use of therapeutic vaccines in TB was first suggested by Robert Koch in 1890. He believed that the use of repeated injections of supernatants of *Mtb* cultures—known as old tuberculin—could act as a potential remedy for TB [108]. In the absence of available treatment for TB, his ideas attracted many physicians around the world, and soon the old tuberculin was widely used. Tuberculin immunotherapy failed—in some instances it probably killed patients as a result of an exacerbated immune response [109]. With today's knowledge, it is likely that administration of these antigens to already infected individuals resulted in a cytokine storm, a tissue-damaging, harmful response involving exacerbated release of TNF-α, as well

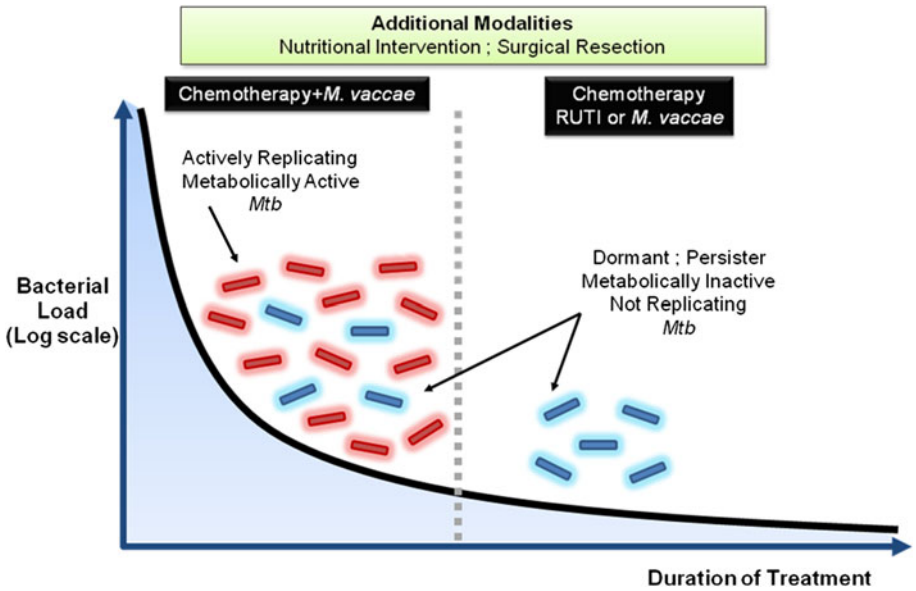

Fig. 2 The current concept of immunotherapy in MDR-TB management

as more down-stream pro-inflammatory cytokines [110]. Yet, no well conducted study has addressed the question whether, and under which circumstances, the old Tuberculin lacked safety.

The introduction of chemotherapy proved highly successful, especially after combination therapy was introduced [111]. With the emergence of drug-resistant TB, effectiveness of chemotherapy was challenged, and immunotherapy has been revisited [112]. To overcome the argument presented by this exacerbated immune response against the use of therapeutic vaccines, a modified concept is needed before they can be reconsidered.

There are two areas in which immunotherapy need to be applied. One, exemplified by *M. vaccae*, is that of immunomodulation, in which the Th2 milieu induced and maintained by active tuberculous infection is corrected toward Th1, increasing the efficacy of chemotherapy. The other is elimination of persisters after a course of chemotherapy or in cases of latent tuberculosis that have never had clinical disease, for which the RUTI vaccine may be most effective.

Immune modulation needs to be applied as soon as diagnosis is confirmed, and chemotherapy started to correct the immune response away from that responsible for the pathology. The mechanism for this does not require antigens specific to *Mtb*, but reversion to Th1 restores cellular immune responses to antigens common to all mycobacteria, some of which are shared with human stress proteins expressed in tissues involved in chronic inflammation [113]. It is through this correction that immunotherapy with *M. vaccae* and some organisms related to it, results in its applicability to a range of chronic inflammatory diseases, many of them not mycobacterial in their etiology [114–121].

Eradication of persistent *Mtb*, which is virtually resistant to chemotherapy (see Fig. 2), is a modern concept. By reducing bacterial load, the cytokine storm which causes the Th2-related exacerbated immune response can be prevented [122]; this is essential for therapeutic vaccination. Indeed for this purpose, the focus should be on therapeutic vaccines that express latency-associated antigens as found in dormant *Mtb*. Such vaccination should assist the host immune system in boosting the immune response directed at these latency antigens, eventually resulting in complete eradication of persisting *Mtb*. They could be used to enhance the immune response during the continuation phase of TB treatment, in which the remaining bacteria are poorly sensitive, if not refractory, to antimycobacterial agents, and potentiate chemotherapy.

The greatest benefits for both types of immunotherapy would be for MDR-TB or XDR-TB as it would potentially improve the relatively low treatment success rate. Therapeutic vaccines do not interfere directly with the causative organism, and hence, they are not involved in the development of drug resistance [123]. Therapeutic vaccination would also be beneficial for drug-sensitive TB as it could potentially shorten the current 6-month standard therapy and help diminish the development of drug resistance. Finally, success in MDR-TB treatment could pave the way for a latency-targeted (post-exposure) vaccine. Indeed, reducing the huge reservoir of *Mtb*—drug-susceptible or not—by vaccination strategies could ultimately accelerate elimination of the disease.

Chemotherapy combined with immunotherapy should be the frontline approach in the management of MDR-TB. Early bactericidal activity of currently available second-line regimens is essential for the elimination of most

replicating metabolically active *Mtb* bacilli in the early phase of treatment.

After significant reduction of the initial bacterial load with chemotherapy and *M. vaccae*, therapeutic vaccine(s) such as RUTI should be introduced in order to overcome the remaining persistent *Mtb* with slow replication rate and reduced drug sensitivity. The timing of immunotherapy initiation with RUTI is critical to prevent the emergence of an exacerbated immune response; chemotherapy, with or without *M. vaccae*, must have significantly reduced the bacterial load in advance.

Clinical, radiological, and bacteriological outcome, for example, using the vital stain fluorescent diacetate [124, 125], must be assessed in order to monitor true reduction in bacterial burden. In vitro susceptibility, fast molecular assays, and PK, using limited sampling of dried blood spots, should be incorporated to enhance the chance of drug treatment success. Eventually, additional modalities, such as nutritional intervention and surgical resection, should also be considered during the course of treatment.

Outlook

The classical approach using combined drug regimens remains the key modality in MDR-TB management. Additional support, such as nutritional intervention (by micronutrients and macronutrients) [126–128], and in selected cases, surgical resection [129, 130] are also important for beneficial outcome. However, considering the current low rate of success as well as the long duration of therapy in MDR-TB treatment, immunotherapy should now be explored in a formal randomized fashion. Current understanding of immune responses against *Mtb* should lead the way to further developing the concept of immunotherapy. The immunogenicity and safety of two therapeutic TB vaccines, *M. vaccae* and RUTI, have been demonstrated in preclinical work and in human trials. *M. vaccae* evokes optimal therapeutic results when given in multiple doses, while RUTI should probably best be given after significant reduction of bacterial load. For the RUTI product, further safety studies are necessary to reduce the risk of a pathologic immune response. *M. vaccae* restores Th1/Th2 balance, while RUTI exposes the host with specific sets of inactivated latency or dormancy antigens. Hence, administration of both vaccines in combination might be worth pursuing. Indeed, immunotherapy is now ready for prime time.

References

1. Keshavjee S, Farmer PE (2012) Tuberculosis, drug resistance, and the history of modern medicine. N Engl J Med 367(10):931–936
2. Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen GM, Dooley SW (1993) The emergence of drug-resistant tuberculosis in New York City. N Engl J Med 328(8):521–526
3. Edlin BR, Tokars JI, Grieco MH, Crawford JT, Williams J, Sordillo EM, Ong KR, Kilburn JO, Dooley SW, Castro KG (1992) An outbreak of multidrug-resistant tuberculosis among hospitalized patients with the acquired immunodeficiency syndrome. N Engl J Med 326(23):1514–1521
4. World Health Organization (1994) Tuberculosis: a global emergency. WHO, Geneva
5. Raviglione M, Marais B, Floyd K et al (2012) Scaling up interventions to achieve global tuberculosis control: progress and new developments. Lancet 379(9829):1902–1913
6. Swaminathan S, Ramachandran R, Baskaran G, Paramasivan CN, Ramanathan U, Venkatesan P, Prabhakar R, Datta M (2000) Risk of development of tuberculosis in HIV-infected patients. Int J Tuberc Lung Dis 4(9):839–844
7. World Health Organization (2011) Global tuberculosis control. WHO, Geneva
8. Nathanson E, Nunn P, Uplekar M, Floyd K, Jaramillo E, Lönnroth K, Weil D, Raviglione M (2010) MDR tuberculosis—critical steps for prevention and control. N Engl J Med 363(11):1050–1058
9. Skrahina A, Hurevich H, Zalutskaya A, Sahalchyk E, Astrauko A, van Gemert W, Hoffner S, Rusovich V, Zignol M (2012) Alarming levels of drug-resistant tuberculosis in Belarus: results of a survey in Minsk. Eur Respir J 39(6):1425–1431
10. World Health Organization (2010) Multidrug and extensively drug-resistant TB (M/XDR TB)—Global Report on Surveillance and Response 2010. WHO, Geneva
11. Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, Zeller K, Andrews J, Friedland G (2006) Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet 368(9547):1575–1580
12. Zhao Y, Xu S, Wang L et al (2012) National survey of drug-resistant tuberculosis in China. N Engl J Med 366(23):2161–2170
13. Liang L, Wu Q, Gao L et al (2012) Factors contributing to the high prevalence of multidrug-resistant tuberculosis: a study from China. Thorax 67(7):632–638
14. Olson EN (2004) Undermining the endothelium by ablation of MAPK-MEF2 signaling. J Clin Invest 113(8):1110–1112
15. Chaisson RE, Nuermberger EL (2012) Confronting multidrug-resistant tuberculosis. N Engl J Med 366(23):2223–2224
16. Ahuja SD, Ashkin D, Avendano M et al (2012) Multidrug resistant pulmonary tuberculosis treatment regimens and patient outcomes: an individual patient data meta-analysis of 9,153 patients. PLoS Med 9(8):e1001300
17. Diel R, Rutz S, Castell S, Schaberg T (2012) Cost of illness of tuberculosis in Germany. Eur Respir J 40(1):143–151
18. Orenstein EW, Basu S, Shah NS, Andrews JR, Friedland GH, Moll AP, Gandhi NR, Galvani AP (2009) Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. Lancet Infect Dis 9(3):153–161
19. Yew WW, Lange C, Leung CC (2011) Treatment of tuberculosis: update 2010. Eur Respir J 37(2):441–462
20. Leimane V, Dravniece G, Riekstina V, Sture I, Kammerer S, Chen MP, Skenders G, Holtz TH (2010) Treatment outcome of multidrug/extensively drug-resistant tuberculosis in Latvia, 2000–2004. Eur Respir J 36(3):584–593
21. Migliori GB, De Iaco G, Besozzi G, Centis R, Cirillo DM (2007) First tuberculosis cases in Italy resistant to all tested drugs. Euro Surveill Eur Commun Dis Bull 12(5):E070517.1
22. Velayati AA, Masjedi MR, Farnia P, Tabarsi P, Ghanavi J, ZiaZarifi AH, Hoffner SE (2009) Emergence of new forms of

totally drug-resistant Tuberculosis Bacilli: super extensively drug-resistant tuberculosis or totally drug-resistant strains in Iran. Chest 136(2):420–425
23. Udwadia ZF, Amale RA, Ajbani KK, Rodrigues C (2012) Totally drug-resistant tuberculosis in India. Clin Infect Dis 54(4):579–581
24. Chang KC, Leung CC, Grosset J, Yew WW (2011) Treatment of tuberculosis and optimal dosing schedules. Thorax 66(11):997–1007
25. Faustini A (2006) Risk factors for multidrug resistant tuberculosis in Europe: a systematic review. Thorax 61(2):158–163
26. Pasipanodya JG, Srivastava S, Gumbo T (2012) Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. Clin Infect Dis 55(2):169–177
27. Axelsson-Robertson R, Magalhaes I, Parida SK, Zumla A, Maeurer M (2012) The Immunological footprint of *Mycobacterium tuberculosis* T-cell epitope recognition. J Infect Dis 205(suppl 2):S301–S315
28. Gupta A, Kaul A, Tsolaki AG, Kishore U, Bhakta S (2012) *Mycobacterium tuberculosis*: immune evasion, latency and reactivation. Immunobiology 217(3):363–374
29. van Altena R, Duggirala S, Gröschel MIP, van der Werf TS (2011) Immunology in tuberculosis: challenges in monitoring of disease activity and identifying correlates of protection. Curr Pharm Des 17(27):2853–2862
30. Kleinnijenhuis J, Oosting M, Joosten LAB, Netea MG, van Crevel R (2011) Innate immune recognition of *Mycobacterium tuberculosis*. Clin Dev Immunol 2011:1–12
31. Dheda K, Schwander SK, Zhu B, van Zyl-Smit RN, Zhang Y (2010) The immunology of tuberculosis: from bench to bedside. Respirology 15(3):433–450
32. Fabri M, Stenger S, Shin D-M et al (2011) Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. Sci Transl Med 3(104):104ra102
33. Schaible UE, Sturgill-Koszycki S, Schlesinger PH, Russell DG (1998) Cytokine activation leads to acidification and increases maturation of *Mycobacterium avium*-containing phagosomes in murine macrophages. J Immunol 160(3):1290–1296
34. Liu PT, Modlin RL (2008) Human macrophage host defense against *Mycobacterium tuberculosis*. Curr Opin Immunol 20(4):371–376
35. Welin A, Lerm M (2012) Inside or outside the phagosome? The controversy of the intracellular localization of *Mycobacterium tuberculosis*. Tuberc (Edinb) 92(2):113–120
36. Rohde KH, Abramovitch RB, Russell DG (2007) *Mycobacterium tuberculosis* invasion of macrophages: linking bacterial gene expression to environmental cues. Cell Host Microbe 2(5):352–364
37. Russell DG (2001) *Mycobacterium tuberculosis*: here today, and here tomorrow. Nat Rev Mol Cell Biol 2(8):569–577
38. Melo RCN, Dvorak AM (2012) Lipid body–phagosome interaction in macrophages during infectious diseases: host defense or pathogen survival strategy? PLoS Pathog 8(7):e1002729
39. Peyron P, Vaubourgeix J, Poquet Y et al (2008) Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for *M. tuberculosis* persistence. PLoS Pathog 4(11):e1000204
40. Pandey AK, Sassetti CM (2008) Mycobacterial persistence requires the utilization of host cholesterol. Proc Natl Acad Sci USA 105(11):4376–4380
41. Colombo MI, Beron W, Stahl PD (1997) Calmodulin regulates endosome fusion. J Biol Chem 272(12):7707–7712
42. Maniak M, Rauchenberger R, Albrecht R, Murphy J, Gerisch G (1995) Coronin involved in phagocytosis: dynamics of particle-induced relocalization visualized by a green fluorescent protein Tag. Cell 83(6):915–924
43. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR (1993) An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. J Exp Med 178(6):2249–2254
44. Kaufmann SHE (2010) Novel tuberculosis vaccination strategies based on understanding the immune response. J Int Med 267(4):337–353
45. Herbst S, Schaible UE, Schneider BE (2011) Interferon gamma activated macrophages kill mycobacteria by nitric oxide induced apoptosis. PLoS ONE 6(5):e19105
46. Ní Cheallaigh C, Keane J, Lavelle EC, Hope JC, Harris J (2011) Autophagy in the immune response to tuberculosis: clinical perspectives. Clin Exp Immunol 164(3):291–300
47. Hanekom WA, Dockrell HM, Ottenhoff THM, Doherty TM, Fletcher H, McShane H, Weichold FF, Hoft DF, Parida SK, Fruth UJ (2008) Immunological outcomes of new tuberculosis vaccine trials: WHO panel recommendations. PLoS Med 5(7):e145
48. Rook GAW, Hernández-Pando R, Dheda K, Teng Seah G (2004) IL-4 in tuberculosis: implications for vaccine design. Trends Immunol 25(9):483–488
49. VanHeyningen TK, Collins HL, Russell DG (2001) IL-6 produced by macrophages infected with Mycobacterium species suppresses T cell responses. J Immunol 158(1):330–337
50. Hett EC, Rubin EJ (2008) Bacterial growth and cell division: a mycobacterial perspective. Microbiol Mol Biol Rev 72(1):126–156
51. Cardona P-J (2006) RUTI: a new chance to shorten the treatment of latent tuberculosis infection. Tuberc (Edinb) 86(3–4):273–289
52. Gengenbacher M, Kaufmann SHE (2012) *Mycobacterium tuberculosis*: success through dormancy. FEMS Microbiol Rev 36(3):514–532
53. Zhang Y, Yew WW, Barer MR (2012) Targeting persisters for tuberculosis control. Antimicrob Agents Chemother 56(5):2223–2230
54. Cardona P-J (2010) Revisiting the natural history of tuberculosis. Arch Immunol Ther Exp (Warsz) 58(1):7–14
55. Barry CE, Boshoff HI, Dartois V, Dick T, Ehrt S, Flynn J, Schnappinger D, Wilkinson RJ, Young D (2009) The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. Nat Rev Microbiol 7(12):845–855
56. Locht C, Rouanet C, Hougardy J-M, Mascart F (2007) How a different look at latency can help to develop novel diagnostics and vaccines against tuberculosis. Expert Opin Biol Ther 7(11):1665–1677
57. Neyrolles O, Hernández-Pando R, Pietri-Rouxel F et al (2006) Is adipose tissue a place for *Mycobacterium tuberculosis* persistence? PLoS ONE 1(1):e43
58. Hernandez-Pando R, Jeyanathan M, Mengistu G, Aguilar D, Orozco H, Harboe M, Rook GA, Bjune G (2000) Persistence of DNA from *Mycobacterium tuberculosis* in superficially normal lung tissue during latent infection. Lancet 356(9248):2133–2138
59. Barrios-Payan J, Saqui-Salces M, Jeyanathan M, Alcantara-Vazquez A, Castanon-Arreola M, Rook G, Hernandez-Pando R (2012) Extrapulmonary locations of *Mycobacterium tuberculosis* DNA during latent infection. J Infect Dis 206(8):1194–1205
60. Clay H, Volkman HE, Ramakrishnan L (2008) Tumor necrosis factor signaling mediates resistance to mycobacteria by inhibiting bacterial growth and macrophage death. Immunity 29(2):283–294
61. Lin PL, Myers A, Smith L et al (2010) Tumor necrosis factor neutralization results in disseminated disease in acute and latent *Mycobacterium tuberculosis* infection with normal granuloma

structure in a cynomolgus macaque model. Arthritis Rheum 62(2):340–350
62. Iliopoulos A, Psathakis K, Aslanidis S, Skagias L, Sfikakis PP (2006) Tuberculosis and granuloma formation in patients receiving anti-TNF therapy. Int J Tuberc Lung Dis 10(5):588–590
63. Bru A, Cardona P-J (2010) Mathematical modeling of tuberculosis bacillary counts and cellular populations in the organs of infected mice. PLoS ONE 5(9):e12985
64. Cardona P-J, Ivanyi J (2011) The secret trumps, impelling the pathogenicity of tubercle bacilli. Enferm Infecc Microbiol Clin 29(Suppl 1):14–19
65. Ramakrishnan L (2012) Revisiting the role of the granuloma in tuberculosis. Nat Rev Immunol 12(5):352–366
66. Volkman HE, Pozos TC, Zheng J, Davis JM, Rawls JF, Ramakrishnan L (2010) Tuberculous granuloma induction via interaction of a bacterial secreted protein with host epithelium. Science 327(5964):466–469
67. Black GF, Thiel BA, Ota MO et al (2009) Immunogenicity of novel DosR regulon-encoded candidate antigens of *Mycobacterium tuberculosis* in three high-burden populations in Africa. Clin Vaccine Immunol 16(8):1203–1212
68. Schnappinger D, Schoolnik GK, Ehrt S (2006) Expression profiling of host pathogen interactions: how *Mycobacterium tuberculosis* and the macrophage adapt to one another. Microb Infect 8(4):1132–1140
69. Garton NJ, Waddell SJ, Sherratt AL et al (2008) Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum. PLoS Med 5(4):e75
70. Chegou NN, Black GF, Loxton AG et al (2012) Potential of novel *Mycobacterium tuberculosis* infection phase-dependent antigens in the diagnosis of TB disease in a high burden setting. BMC Infect Dis 12(1):10
71. Chegou NN, Essone PN, Loxton AG et al (2012) Potential of host markers produced by infection phase-dependent antigen-stimulated cells for the diagnosis of tuberculosis in a highly endemic area. PLoS ONE 7(6):e38501
72. Boon C, Dick T (2010) How *Mycobacterium tuberculosis* goes to sleep: the dormancy survival regulator DosR a decade later. Futur Microbiol 7(4):513–518
73. Wayne LG, Sohaskey CD (2001) Nonreplicating persistence of *Mycobacterium tuberculosis*. Annu Rev Microbiol 55:139–163
74. Keren I, Minami S, Rubin E, Lewis K (2011) Characterization and transcriptome analysis of *Mycobacterium tuberculosis* persisters. mBio 2(3):e00100–e00111
75. Manganelli R, Voskuil MI, Schoolnik GK, Smith I (2001) The *Mycobacterium tuberculosis* ECF sigma factor sigmaE: role in global gene expression and survival in macrophages. Mol Microbiol 41(2):423–437
76. Aagaard C, Hoang T, Dietrich J, Cardona P-J, Izzo A, Dolganov G, Schoolnik GK, Cassidy JP, Billeskov R, Andersen P (2011) A multistage tuberculosis vaccine that confers efficient protection before and after exposure. Nat Med 17(2):189–194
77. Mitchison D, Davies G (2012) The chemotherapy of tuberculosis: past, present and future [State of the art]. Int J Tuberc Lung Dis 16(6):724–732
78. Lienhardt C, Raviglione M, Spigelman M, Hafner R, Jaramillo E, Hoelscher M, Zumla A, Gheuens J (2012) New drugs for the treatment of tuberculosis: needs, challenges, promise, and prospects for the future. J Infect Dis 205(suppl 2):S241–S249
79. Gler MT, Skripconoka V, Sanchez-Garavito E et al (2012) Delamanid for multidrug-resistant pulmonary tuberculosis. N Engl J Med 366(23):2151–2160
80. Diacon AH, Donald PR, Pym A et al (2012) Randomized pilot trial of eight weeks of bedaquiline (TMC207) treatment for multidrug-resistant tuberculosis: long-term outcome, tolerability, and effect on emergence of drug resistance. Antimicrob Agents Chemother 56(6):3271–3276
81. Alffenaar JWC, van der Laan T, Simons S, van der Werf TS, van de Kasteele PJ, de Neeling H, van Soolingen D (2011) Susceptibility of clinical *Mycobacterium tuberculosis* isolates to a potentially less toxic derivate of linezolid, PNU-100480. Antimicrob Agents Chemother 55(3):1287–1289
82. Williams K, Minkowski A, Amoabeng O, Peloquin CA, Taylor D, Andries K, Wallis RS, Mdluli KE, Nuermberger EL (2012) Sterilizing activities of novel combinations lacking first- and second-line drugs in a murine model of tuberculosis. Antimicrob Agents Chemother 56(6):3114–3120
83. Pranger AD, Kosterink JGW, van Altena R, Aarnoutse RE, van der Werf TS, Uges DRA, Alffenaar J-WC (2011) Limited-sampling strategies for therapeutic drug monitoring of moxifloxacin in patients with tuberculosis. Ther Drug Monit 33(3):350–354
84. Pranger AD, van Altena R, Aarnoutse RE, van Soolingen D, Uges DRA, Kosterink JGW, van der Werf TS, Alffenaar JWC (2011) Evaluation of moxifloxacin for the treatment of tuberculosis: 3 years of experience. Eur Respir J 38(4):888–894
85. Alffenaar J-WC, van Altena R, Harmelink IM, Filguera P, Molenaar E, Wessels AMA, van Soolingen D, Kosterink JGW, Uges DRA, van der Werf TS (2010) Comparison of the pharmacokinetics of two dosage regimens of linezolid in multidrug-resistant and extensively drug-resistant tuberculosis patients. Clin Pharmacokinet 49(8):559–565
86. Alffenaar J-WC, Kosterink JGW, van Altena R, van der Werf TS, Uges DRA, Proost JH (2010) Limited sampling strategies for therapeutic drug monitoring of linezolid in patients with multidrug-resistant tuberculosis. Ther Drug Monit 32(1):97–101
87. Alffenaar JWC, van Altena R, Bökkerink HJ, Luijckx GJ, van Soolingen D, Aarnoutse RE, van der Werf TS (2009) Pharmacokinetics of moxifloxacin in cerebrospinal fluid and plasma in patients with tuberculous meningitis. Clin Infect Dis 49(7):1080–1082
88. Srivastava S, Pasipanodya JG, Gumbo T (2012) Reply to Alffenaar. J Infect Dis 205(11):1766
89. McShane H (2011) Tuberculosis vaccines: beyond bacille Calmette-Guerin. Philos Trans R Soc Lond B Biol Sci 366(1579):2782–2789
90. Kaufmann SH, Hussey G, Lambert P-H (2010) New vaccines for tuberculosis. Lancet 375(9731):2110–2119
91. Zwerling A, Behr MA, Verma A, Brewer TF, Menzies D, Pai M (2011) The BCG World Atlas: a database of global BCG vaccination policies and practices. PLoS Med 8(3):e1001012
92. Ottenhoff THM, Kaufmann SHE (2012) Vaccines against tuberculosis: where are we and where do we need to go? PLoS Pathog 8(5):e1002607–e1002612
93. Maleszewska M, Moonen J-RAJ, Huijkman N, van de Sluis B, Krenning G, Harmsen MC (2012) IL-1β and TGFβ2 synergistically induce endothelial to mesenchymal transition in an NFκB-dependent manner. Immunobiology. doi:10.1016/j.imbio.2012.05.026
94. Beresford B, Sadoff JC (2010) Update on research and development pipeline: tuberculosis vaccines. Clin Infect Dis 50(s3):S178–S183
95. Krenning G, Zeisberg EM, Kalluri R (2010) The origin of fibroblasts and mechanism of cardiac fibrosis. J Cell Physiol 225(3):631–637
96. Kaufmann SHE (2010) Future vaccination strategies against tuberculosis: thinking outside the box. Immunity 33(4):567–577
97. World Health Organization (2011) Global Plan to Stop TB. WHO, Geneva
98. Skeiky YAW, Alderson MR, Ovendale PJ et al (2004) Differential immune responses and protective efficacy induced by

components of a tuberculosis polyprotein vaccine, Mtb72F, delivered as naked DNA or recombinant protein. J Immunol 172(12):7618–7628
99. Grange JM, Brunet LR, Rieder HL (2011) Immune protection against tuberculosis—when is immunotherapy preferable to vaccination? Tuberc (Edinb) 91(2):179–185
100. Suthar AB, Lawn SD, del Amo J et al (2012) Antiretroviral therapy for prevention of tuberculosis in adults with HIV: a systematic review and meta-analysis. PLoS Med 9(7):e1001270
101. von Reyn CF, Mtei L, Arbeit RD et al (2010) Prevention of tuberculosis in Bacille Calmette–Guérin-primed, HIV-infected adults boosted with an inactivated whole-cell mycobacterial vaccine. AIDS 24(5):675–685
102. Stanford JL, Grange JM (1994) The promise of immunotherapy for tuberculosis. Respir Med 88(1):3–7
103. Yang X-Y, Chen Q-F, Li Y-P, Wu S-M (2011) Mycobacterium vaccae as adjuvant therapy to anti-tuberculosis chemotherapy in never-treated tuberculosis patients: a meta-analysis. PLoS ONE 6(9):e23826
104. Vilaplana C, Montané E, Pinto S, Barriocanal AM, Domenech G, Torres F, Cardona PJ, Costa J (2010) Double-blind, randomized, placebo-controlled Phase I Clinical Trial of the therapeutical antituberculous vaccine RUTI®. Vaccine 28(4):1106–1116
105. Hawkridge T, Mahomed H (2011) Prospects for a new, safer and more effective TB vaccine. Paediatr Respir Rev 12(1):46–51
106. Arjanova VO, Prihoda DN, Yurchenko VL, Sokolenko NI, Frolov MV (2010) Phase 2 trial of V-5 Immunitor (V5) in patients with chronic hepatitis C Co-infected with HIV and *Mycobacterium tuberculosis*. J Vaccines Vaccin 01(01):1–5
107. Butov DA, Pashkov YN, Stepanenko AL, Choporova AI, Butova TS, Batdelger D, Jirathitikal V, Bourinbaiar AS, Zaitzeva SI (2011) Phase IIb randomized trial of adjunct immunotherapy in patients with first-diagnosed tuberculosis, relapsed and multi-drug-resistant (MDR) TB. J Immune Based Ther Vaccines 9(1):3
108. Koch R (1891) A further communication on a remedy for tuberculosis. BMJ 1(1568):125–127
109. Gradmann C (2001) Robert Koch and the pressures of scientific research: tuberculosis and tuberculin. Med Hist 45(1):1–32
110. Die Atiologie der Tuberculose. Facsimile of the original contribution by Robert Koch in "Berliner Klinische Wochenschrift" 10 April 1882
111. British Medical Research Council (1972) Controlled clinical trial of short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis. Lancet 1(7760):1079–1085
112. Grange JM (1997) Immunotherapy: a new strategy for tuberculosis control? Respir Med 91(1):1–4
113. Stanford J, Stanford C, Stansby G, Bottasso O, Bahr G, Grange J (2009) The common mycobacterial antigens and their importance in the treatment of disease. Curr Pharm Des 15(11):1248–1260
114. Camporota L, Corkhill A, Long H et al (2003) The effects of *Mycobacterium vaccae* on allergen-induced airway responses in atopic asthma. Eur Respir J 21(2):287–293
115. Fontanella GH, Pascutti MF, Daurelio L, Perez AR, Nocito AL, Wojdyla D, Bottasso O, Revelli SS, Stanford JL (2007) Improved outcome of *Trypanosoma cruzi* infection in rats following treatment in early life with suspensions of heat-killed environmental Actinomycetales. Vaccine 25(17):3492–3500
116. Stansby G, Chan YC, Berwanger CS, Shurey S, Rook GAW, Stanford JL (2002) Prevention of experimental myointimal hyperplasia by immunomodulation. Eur J Vasc Endovasc Surg 23(1):23–28
117. Abbot NC, Beck JS, Feval F, Weiss F, Mobayen MH, Ghazi Saidi K, Dowlati Y, Velayati AA, Stanford JL (2002) Immunotherapy with *Mycobacterium vaccae* and peripheral blood flow in long-treated leprosy patients, a randomised, placebo-controlled trial. Eur J Vasc Endovasc Surg 24(3):202–208
118. Stanford JL, Stanford CA, O'Brien MER, Grange JM (2008) Successful immunotherapy with *Mycobacterium vaccae* in the treatment of adenocarcinoma of the lung. Eur J Cancer 44(2):224–227
119. Marro A, Pirles M, Schiaffino L, Bin L, Dávila H, Bottasso OA, McIntyre G, Ripley PR, Stanford CA, Stanford JL (2011) Successful immunotherapy of canine flea allergy with injected Actinomycetales preparations. Immunotherapy 3(8):971–978
120. Ramu G, Prema GD, Balakrishnan S, Shanker Narayan NP, Stanford JL (1990) A preliminary report on the immunotherapy of psoriasis. Indian Med Gazette 124:381–382
121. Lehrer A, Bressanelli A, Wachsmann V, Bottasso O, Bay ML, Singh M, Stanford C, Stanford J (1998) Immunotherapy with *Mycobacterium vaccae* in the treatment of psoriasis. FEMS Immunol Med Microbiol 21(1):71–77
122. Gil O, Guirado E, Gordillo S, Díaz J, Tapia G, Vilaplana C, Ariza A, Ausina V, Cardona P-J (2006) Intragranulomatous necrosis in lungs of mice infected by aerosol with *Mycobacterium tuberculosis* is related to bacterial load rather than to any one cytokine or T cell type. Microb Infect 8(3):628–636
123. Uhlin M, Andersson J, Zumla A, Maeurer M (2012) Adjunct immunotherapies for tuberculosis. J Infect Dis 205(suppl 2):S325–S334
124. Van Deun A, Maug AKJ, Hossain A, Gumusboga M, de Jong BC (2012) Fluorescein diacetate vital staining allows earlier diagnosis of rifampicin-resistant tuberculosis. Int J Tuberc Lung Dis 16(9):1174–1179
125. Hamid Salim A, Aung K, Hossain MA, Van Deun A (2006) Early and rapid microscopy-based diagnosis of true treatment failure and MDR-TB. Int J Tuberc Lung Dis 10(11):1248–1254
126. Warmelink I, ten Hacken NH, van der Werf TS, van Altena R (2010) Weight loss during tuberculosis treatment is an important risk factor for drug-induced hepatotoxicity. Br J Nutr 105(03):400–408
127. Warmelink I, van Altena R, ten Hacken N, van der Werf T, van der Veer E (2011) Nutritional status and vitamin D3 during antimicrobial treatment. Lancet 377(9775):1407–1408
128. Campbell GR, Spector SA (2012) Vitamin D inhibits human immunodeficiency virus type 1 and *Mycobacterium tuberculosis* infection in macrophages through the induction of autophagy. PLoS Pathog 8(5):e1002689
129. Kempker RR, Vashakidze S, Solomonia N, Dzidzikashvili N, Blumberg HM (2012) Surgical treatment of drug-resistant tuberculosis. Lancet Infect Dis 12(2):157–166
130. Iddriss A, Padayatchi N, Reddy D, Reddi A (2012) Pulmonary resection for extensively drug resistant tuberculosis in KwazuluNatal, South Africa. Ann Thorac Surg 94(2):381–386