Adjunct Immunotherapies for Tuberculosis

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The continued spread of multidrug-resistant (MDR) tuberculosis and extensively drug-resistant tuberculosis poses a major threat to global tuberculosis control. Treatment is complex and requires longer use of more-expensive, less effective, and toxic anti-tuberculosis drugs, which results in high morbidity and mortality. The poor treatment outcomes and the slow progress in the development and evaluation of new tuberculosis drugs have given rise to the development of adjunct immunotherapy. The host immune system is a critical factor both for containment and cure of Mycobacterium tuberculosis infection. Augmentation or dampening of proinflammatory responses can be of value in the treatment of individuals who have nonproductive M. tuberculosis infection with inflammation-induced tissue damage. The use of immunotherapy with interleukin 2, interferon γ, and interleukin 7 as an adjunct to drug treatment may improve success rates for treatment of MDR tuberculosis, shorten treatment time for drug-sensitive tuberculosis, and improve the immunity of individuals by enhancing M. tuberculosis elimination to prevent recurrence of disease. A broad range of immunological treatments, including cytokine treatment or cell-based therapy, is now available, although not all have been evaluated in humans. This review gives a critical overview of current adjunct immunotherapies for active tuberculosis, which are at various stages of development.

Treatment of drug-resistant tuberculosis is cost intensive, associated with poor treatment outcomes [1–3], and linked with high incidence of adverse events [3, 4]. There is an urgent need for alternative adjunct therapies for treatment of multidrug-resistant (MDR) tuberculosis and extensively drug-resistant (XDR) tuberculosis: Mycobacterium tuberculosis strains resistant to all anti-tuberculosis drugs have been reported [5], and the number of cases of MDR tuberculosis is alarmingly increasing worldwide, with MDR detected in up to 35% of newly diagnosed cases and in 76.5% of patients who had previously been treated for tuberculosis. XDR tuberculosis was identified in 14% of patients with MDR, with patients <35 years old exhibiting an odds of MDR tuberculosis that was 2 times that for individuals aged >35 years [6]. The deleterious outcome of MDR tuberculosis and XDR tuberculosis, each with a mortality >30% of patients in the first year after diagnosis, is due to several factors, including chronic infection, inflammation, and immune exhaustion. Poor immune responses, particularly impaired T-helper 1 (Th1) cell responses, in patients with drug-resistant tuberculosis contribute to treatment failures [7–10].

The continued spread of MDR tuberculosis and XDR tuberculosis poses a major threat to global tuberculosis control. Treatment of MDR tuberculosis and XDR tuberculosis is complex and requires longer use of expensive, less effective, and toxic anti-tuberculosis drugs, resulting in high mortality. The poor treatment outcomes and the slow progress in development and evaluation of new tuberculosis drugs and drug regimens for drug-resistant tuberculosis have given rise to the development of several immunotherapies for potential use as adjuncts to conventional anti-tuberculosis
**Table 1. Immunotherapeutic Agents for Potential Use in Adjunct Tuberculosis Treatment**

<table>
<thead>
<tr>
<th>Immunotherapeutic Approach</th>
<th>Product/Agent</th>
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<tbody>
<tr>
<td>Influence a favorable immune response</td>
<td>Anti-interleukin 4 neutralizing antibodies</td>
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<tr>
<td></td>
<td>High-dose intravenous immunoglobulin</td>
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<td></td>
<td>16α-bromodeoxypiandrostenedione (HE2000)</td>
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<td></td>
<td>Heat-killed environmental mycobacterial preparations (Mycobacterium vaccae, M.w)</td>
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<tr>
<td></td>
<td>RUTI (Mycobacterium tuberculosis liposomal preparation)</td>
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<tr>
<td></td>
<td>DNA vaccine (with HSP65, Ag85, MPT-64, and MPT-83)</td>
</tr>
<tr>
<td></td>
<td>γ-glutamyl-tryptophan (SCV-07 SciCLone)</td>
</tr>
<tr>
<td></td>
<td>Dherelo (plant extracts)</td>
</tr>
<tr>
<td></td>
<td>Corticosteroids (reduces TNF-α levels)</td>
</tr>
<tr>
<td></td>
<td>Thalidomide (reduces TNF-α levels)</td>
</tr>
<tr>
<td></td>
<td>Etanercept (anti–TNF-α; reduces levels of terminally differentiated T cells, which express TNF on their cell surface, and neutralizes TNF-α [96, 97])</td>
</tr>
<tr>
<td></td>
<td>Recombinant human cytokines</td>
</tr>
<tr>
<td></td>
<td>Interleukin 2</td>
</tr>
<tr>
<td></td>
<td>Interleukin 7</td>
</tr>
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<td></td>
<td>Interleukin 15</td>
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<tr>
<td></td>
<td>Interleukin 27</td>
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<tr>
<td></td>
<td>Interleukin 12</td>
</tr>
<tr>
<td></td>
<td>Interferon γ</td>
</tr>
<tr>
<td></td>
<td>Recombinant granulocyte-macrophage colony-stimulating factor</td>
</tr>
</tbody>
</table>

The table is adapted from [11] and [12] and lists immunological compounds and immune-response modifiers that may aid to restore and achieve a clinically effective anti–M. tuberculosis immune response. Note that the compounds are not only suitable for the use as adjunct treatment for active tuberculosis. **Abbreviation:** TNF-α, tumor necrosis factor α.

Use of biological therapy: why and when

There are different clinical situations in which adjunct immunotherapy options may be considered, such as for individuals with inborn or acquired immune defects. The Mendelian susceptibility to mycobacterial diseases is primarily seen in children, but adult cases are being increasingly reported [13, 14]. The most common genetic etiologies for the Mendelian susceptibility to mycobacterial diseases are interleukin 12Rb1 or interferon γ (IFN-γ) receptor deficiencies [13]. Patients with these conditions are prime candidates for adjuvant immunotherapy in the form of exogenous cytokines to overcome nominal deficits in levels of these molecules [15]. IFN-γ has been successfully administrated to some of these individuals in smaller case studies [15–18].

Arguably, the 3 most pressing clinical indications for using adjunct immunotherapy for tuberculosis are as follows: (1) to facilitate increased cure rates among patients with MDR tuberculosis for whom results of standard therapy are suboptimal, (2) to reduce hospital costs by shortening the duration of drug-susceptible tuberculosis, and (3) to decrease potential tissue damage and morbidity by downregulating a clinically non-productive host immune response. In all 3 situations, active tuberculosis (particularly in individuals with human immunodeficiency virus infection) is targeted. Timing to speed up clearance of the infection is crucial, and we will therefore focus on cytokines and other biological response modifiers that are already in clinical use and could therefore be quickly moved into clinical trials.

**Cytokines as Adjunct Therapies: Completed and Ongoing Clinical Trials**

Protective T-cell immunity against *M. tuberculosis* at the site of infection is mediated by Th1 cytokines, such IFN-γ and interleukin 2 (IL-2) [19]. Lack of clinically effective Th1 responses has been incriminated in tuberculosis pathogenesis; therefore, IL-2 injection has been deemed to be a natural adjunct therapy for tuberculosis. The first randomized trial showed, in contrast to the expected outcome, a slightly prolonged time to negative sputum conversion in the IL-2–treated group [20]. It was postulated that IL-2 increased the number of regulatory T cells (Tregs), resulting in adverse effects on the anti–M. tuberculosis immune response [21]. IL-2 is already a standard option for adjunct treatment of malignant melanoma and renal cancer [22, 23] and could therefore be moved rapidly into a clinical setting for treatment of tuberculosis if clinical benefits could be demonstrated, perhaps by inducing a compartmentalized IL-2 response through an aerosolized route. Aerosolized IL-2 has been used in the treatment of lung metastases from primary kidney cancer [24].

Preliminary studies concerning treatment of MDR tuberculosis with aerosolized IFN-γ showed initially promising results [25]. It has been shown that nebulized IFN-γ, in combination with standard treatment, can both decrease constitutional symptoms, such as night sweats and fever, and increase the rate of *M. tuberculosis* clearance from sputum [26]. One likely mode of IFN-γ action is to induce the release of IP-10, which is associated with increased lymphocyte recruitment and decreased inflammation mediated by neutrophils. This results in limited immune-mediated tissue damage and an improved drug therapy [11, 12] (Table 1). This review highlights needs, underlying mechanisms, unanswered issues, and progress in the field of adjunct immunotherapies for tuberculosis.
time to M. tuberculosis clearance [27, 28] (Table 2). In vitro models were employed to study in greater detail IFN-γ–mediated actions on human macrophages. IFN-γ–induced activation of macrophages limits M. tuberculosis growth in 40% human plasma under 5%–10% (physiologic) oxygen in the presence of granulocyte-macrophage colony-stimulating factor and/or tumor necrosis factor α (TNF-α), followed by culture in IFN-γ. This could not be achieved using fetal bovine serum in 20% oxygen along with higher cytokine concentrations and premature IFN-γ exposure. These data warrant scrutiny of the experimental models used for in vitro testing of cytokines as adjunct tuberculosis therapy [29].

IFN-γ and TNF-α are the 2 cytokines whose critical roles in the control of human tuberculosis are best documented by (1) genetic deficiencies, (2) acquired predisposition to mycobacterial infection associated with autoantibodies directed to IFN-γ [30], and (3) administration of therapeutic agents that neutralize TNF-α in the treatment of inflammatory diseases [31]. A recent systematic review of adjunct IFN-γ therapy (subcutaneous, intramuscular, and aerosolized formulations) for the treatment of pulmonary tuberculosis identified 9 international trials. Meta-analysis of 3 trials showed significant improvement concerning sputum negativity after 2 months of intramuscular formulation. Statistically relevant differences for the subcutaneous and aerosolized IFN-γ formulations were found for tuberculosis-associated symptoms (ie, fever and night sweats). However, the number of patients enrolled was too small to provide more-definitive statements concerning safety and efficacy [32].

More-recent data from murine models showed that effective resistance to M. tuberculosis requires the host to contain bacterial replication and prevent deleterious immune responses [33]. IFN-γ has been identified as having a central role in this step: IFN-γ is crucial for macrophage activation and tissue inflammation, and these functions can be dissociated. IFN-γ–negative CD4+ T cells have been shown to retain antimicrobial capacity, yet they lost the capacity to suppress tissue inflammation. IFN-γ reduced interleukin 17 (IL-17) production, which is responsible for neutrophil recruitment and activation. Nandi and Behar [33] suggested that an increased number of neutrophils could turn out to be a biologically relevant biomarker of nonproductive Th1 immunity (or loss of IFN-γ responsiveness). These data show the multifaceted functions of IFN-γ in tuberculosis therapy and underline the need to identify relevant biomarkers for gauging response to therapy and to define the most suitable time point for immune intervention [33].

IMMUNOTHERAPY: TIPPING THE BALANCE BY REDUCING INFLAMMATION

There is a broad array of data regarding the application of exogenous cytokines or costimulatory alternatives that could be used as immunostimulatory agents to augment and expand anti–M. tuberculosis cellular immune responses (Table 2). Both the dosing and the timing of exogenous cytokine therapy need to be examined (see the discussion above about the different actions of IFN-γ on the immune response in tuberculosis, which depend on dosing and timing). A more individualized immunological profile is required to choose the most suitable reagent and time frame for therapy. It is possible that too much added “inflammation” in the form of exogenous agents (eg, IFN-γ, interleukin 18, and TNF-α) might be deleterious, contribute to immunopathology, or lead to exhaustion of the existing immune response [34]. It has been postulated that there is a safeguard mechanism that protects against autoimmune diseases by putting a limit on the amount of stimulation lymphocytes can “manage” before going into anergy. The molecules that trigger this “emergency brake” are PD-1 and KLRG1 [35, 36]. Thus, certain clinical situations may require an increase in inflammation, whereas others require dampening of the immune response, at least at the site of infection. This might be of paramount importance for tipping an existing immunological imbalance in order to stop organ damage by overt inflammation and to refocus the immune response toward clinically and biologically relevant immuno-reactivity.

One possible approach is the infusion of autologous immunomodulatory mesenchymal stromal cells (MSCs) (Skrahin et al, unpublished data). Nine patients with MDR tuberculosis were enrolled in a study to evaluate this approach; all received individualized therapy after drug-susceptibility testing, in accordance with World Health Organization standards. The patients were infused with bone marrow–derived MSCs and followed clinically and experimentally for 6 months. Five patients were cured, and 4 patients showed disease stabilization. Several mechanisms may account for the beneficial role of MSCs on tuberculosis treatment. A direct, protective, regenerative effect of the MSCs on lung tissue may be clinically relevant for patients. Other beneficial effects of the MSCs on overt chronic inflammation could include the overall down-tuning of inflammation-related genes and the upregulation of genes involved in phagocytosis, which was shown in a murine sepsis model [37].

Other approaches may also be used to limit excess inflammation. Intravenous immunoglobulin (IVIG) is routinely used as a supplemental treatment for patients with primary or secondary immunodeficiencies [38]. The effects of IVIG are likely due to mechanisms associated with its variable F(ab)2 and constant Fc regions. Beneficial effects of IVIG have been observed in patients with infectious diseases who received IVIG doses that were greater than those associated with standard immunodeficiency treatment protocols [39]. The detailed effects of IVIG on tuberculosis are ill defined. A high concentration of exogenous immunoglobulin G molecules may
<table>
<thead>
<tr>
<th>Agent</th>
<th>Disease Species</th>
<th>Theoretical Mode(s) of Action</th>
<th>Outcome(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-2</strong></td>
<td>Tuberculosis Human</td>
<td>Increased Th1 responses</td>
<td>Prolonged time to negative sputum conversion in the IL-2–treated group, most likely due to Treg expansion</td>
<td>[20]</td>
</tr>
<tr>
<td><strong>IFN-γ</strong></td>
<td>Tuberculosis Human</td>
<td>Induces IP-10, which is associated with increased recruitment of lymphocytes and decreased inflammation mediated by neutrophils; limits immune-mediated tissue damage; decreases time to tuberculosis clearance</td>
<td>Nebulized IFN-γ in combination with standard treatment decreases symptoms (ie, night sweats, fever) and increases the rate of tuberculosis clearance from sputum</td>
<td>[25, 26]</td>
</tr>
<tr>
<td><strong>Thalidomide</strong></td>
<td>Tuberculosis Human</td>
<td>Suppresses TNF-α production by macrophages and reduces systemic inflammation, increases IFN-γ, IL-2, and IL-12 levels, has a costimulatory effect on T cells</td>
<td>Shows promising effects against severe, overt inflammatory reactions associated with tuberculosis</td>
<td>[46, 47]</td>
</tr>
<tr>
<td><strong>Mycobacterium vaccae</strong></td>
<td>Tuberculosis Human</td>
<td>Directs CD8+ T cells against epitopes common among mycobacterial strains and/or alters the T cell response toward a Th1 prototype</td>
<td>Several larger double-blind randomized trials failed to show effects of the vaccine</td>
<td>[98–102]</td>
</tr>
<tr>
<td><strong>RUTI vaccine</strong></td>
<td>Tuberculosis Human</td>
<td>Combined with an initial period of chemotherapy, the rationale is to reactivate latent <em>M. tuberculosis</em> bacilli and make them more susceptible to killing by vaccine-created antigen-specific T cells; will contribute new T cell epitopes that were earlier neglected by the natural immune repertoire</td>
<td>Some efficacy in controlling tuberculosis after an initial short burst of chemotherapy in mice and guinea pigs</td>
<td>[103]</td>
</tr>
<tr>
<td><strong>Mesenchymal stem cells</strong></td>
<td>Tuberculosis Human</td>
<td>Has a direct, protective, regenerative effect on lung tissue, downregulates overt chronic inflammation, upregulates genes involved in phagocytosis</td>
<td>Of 9 study patients, 5 were cured, and 4 showed disease stabilization</td>
<td>Skrahin et al (unpublished data)</td>
</tr>
<tr>
<td><strong>IVIG</strong></td>
<td>Tuberculosis/ bacterial infection Murine</td>
<td>Mechanisms are associated with variable F(ab)2 and constant Fc regions</td>
<td>Reduces unproductive inflammation, with multiple effects on innate and adaptive cellular immune responses</td>
<td>[40]</td>
</tr>
<tr>
<td><strong>IL-7</strong></td>
<td>Tuberculosis Human</td>
<td>Increases T cell memory responses, downregulates immune-suppressive TGFbeta production, has positive effect on IL-17 production at the site of infection via γδ T cells</td>
<td>Showed a survival advantage in <em>M. tuberculosis</em> infection if coadministered with BCG vaccine</td>
<td>[91, 92]</td>
</tr>
<tr>
<td><strong>IL-7</strong></td>
<td>Tuberculosis Human</td>
<td>Restores immune responsiveness, mediates protection from apoptosis, contributes to dendritic cell activation, aids in immune memory formation</td>
<td>Has been successfully used to treat HIV infection and patients after HSCT; speeds up immune reconstitution in patients after HSCT, and a similar effect may be helpful in tuberculosis</td>
<td>[85, 86]</td>
</tr>
</tbody>
</table>
outcompete existing circulating endogenous antibodies and consequently dampen clinically unproductive inflammation [40].

**IMMUNE-RESPONSE MODIFIERS IN TUBERCULOSIS: THALIDOMIDE**

While results with IFN-γ have been encouraging, indirect ways to modulate anti-tuberculosis immune responses have been explored using chemical compounds. Thalidomide was initially used as a tranquilizer [41]. Later discoveries have shown its potent anti-inflammatory and immunomodulatory effects [42]. Thalidomide suppresses TNF-α production in macrophages and thereby reduces systemic inflammation [43], whereas it increases levels of other cytokines (eg, IFN-γ, IL-2, and interleukin 12 [IL-12]) [44]. Thalidomide has a costimulatory effect on T cells, preferentially targeting CD8+ T cells [45]. More-comprehensive studies regarding the use of thalidomide to treat tuberculosis are not available at this point, yet clinical data suggested that thalidomide shows promising effects against severe, overt inflammatory reactions associated with tuberculosis, particularly in the central nervous system [46, 47]. Thalidomide-mediated associations are associated with age, and most clinical data are from children. Of note, the clinical presentation of tuberculosis is different in children as compared with adults [48] in part because the immune responses in children and adults are qualitatively and quantitatively different. Children show higher T-helper cell 17 (Th17) responses in the peripheral circulation and favor T-helper cell 2 responses (characterized by interleukin 4 and interleukin 5 responses), as reviewed in a recent meeting report [49].

**TIMING IS IMPORTANT**

The timing of all immunotherapies might be of vital importance, and therefore individualized diagnosis and pattern-recognition analysis is required to choose the best strategy. Perhaps an extreme example that shows the importance of the timing of biological therapy and immunomodulation is the use of IFN-γ to treat patients with sepsis syndrome. Intuitively, IFN-γ treatment should worsen the cytokine storm. Yet if IFN-γ is applied in a specific window of immune paralysis during the clinical course of sepsis, defined by downregulation of HLA-DR on monocytes in peripheral blood mononuclear cells, patients show increased survival [50]. At this specific time point only, IFN-γ helps turn the unsuccessful immune response into a clinically measurable reaction associated with increased survival.

Another relevant example regarding timing is the double-edged sword of IL-17 in tuberculosis. During primary tuberculosis, both IL-17 and Th1 responses are induced at the site of infection [51]. This is of great importance for inducing the expression of chemokines that promote further cell recruitment and granuloma organization [52, 53]. During the
chronic phase of *M. tuberculosis* infection, a delicate balance of Th1 and Th17 responses appears to be required to control bacterial growth and limit immunopathology. If the Th17 response is continually present, then excessive inflammation may take place, leading to a continued extensive neutrophil recruitment and subsequent tissue damage [54]. Thus, the regulation of Th17 responses during the different stages of tuberculosis is essential to promote clinically relevant anti-tuberculosis responses that prevent extensive immunopathology.

**FOCUS OF ATTENTION: ACCESS TO THE SITE OF *M. tuberculosis* INFECTION**

While chronic tuberculosis is localized to the lungs, it might be beneficial to directly apply in situ immunotherapy that influences activities at the site of infection. Several candidate molecules may be the target for local intervention. For instance, transforming growth factor β (TGF-β) might have an inhibitory effect on the effective local tuberculosis response [55]. Additionally, TGF-β is capable of inducing collagen synthesis and promoting fibrosis, a common abnormality in advanced pulmonary tuberculosis [56]. An appealing strategy to influence the TGF-β levels is to locally enhance the levels of IL-7. IL-7 will then decrease TGF-β levels, decrease T cell apoptosis, and selectively induce proliferation in non-Tregs [57]. A viable therapeutic option is to counteract the adverse effects of interleukin 10 (IL-10) on antigen processing and presentation in macrophages [58]. For in situ immunotherapy to be effective, an efficient way of delivering the therapeutic compounds has to be used. It would be tempting to combine intravenous infusion of MSCs, which can home to the inflamed tissue in the lung, with vectors carrying IL-7 or other potentially beneficial agents [59] to counteract TGF-β expression in situ and thereby promote immunosuppressive effects and aids to restructure lung tissue. Other prospective methods for delivering agent delivery involve liposomes (reviewed in [60]) or nonimmunogenic, inert vehicles, such as nonviral hyaluronan nanoparticles [61].

**RESCUE FROM EXHAUSTION: ADJUNCT IMMUNOTHERAPIES AND THE NEED FOR IMMUNE PROFILING**

While tuberculosis, particularly MDR tuberculosis, can be a lifelong disease, there is a risk that repeated antigenic stimulation will drive *M. tuberculosis*–specific T cells into exhaustion or anergy [62, 63]. We discuss here the use of immunotherapeutic approaches for treatment of active tuberculosis; cytokines and immune-response modifiers may be viable options on the basis of limited data concerning IL-2 and/or IFN-γ treatments. While these treatments may help to achieve faster *M. tuberculosis* clearance, they may have different effects in the long run on the basis of the following observations. First, adjunct immunotherapies will most likely be applied while the patient is taking anti-tuberculosis drugs. At this time, we have no robust data on how anti-tuberculosis drugs act on immunoreactivity in general. Several antibiotics have been shown to exert anti-inflammatory properties [64, 65], and we recently learned from the cancer field that “standard” anticancer drugs have potent immunostimulatory properties crucial for response to (cancer) therapy. These novel data show the additive value of standard chemotherapy and underline the need for a multimodal approach in biological therapy adjunct to the standard treatment modalities [66]. Consequently, the standard drugs in tuberculosis would need to be tested for their interface with an ongoing anti-tuberculosis immune response. Second, although this report is focused on the treatment of active tuberculosis, any treatment with cytokines will have proximal effects on anti-tuberculosis immune responses. For example, IL-2 treatment may be beneficial in tuberculosis, yet it also carries the risk of inducing activation-induced cell death in antigen-specific T cells (reviewed in [67]); this may have consequences for the establishment of long-lived immune memory responses directed against *M. tuberculosis* antigens. Third, the immune response in individuals at the time of adjunct therapy needs to be better defined to allow a more targeted approach. Cytokine-release assays gauging levels of IL-2, IFN, TNF, interleukin 10, and IL-17 produced by CD4+ and CD8+ T cells may be helpful to evaluate the ex vivo response to maximal T-cell stimulation and to defined *M. tuberculosis* antigens. The profile of a clinically protective cellular immune response directed against defined target antigens has yet to be defined. Particularly the cytokine production of CD4+ and CD8+ T-cells has to be considered. A recent report demonstrated that cellular immune responses in individuals with active tuberculosis lack IL-2 and IFN-γ responses and are only capable of producing TNF-α in response to ESAT-6 in individuals with latent tuberculosis [68]. Although the purpose of the study by Harari et al [68] was to design diagnostic tests that were biologically more meaningful, a similar testing platform could be used to gauge immune responses, which will help profile the efficacy of immunotherapy for tuberculosis and provide useful markers for guiding novel treatment modalities. Standard immunomonitoring protocols that will allow multicenter studies are need in the field of tuberculosis therapies. For instance, the MIATA (Minimal Information About T Cell Assays) initiative, launched in October 2009, aims to achieve a broad consensus on which T cell assay parameters should be systemically reported in scientific articles about the use of immunotherapy in patients with cancer (http://www.cancerresearch.org), and a similar approach could be adopted for the field of infectious diseases.

Robust immunomonitoring will also help to substantiate the observation that individuals with MDR tuberculosis may
have a different type of immune response (eg, the Th1 response might be absent), compared with individuals with drug-susceptible tuberculosis, and would therefore require a different therapeutic approach [9, 69]. Similarly, if high number of Tregs is present in patients with tuberculosis [70, 71], removal of Tregs could be helpful if a stronger immune response is required; trials concerning Treg removal are currently underway for other clinical indications [72].

The role of immunotherapy may be 2-fold in the context of immune exhaustion: immunotherapeutic strategies may help revert an unsuccessful immune response back to a productive response and/or may induce long-lasting immune memory capable of providing continuous protection. *M. tuberculosis* antigen processing and presentation is key in this matter (as Brighenti and Andersson assert in this supplement of the *The Journal of Infectious Diseases*) because *M. tuberculosis* antigens can be delivered via *M. tuberculosis*-infected macrophages [73] or other antigen-presenting cells. *M. tuberculosis* antigens can formally be presented after cross-presentation of killed *M. tuberculosis*-infected antigen-presenting cells, which are then engulfed and subsequently presented [74]; by generation of exosomes [75]; or via autophagy [76]. While autophagy is believed to be deleterious in cancer [77], it may help induce stronger cellular immune responses in *M. tuberculosis* infection [78]. More-careful studies examining drugs affecting autophagy are warranted. For example, rapamycin [79] and antidepressants [80] induce autophagy, while other drugs, including hydroxychloroquine [81], block autophagy.

In mycobacterial disease, the T-cell receptor zeta is downregulated in immune cells at the site of infection, resulting in T-cell anergy [82, 83]. The T-cell receptor zeta chain is essential for signaling in T cells, as well in some natural killer cell subsets. The absence of the CD3 zeta chain “blinds” immune effector cells, leading to bona fide nonresponsiveness of the T-cell receptor: the signal, elicited on engagement of the MHC-presented peptide with the T-cell receptor, is not transduced into the cytoplasm. This phenomenon, also seen in different tumors, can be reverted with exogenous IL-2 [84]. In tuberculosis, other cytokines, such as IL-7, might prove to be more attractive since IL-2 also risks inducing additional Tregs [20, 21].

**FUTURE POSSIBLE IMMUNOTHERAPIES: LESSONS FROM MEN AND MICE**

Even though the continuous struggle against tuberculosis has achieved numerous new insights in bacteriology, immunology, and immunopathology, a high number of potential therapy options remain to be tested. In humans, IL-7 has been used successfully to improve treatment of human immunodeficiency virus and hepatitis C virus infections [85, 86] and has helped to speed up immune reconstitution in patients after hematopoietic stem cell transplantation [87]. IL-7 is known to restore immune responsiveness [88], mediate protection from apoptosis in immune cells [89], and contribute to dendritic cell activation [90]. IL-7 could therefore represent a reasonable candidate for testing in a phase 1 clinical trial for treatment of tuberculosis. Several studies in mice showed a survival advantage in *M. tuberculosis* infection if IL-7 or interleukin 15 is coadministered with BCG vaccine [91, 92]. This effect can most likely be attributed to the positive effects of these cytokines on T-cell memory responses [91]. In a nonhuman primate model, upregulation of IL-7 production in situ (ie. in lung tissue) has been demonstrated in animals who showed increased survival after challenge with virulent *M. tuberculosis*. This would be in line with a beneficial role of IL-7 in human tuberculosis [57]. Other advantageous effects with IL-7 coadministration are its positive effect on IL-17 production at the site of infection mediated via γδ T cells [93]. Dermal γδ T cells are dependent on IL-7 for their survival. During mycobacterial infection, dermal γδ T cells are a considerable source of IL-17 [94]. Absence of dermal γδ T cells is associated with decreased expansion of *M. tuberculosis*-specific CD4+ T cells in skin-draining lymph nodes [93]. Thus, IL-7-driven activation of γδ T cells may help increase IL-17 levels and support CD4+ adaptive T-cell responses. This example also shows that cytokine-based therapies are complex and require a detailed analysis of the immunopathology in order to be useful for adjunct therapy in tuberculosis.

More recently, interleukin 24 (IL-24) has been shown to be downregulated in sera of patients with tuberculosis [95]. IL-24 is a tumor suppressor and member of the IL-10 family of cytokines. The cytokine activates CD8+ T cells in mice, resulting in increased IFN-γ production. In addition, IL-24 has been shown to activate neutrophils and subsequently increase their IL-12 production, a profile which could help promote better anti-*M. tuberculosis* immune responses. In a mouse model, exogenous IL-24 had a protective effect against tuberculosis infection [95]. Thus, IL-24 may be one of several interesting candidates for testing in ex vivo human material, followed by testing in nonhuman primate models of tuberculosis.

**CONCLUSIONS**

Adjunct immunotherapies will need to be evaluated in randomized, placebo-controlled trials. Timing of these therapies is critical, and biologically and clinically relevant markers need to be developed to select patients who will benefit from custom-tailored therapy. Immunotherapeutic interventions may at first be expensive, and their clinical benefits would need to be examined in detail. However, although current treatment modalities for MDR tuberculosis and XDR tuberculosis are very costly in terms of price, MDR tuberculosis and XDR tuberculosis are costly in terms of lives lost. If biological therapy works, then appropriate means for translational use would need to be.
strategically developed. Biological therapies have been implemented in the fields of cancer and autoimmune diseases, and the lessons learned in these settings will, in the near future, benefit the quest for novel treatment modalities for tuberculosis.

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Notes

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