

## Immunotherapy for Tuberculosis: what's the better choice?

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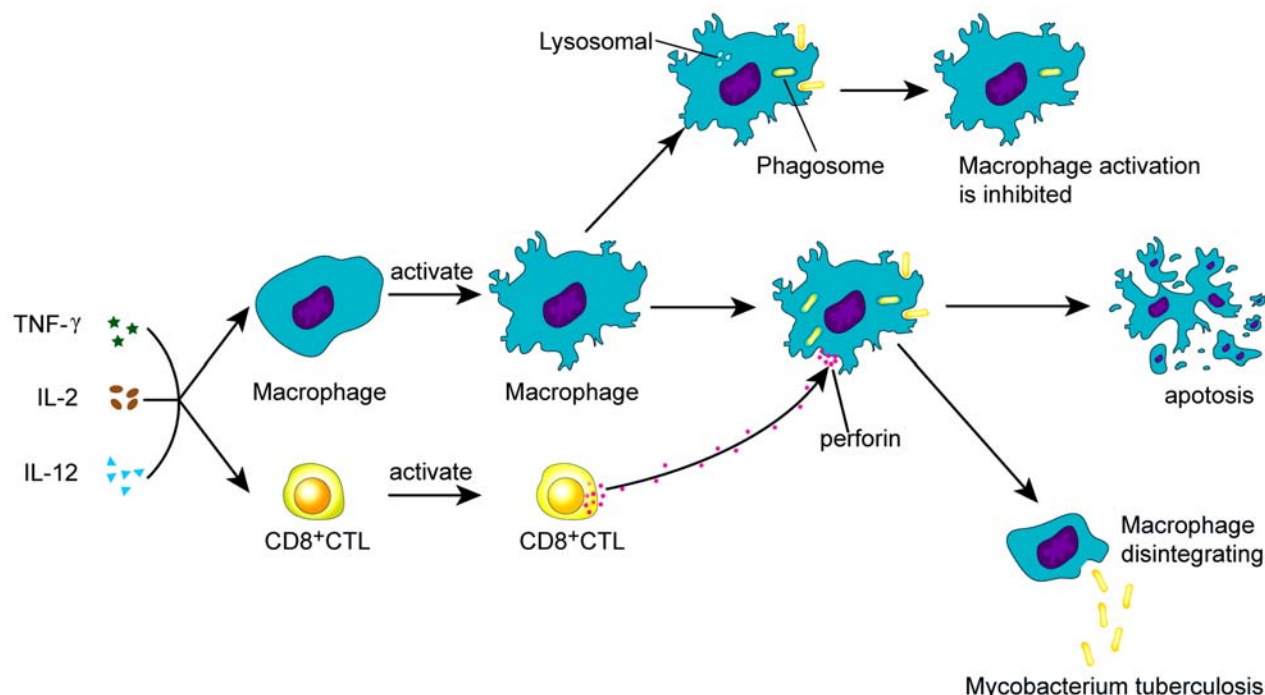
## 1. ABSTRACT

A Th1/Th2 imbalance in tuberculosis (TB) patients caused by a decreased Th1 response and an increased Th2 response is a significant factor in the pathogenesis and development of TB. Protective immune responses to TB include bacteriostatic and bactericidal responses. Unfortunately, however, immunoprotection and immune pathology co-exist in TB patients. Immunotherapy for TB principally aims to restore the Th1/Th2 balance by enhancing the Th1 response and suppressing the excessive Th2 response. Immunotherapy for TB can be classified into three categories: immune-enhancing therapy using cytokines, immunosuppressive therapy, and immunomodulatory therapy. Immunomodulatory therapy targets the Th1/Th2 imbalance and includes cytokine regulation therapy, antibody regulation therapy, a multi-dose heat-inactivated *Mycobacterium vaccae* vaccine, thymosin hormones and a DNA vaccine. A new approach in supplementary TB immunotherapy is to simultaneously up-regulate the Th1 response and down-regulate the Th2 response. While immunotherapy can contribute to TB treatment, it may also cause immunopathological injury. Therefore, immunotherapy needs to be improved and further studied to maximize its potential.

## 2. ADVANCES IN IMMUNOTHERAPY FOR TUBERCULOSIS TREATMENT

### 2.1. Imbalance of the Th1/Th2 response

T lymphocytes mediate the protective immune response to *Mycobacterium tuberculosis* (*M. tuberculosis*) infection in humans. This immune response is dependent on T helper (Th) 1 cytokines, such as IFN- $\gamma$ , IL-2, IL-12, and this is the basis for the activation of macrophages and CD8<sup>+</sup> cytotoxic T lymphocytes (CTL). Activation of macrophages is important for the control of *M. tuberculosis* infections since this remarkably enhances the ability of macrophages to engulf and kill *M. tuberculosis*. *M. tuberculosis* can counter the classic Th1-mediated macrophage activation by inhibiting phagosome maturation, lysosome fusion, and MHC antigen presentation, resulting in its survival. When this occurs, activated CD8<sup>+</sup> CTL are the key factor in the compensatory bactericidal mechanism. Activated CD8<sup>+</sup> CTL can directly engulf and kill *M. tuberculosis*-infected macrophages. Alternatively, activated CD8<sup>+</sup> CTL can destroy these macrophages by releasing granzyme, resulting in the release and killing of intracellular *M. tuberculosis* (Figure 1). Thus, this combined immune response driven by Th1 cytokines involving both



**Figure 1.** The Th1 immune response to *Mycobacterium tuberculosis* in humans. Th1 cytokines, such as IFN- $\gamma$ , IL-2, IL-12, activate macrophages and CD8<sup>+</sup> cytotoxic T lymphocytes (CTL). The ability of activated macrophages to engulf and kill *M. tuberculosis* is remarkably enhanced. *Mycobacterium* can counter the classic Th1-mediated macrophage activation through inhibiting phagosome maturation or lysosome fusion, resulting in survival of the bacteria. In this case, activated CD8<sup>+</sup> CTL can directly engulf or kill mycobacterium-infected macrophages, or destroy these macrophages using granzyme, resulting in intracellular mycobacterium release and death.

macrophages and CD8<sup>+</sup> CTL is ideal for killing *M. tuberculosis*. This dual mechanism is also the immunological basis of TB treatment by Th1 cytokines (1). In addition, Th2 cytokines, including IL-4, IL-5, IL-10 and TGF- $\beta$ , can inhibit the protective response of Th1. Thus, Th1 and Th2 interact and inhibit each other to maintain the normal immune response, thereby killing *M. tuberculosis* without pathological damage caused by an excessive immune response (2). Interestingly, a Th1/Th2 imbalance was found in TB patients with decreased Th1 cytokines and increased Th2 cytokines in the circulation, while Th1 cytokines in infected tissues were over-expressed (3). Therefore, a Th1/Th2 imbalance is significant in the pathogenesis and development of TB.

A recent study found that infection with a single high dose of *M. tuberculosis*, as well as infection with worms or non-tuberculous mycobacteria, can induce a Th2 response characterized by elevated levels of IL-4, while a low dose *M. tuberculosis* infection can stimulate a Th1 response (4).

## 2.2. Co-existence of bacteriostatic and bactericidal responses

The protective immune response includes bacteriostatic and bactericidal responses. *M. tuberculosis* invading alveoli are predominantly killed by macrophages of the innate immune system. Some bacteria can escape to become Okazaki's primary tuberculosis, which is characterized by polymorphonuclear neutrophil infiltration and

is followed by rising monocyte and lymphocyte counts and the formation of a granuloma. The granuloma, as a physical barrier, can prevent the spread of infection. Despite this protective effect, the bacteriostatic response can antagonize chemotherapy because *M. tuberculosis* hidden in granulomas goes into hibernation in this unfavorable environment, resulting in the evasion of chemotherapeutic killing. This is a source of recurrent infections, and is one reason why TB treatment regimes are long and difficult.

## 2.3. Co-existence of immunoprotection and immune pathology

The pathology of TB is the result of an interaction between *M. tuberculosis* and the immune response of the host, in which cell-mediated immunity is dominated by the anti-*M. tuberculosis* immune response. However, an excessive immune response could cause pathological injury, including tissue damage, chronic lung function deterioration, pulmonary fibrosis, bronchial distortion, expansion and even lung damage; these processes result in disease progression. In this situation, even if the infection has been cleared, the morbidity and mortality of TB will still increase due to these complications (5).

## 4. TB IMMUNOTHERAPY

The purposes of immunotherapy for TB are as follows: to enhance the treatment success of multi-drug

resistant tuberculosis (MDR-TB) and extremely drug-resistant tuberculosis (XDR-TB); to shorten the treatment course for chemo-sensitive TB; to reduce TB recurrence after chemotherapy by improving immunity; and to alleviate immune pathological damage (6). The immunotherapy strategy for TB includes restoring the Th1/Th2 balance by enhancing the Th1 response and suppressing the excessive Th2 response, by altering the dominant bacteriostatic response to a bactericidal response, and by regulating the immune response to inhibit immune pathological damage. Based on this strategy, immunotherapy for TB can be classified into three categories including immune-enhancing therapy with cytokines, immunosuppressive therapy, and immunomodulatory therapy.

### 3.1. Immune-Enhancing Therapy With Cytokines

In immune-enhancing therapy, cytokines such as recombinant human  $\gamma$ -interferon (rh-IFN- $\gamma$ ), recombinant human interleukin-2 (rh-IL-2) and recombinant human interleukin-12 (rh-IL-12) are used.

### 3.2. Recombinant human $\gamma$ -interferon (rh-IFN- $\gamma$ )

IFN- $\gamma$  is an important Th1 cytokine. In a mouse model of TB, IFN- $\gamma$  was found to improve the ability of immune cells to engulf and kill *M. tuberculosis*. Thus, IFN- $\gamma$  is a promising treatment for drug-resistant TB or MDR-TB (7). However, a recent study found that IFN- $\gamma$  was not as efficacious as expected. Condos et al. (8) and Koh et al. (9) found that inhalation of IFN- $\gamma$  during treatment of MDR-TB only reduced the quantity of *M. tuberculosis* in the short-term without completely eradicating the infection, even when the inhaled treatment was continued for 6 months. Two multicenter randomized controlled trials investigating inhaled INF- $\gamma$  as a supplementary therapy for MDR-TB, or subcutaneous injections of INF- $\gamma$  for cavitary pulmonary tuberculosis which is sensitive to chemotherapy, were prematurely terminated due to lack of efficacy (8).

The unsuccessful use of IFN- $\gamma$  as a supplementary therapy for MDR-TB could be attributed to factors including the usage or dosage of IFN- $\gamma$ , the limited number of patients enrolled in the study, and the differences between the human and mouse immune systems. However, we think that the most significant factor is that the subjects were not IFN- $\gamma$ -deficient. This would result in ineffective exogenous IFN- $\gamma$  supplementation because IFN- $\gamma$  adjuvant therapy is applicable to patients with IFN- $\gamma$  deficiency but without IFN- $\gamma$  receptor deficiency (10,11). Researchers have found that the level of inducible NO synthase (iNOS) in macrophages of bronchoalveolar lavage fluid from TB patients who inhaled IFN- $\gamma$  was not increased compared to TB patients treated without inhaled IFN- $\gamma$ . This indicates that exogenous supplementation of IFN- $\gamma$  does not significantly up-regulate the Th1 response in alveolar macrophages whose IFN- $\gamma$ -induced gene has already been up-regulated by *M. tuberculosis* stimulation.

When IFN- $\gamma$  supplementary therapy is used for TB patients with IFN- $\gamma$  deficiency, patients with localized pulmonary TB should inhale rh-IFN- $\gamma$ , while patients with

systemic disseminated TB should have subcutaneous or intramuscular injections of rh-IFN- $\gamma$ .

### 3.3. Recombinant human interleukin-2 (rh-IL-2)

IL-2 is also an important Th1 cytokine, and has been studied in the treatment of *M. tuberculosis* infections. It has been found that IL-2 can reduce the number of acid-fast bacilli (AFB) in the sputum of MDR-TB patients (12). However, another study carried out in Uganda showed that in the treatment of chemotherapy-sensitive TB, patients in the IL-2 intervention group had more AFB in the sputum and a longer period before sputum AFB examination became negative, as well as no obvious improvement in their symptoms (13). Interestingly, this study also found that IL-2 could progressively reduce IFN- $\gamma$  production, as well as reduce the skin test response. On the basis of previous studies, we speculate that the difference between the results of these two studies is due to the role of IL-2 in the immune response to TB. On one hand, IL-2 could promote a bacteriostatic response by promoting granuloma formation. On the other hand, IL-2 could down-regulate the immune response by stimulating the proliferation of T regulatory cells during treatment, resulting in decreased bacterial clearance (14). Therefore, IL-2 may be useful in MDR-TB treatment.

### 3.4. Recombinant human interleukin-12 (rh-IL-12)

In the Th1-mediated immune response to TB, IL-12 and IFN- $\gamma$  constitute an "IFN- $\gamma$ -IL-12 immune axis" in a positive feedback cycle (15). In this positive feedback, only a high dose of IL-12 can cause a significant increase in IFN- $\gamma$  secretion. Due to the side effects of a high-dose IL-12 such as hematopoietic toxicity, liver damage and muscle necrosis, the therapeutic window of IL-12 is narrow and its clinical application is limited (16). A clinical trial of IL-12 supplementary treatment in TB has not yet been reported.

Treatment with IL-12 works by strengthening the Th1 immune response. However, recent studies failed to find the expected therapeutic effect through simply strengthening the Th1 immune response. This may be due to the so-called Ceiling-like effect. In the Ceiling-like effect, since an active Th1 immune response already exists in the local lesion, the additional protective effect induced by exogenous Th1 cytokines is limited. Furthermore, it is likely to increase the Th2 immune response by a feedback mechanism, leading to immunopathological damage. Therefore, we suggest that exogenous Th1 cytokines would be useful for TB patients deficient in Th1 cytokines, rather than those with a receptor deficiency.

### 3.5. Immunosuppressive Therapy

Immunosuppressive therapy is relevant for TB patients with a strong immune response, such as patients with caseous pneumonia, scrofula, tuberculous meningitis, tuberculous pericarditis, tuberculous peritonitis, a strongly positive PPD skin test, or significantly increased tumour necrosis factor alpha (TNF- $\alpha$ ) levels. For these patients, immunosuppressive therapy could suppress the immune response to prevent *M. tuberculosis* from becoming dormant, while reducing immunopathological injury. A

short-term and potent anti-TB treatment should be administered early (17).

Recently, a TNF- $\alpha$  inhibitor has shown promise as an immunosuppressive therapy for TB because TNF- $\alpha$  plays an important role in promoting and maintaining granulomas during the process of anti-TB protective immunity (18). Researchers have found that infliximab, a monoclonal antibody against TNF- $\alpha$ , can control the progression of inflammatory autoimmune diseases such as rheumatoid arthritis and Crohn's disease, but it increased the TB relapse rate of these patients (19). It may be that the inhibition of TNF- $\alpha$  results in failed formation and maintenance of granulomas, and the release and spread of latent *M. tuberculosis* (19). In theory, inhibiting TNF- $\alpha$  can alleviate the clinical symptoms in patients with advanced TB and reduce the formation of granulomas because TNF- $\alpha$  is associated with pathological lung injury and weight loss in these patients. Moreover, since increased levels of TNF- $\alpha$  can promote replication of human immunodeficiency virus (HIV) and apoptosis of T lymphocytes, treatment with a TNF- $\alpha$  inhibitor could be considered for patients co-infected with HIV and TB (19). In the following section, we review the major drugs that inhibit TNF- $\alpha$ .

### 3.6. Thalidomide and its analogues

Thalidomide is a strong TNF- $\alpha$  inhibitor that can improve clinical symptoms in TB patients and increase their weight (20). However, high-dose (24 mg/kg/d, oral route) thalidomide is not recommended for the treatment of children with severe tuberculous meningitis, since these patients did not benefit from this medicine after one month of treatment but suffered serious side effects, including death. As thalidomide can cause teratogenicity and peripheral neuropathy, thalidomide analogues with greater efficacy and fewer side effects, such as the SelCiDs, have been synthesized. However, their efficacy in clinical applications is still unclear (20).

### 3.7. Etanercept

Etanercept is a soluble TNF- $\alpha$  receptor, and it can prevent the formation of granulomas. In a study on patients with TB and HIV infections receiving standard anti-TB treatment, patients who additionally received etanercept treatment on the fourth day had a negative *M. tuberculosis* sputum test earlier than the control group, their pulmonary infiltration foci were absorbed better, and their rate of cavity closure was higher. This study also showed that etanercept was well tolerated with no serious side effects. Though the number of subjects in this study was limited, these results are encouraging and further study is warranted (17).

### 3.8. High-dose prednisolone

Prednisolone can decrease the TNF- $\alpha$  levels. However, no beneficial effects in the long-term were shown after conventional-dose prednisolone treatment in lung and pleural TB (21). Since a previous study demonstrated that a high dose of prednisolone is required to reduce the level of TNF- $\alpha$  by over 50%, treatment of patients with TB and HIV-1 infection ( $CD4^+$  T lymphocyte  $>200$  /mm<sup>3</sup>) with a high dose of prednisolone (2.75

mg/kg/d) was studied (22). In this study, the average cumulative dose for each patient was over 6500 mg. The results showed that patients in the high-dose prednisolone treatment group had a quicker clearance of *M. tuberculosis* in sputum compared with controls in the first month, but no significant difference of *M. tuberculosis* clearance in sputum or survival rates between the two groups in the second month. In addition, high-dose prednisolone can cause serious side effects such as severe hypertension, hyperglycemia, and electrolyte imbalance. Thus, high-dose prednisolone is not clinically recommended for TB therapy at present (22). Even so, we suggest further study of the use of inhaled glucocorticoids for pulmonary TB, since these have less severe side-effects than systemic corticosteroids.

In summary, even though immunosuppressive therapy has shown potential for quickly clearing *M. tuberculosis*, reducing immunopathological injury and improving clinical symptoms, there has not been a practical, effective and safe agent or treatment plan. Moreover, the effect and safety of SelCiDs, etanercept and inhaled corticosteroids needs to be studied further.

### 3.9. Immunomodulatory Therapy

Immunomodulatory therapy can rebalance Th1/Th2 levels. Since the desired therapeutic effect was not achieved by simply strengthening the Th1 response, new treatment strategies could be investigated that down-regulate the Th2 response, possibly with the simultaneous up-regulation of the Th1 response. It seems that simultaneously up-regulating the Th1 response and down-regulating the Th2 response is promising.

### 3.10. Cytokine regulation therapy

Cytokine regulation therapy involves down-regulating the immunosuppressive effects of Th2 cytokines including IL-4, TGF- $\beta$  and IL-10. To down-regulate IL-4, anti-IL-4 antibodies can be used to significantly reduce the *M. tuberculosis* load in TB patients. When combined with IFN- $\gamma$  and IgA, it can prevent recurrence of TB in mice after chemotherapy (23). Anti-IL-4 treatment is applicable to TB patients with high IL-4 levels, such as patients who have a lung cavity, high exposure to environmental *M. tuberculosis*, a worm infection, or who are at high risk of exposure to high-dose and highly virulent *M. tuberculosis* (such as those with close contact with TB patients with positive *M. tuberculosis* sputum tests) (23). However, clinical studies into the use of anti-IL-4 antibodies or soluble IL-4 receptor in TB therapy have not yet been reported.

In addition to IL-4 antibodies, soluble TGF- $\beta$  receptors can play a role in TB therapy. However, they caused aggravation of lung inflammation and immunopathological injury in these patients. Thus, directly inhibition of TGF- $\beta$  is not appropriate (24).

The regulation of IL-10 has been also studied in TB therapy. A study showed that blocking the IL-10 receptor can enhance the effect of chemotherapy, while only blocking the IL-10 receptor was not effective (25).

However, no clinical trial investigating IL-10 antibodies for supplementary TB therapy has been reported.

### 3.11. Antibody regulation therapy

Antibody regulation therapy has focused on novel ways of using antibodies, and using new kinds of antibody, to regulate the anti-TB immune response of the host. Researchers found that high-dose intravenous immunoglobulin (IVIg) could stimulate a significant anti-TB effect through regulating CD8<sup>+</sup> T cells and down-regulating the pathological immune response (this therapeutic effect of high-dose IVIg was not observed in nude mice with a T lymphocyte deficiency) (26). Though high-dose IVIg can stimulate an anti-TB response, it may also aggravate TB. IgG in high-dose IVIg contains completely sialylated Fc oligosaccharides, which differs from the unsialylated Fc oligosaccharides found in TB patients. The IgG in high-dose IVIg has a proinflammatory effect and can induce immunopathological injury, resulting in exacerbation of TB (26). Unfortunately, clinical trials of high-dose IVIg in the treatment of TB have not yet been reported.

In a study based on an animal model, a monoclonal antibody (MoAb) SMITB14 of the IgG1 subclass was protective against *M. tuberculosis* infection in terms of increased long-term survival, reduction in bacterial load, acceleration of antigen removal, and prevention of infection and spread (27). However, the activity of this MoAb has not yet been demonstrated in humans.

In summary, since antibodies are expensive and are typically only effective in treating extracellular bacteria, their use should only be considered in the following special cases. First, antibodies can be used prophylactically for infants exposed to TB by their mothers, or children exposed to patients with active TB. These children, especially infants exposed to MDR-TB and XDR-TB patients, should be given antibody prophylactically in addition to isoniazid before their BCG vaccination becomes effective. Second, antibodies can be used as supplementary treatment for patients with immune deficiency, such as patients with acquired immune deficiency syndrome. Third, antibodies can be used as supplementary medicines for XDR-TB infections.

### 3.12. Multi-dose heat-inactivated *Mycobacterium vaccae* vaccine (*M. vaccae*)

Multi-dose heat-inactivated *Mycobacterium vaccae* (*M. vaccae*) has a bidirectional immunomodulatory effect (29). A meta-analysis combining the results of 11 Chinese studies showed that multiple doses of *M. vaccae* (once a week, at least five times) as a supplementary treatment for MDR-TB was effective (29). A recent study carried out in Tanzania showed that *M. vaccae*-treated patients with HIV infection had a significantly lower burden of TB than controls. In contrast, the Cochrane Collaboration showed that there was no significant benefit from *M. vaccae* to TB patients. In 2010, a meta-analysis they published suggested that early studies supporting the benefits of *M. vaccae* may have suffered from

methodological flaws, and that their results were too optimistic (21).

### 3.13. Thymosin hormones

Thymosin hormones include thymosin, thymopentin and thymosin $\alpha$ 1. The latter two are synthetic compounds and have strong immunological activities. Since only a few studies of thymosin hormones in TB therapy have been reported and they involved only a small number of subjects, their clinical efficacy remains uncertain.

### 3.14. DNA vaccine

A mycobacterial heat shock protein 65 (MHSP65) DNA vaccine has been shown to effectively strengthen the efficacy of CTL and inhibit the IL-4 response (32).

In summary, it may be a better strategy to simultaneously up-regulate the Th1 response and down-regulate the Th2 response than solely strengthening the Th1 response, and this may become a new strategy for TB supplementary immunotherapy in future. However, bidirectional immunoregulating medicines such as the IFN- $\gamma$ /IgA/IL-4 antibody combination therapy, high-dose IVIg and the MHSP65 DNA vaccine have not yet been validated by clinical trials, while the efficacy of multi-dose *M. vaccae* and thymosin hormones remains controversial. Large sample, multi-center, randomized controlled trials are expected.

## 4. OVERALL ASSESSMENT OF SUPPLEMENTARY IMMUNOTHERAPY IN TUBERCULOSIS

Supplementary immunotherapies undoubtedly have a role in TB treatment since previous studies have shown that immunotherapy has the potential to improve the prognosis of TB patients, even patients with MDR-TB or XDR-TB, and may shorten the course of chemotherapy (20). Even so, it seems that supplementary immunotherapy for TB has not achieved the expected results. Furthermore, it is hard to determine whether supplementary immunotherapy for TB is effective because current evidence is insufficient and the number of reported clinical trials is limited. Of those trials, the randomized controlled and large sample studies account for only one-fifth. Therefore, further RCT studies with large sample sizes are required to confirm the effects of immunomodulatory therapies.

Moreover, immunotherapy should be comprehensively considered in the damage-response framework, since as well as contributing to TB therapy it can cause immunopathological injury. Therefore, immunotherapy for TB should be aimed at restoring the immune imbalance. It should be remembered that up-regulating the immune response in patients who are immunocompromised, such as those co-infected with HIV, is likely to cause dissemination of the TB. In contrast, down-regulating the immune response could be considered in TB patients with an excessive immune response, such as TB patients with a strongly positive tuberculin test, caseous pneumonia, or lymph node tuberculosis. For these patients,

adding immunosuppressive agents could be considered in order to reduce the strong antibacterial immune response that antagonizes chemotherapy and to accelerate the killing and removal of bacteria.

In conclusion, immunotherapy has a potential role in TB therapy, and needs to be improved in future.

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**Abbreviations:** TB: tuberculosis; Th: T helper; M. tuberculosis: Mycobacterium tuberculosis; CTL: cytotoxic T lymphocytes; rh-IL-2: interleukin-2; rh-IFN- $\gamma$ : recombinant human  $\gamma$ -interferon; iNOS: inducible NO synthase; rh-IL-2: Recombinant human interleukin-2; AFB: acid-fast bacilli; TNF- $\alpha$ : tumour necrosis factor alpha; HIV: human immunodeficiency virus; IVIg: intravenous immunoglobulin; MoAb: monoclonal antibody; M. vaccae: Mycobacterium vaccae vaccine

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