

Title: Immunotherapy of tuberculosis
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Introduction

The increase in global TB burden during past decade has heightened interest in innovative approaches to shorten treatment and improve outcomes. There are several potential roles for immunotherapy in TB treatment in this context.

- **Improving treatment of MDR and XDR TB.** By containing bacillary replication, immunotherapy could potentially prevent further emergence of resistance, thereby improving treatment outcomes.
- **Ameliorating symptoms.** Tuberculosis results in tissue necrosis and fibrosis that destroys functioning lung tissue. Adjunctive immunotherapy that limited inflammation, necrosis and fibrosis could reduce morbidity and mortality.
- **Preventing deleterious immune activation in TB/HIV co-infection.** In HIV co-infection, an additional role of immunotherapy might be to modulate a host immune response that otherwise promotes T cell activation and HIV expression.
- **Eliminating persisters.** The development of new treatments capable of shortening TB treatment is a major objective of TB drug discovery (1). Immunotherapy that could enhance host responses against slowly replicating persistent tubercle bacilli, a subpopulation not effectively targeted by current therapy, could potentially shorten the required duration of TB treatment and decrease the risk of relapse. Alternatively, if host responses cannot effectively eradicate these persisting bacilli, but instead create the conditions leading to persistence, immunotherapy directed against the granulomatous host response might accelerate the response to treatment by increasing drug bioavailability and enhancing microbial susceptibility.

Cytokine regulation of macrophage activation

Control of *M. tuberculosis* infection occurs at three levels: the isolated macrophage, mixed macrophage and inflammatory cell infiltrates, and mature granulomas (figure 1). As intracellular pathogens, mycobacteria possess the capacity to replicate within the phagocytic cells that comprise the major effector arm of the cellular immune system. Resting human monocytes and macrophages are permissive of intracellular replication of *M. tuberculosis* (2). At this stage, the infection can be affected by factors reflecting innate (natural) immunity. In mice, resistance of resting macrophages to infection with most intracellular pathogens is controlled by the products of a gene on chromosome 1 identified as the *bcg* locus (3). Macrophages of BCG-resistant strains demonstrate increased respiratory burst activity as assessed by peroxide production and enhanced capacity for inhibition of replication of *M. bovis* BCG and *M. intracellulare* (4). The human correlate of this gene may also play a role in determining TB susceptibility (5).

Tumor necrosis factor (TNF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and other macrophage cytokines act in an autocrine fashion to limit intracellular mycobacterial growth (6). These cytokines are produced by macrophages at the site of infection in response to mycobacterial lipoproteins and glycolipids. Other components of the innate immune response, such as natural killer (NK) cells, granulocytes, and antimicrobial peptides may also play a role in mycobacterial resistance (7,8).

Macrophage activation for killing of intracellular *M. tuberculosis* is enhanced by interaction with antigen-specific T cells and local production of interferon (IFN) γ . The recruitment of these cells from the blood and their differentiation and expansion at the site of infection in the lung are critical events in mycobacterial immunity in which TNF, chemokines, IL-12, and IL-2 participate. Mice with targeted disruption of the IFN γ gene or the gene for the IFN γ receptor show increased susceptibility to *M. tuberculosis* and *M. bovis* BCG (9,10). Defects that prevent the clonal expansion and activation of IFN γ -producing T cells, such as deficiencies of IL-12 or IL-18, have similar effects (11,12). Mutations affecting the IFN γ or IL-12 receptors in humans also increase susceptibility to mycobacterial disease (13,14).

Nitric oxide (NO), the production of which is induced by IFN γ , is thought to be the main anti-mycobacterial effector mechanism of activated macrophages (15). Other products of activated macrophages, including superoxide, IL-6 and calcitriol (1,25 dihydroxy vitamin D₃), also restrict intracellular mycobacterial growth (2,16,17). Calcitriol may act in part by simulating production of NO (18).

Cytotoxic T cells contribute toward control of intracellular mycobacterial by a granule-dependent mechanism. Granulysin, a protein found in granules of CTLs, reduce the viability of a broad spectrum of pathogenic bacteria, fungi, and parasites *in vitro*. Granulysin directly kills extracellular mycobacteria, and, in combination with perforin, decreases the viability of intracellular *M. tuberculosis*. (19). However, cytotoxicity directed against host cells per se does not appear to be a major factor in the control of intracellular infection (20).

Granulomas and persistence

In most instances, however, it is believed that the human host response is unable to eradicate infection with *M. tuberculosis*. Granulomas therefore represent a stalemate between host and pathogen – an alternative strategy to physically contain an otherwise virulent pathogen in a microenvironment with reduced oxygen, pH, and micronutrients. In response, mycobacteria undergo profound alterations in metabolism, biosynthesis, and replication, leading to a semi-dormant state. This forms the basis of clinical latency in tuberculosis.

The elucidation of the biology of these sequestered bacilli has become a critical area of research in tuberculosis. Karakousis has examined the biology of dormancy using hollow semipermeable microfibers, which, when implanted subcutaneously in mice, become surrounded by granulomas (21). Mycobacteria contained by these lesions showed stationary CFU counts, decreased metabolic activity, profoundly altered gene expression profiles, and decreased susceptibility to the bactericidal effects of isoniazid.

Granulomas therefore present two contradictory roles in mycobacterial infection: a barrier to dissemination, yet also an impediment to treatment. Thus, alternative strategies for adjuvant immunotherapy might be targeted at the granuloma, maximizing drug penetration and bactericidal effect on persisters (22).

Cytokine dysregulation and TB immunopathogenesis

Specific genetic defects involving the above pathways appear to account for only a small fraction of human tuberculosis cases. Nonetheless, there is substantial evidence

of immune dysregulation in patients with active disease. Up to 25% have a negative tuberculin skin test on initial evaluation (23); this percentage is increased in those with disseminated or miliary disease (24). Up to 60% of patients demonstrate reduced responses to *M. tuberculosis* purified protein derivative (PPD) *in vitro* in terms of T cell blastogenesis, production of IL-2 and IFN γ , and surface expression of interleukin-2 receptors (25,26). This is accompanied by increased production of the regulatory cytokines IL-10, transforming growth factor (TGF) β , and prostaglandin E₂, potentially opening other avenues for therapeutic immune intervention (27,28). Regulatory T cells (Treg), bearing the phenotype CD4⁺ CD25⁺ FoxP3⁺, may be also involved in inhibition of CD4 responses in TB, either through production of IL-10 and TGF β , or through other, undefined mechanisms (29,30).

Immune activation and AIDS/TB co-pathogenesis

Co-infection with HIV is the most potent risk factor for active tuberculosis in a person latently infected with *M. tuberculosis* (31). Tuberculosis is often an early complication of HIV infection, occurring prior to other AIDS-defining illnesses. Prior to the introduction of HIV protease inhibitors, the diagnosis carried an expected mortality of 21% at 9 months, even in those subjects presenting without other AIDS-defining conditions (32). Death was infrequently due to active tuberculosis, however. More often, it resulted from other AIDS-related causes, occurring after the diagnosis of tuberculosis.

Several studies indicate that the adverse interactions of *M. tuberculosis* and HIV are bi-directional, i.e., that tuberculosis affects HIV disease in addition to the better recognized converse interaction. Tuberculosis is characterized by prolonged antigenic stimulation and immune activation, even in HIV-positive subjects (33,34). Antigen induced T cell activation, and expression of proinflammatory cytokines TNF α and other inflammatory cytokines in turn promotes HIV expression by latently infected cells (35,36). *M. tuberculosis* and its proteins and glycolipids directly stimulate HIV replication by mechanisms involving monocyte production of TNF α (37). In the lung, TNF α and HIV-1 RNA are both increased in bronchoalveolar lavage fluid of involved segments of lungs of patients with pulmonary tuberculosis and HIV-1 infection (38). Phylogenetic analysis of V3 sequences demonstrated that HIV-1 RNA present in bronchoalveolar fluid had diverged from plasma, indicating that pulmonary tuberculosis enhances local HIV-1 replication *in vivo*.

These interactions appear to have significant clinical consequences. Plasma HIV viral load increases 5 to 160-fold in HIV-infected persons during the acute phase of tuberculosis (39). New AIDS-defining opportunistic infections occur at a rate 1.4 times that of CD4-matched HIV-infected control subjects without a history of tuberculosis (95% confidence interval: 0.94-2.11) (40). AIDS/TB cases also have a shorter overall survival than control AIDS patients without TB ($p = 0.001$), as well as an increased risk for death (odds ratio = 2.17). Thus, although active tuberculosis may be an independent marker of advanced immunosuppression in HIV-infected patients, it may also act as a cofactor to accelerate the clinical course of HIV infection, potentially offering opportunities for immune-based interventions.

Treatment and testing strategies

To summarize, protective host responses against *M. tuberculosis* are dependent on Th-1 responses mediated primarily by interactions of CD4 T lymphocytes and macrophages. IL-2 and IFN γ are crucial cytokines produced by antigen-responsive T cell that activate macrophages to inhibit intracellular mycobacterial growth and may also act indirectly to enhance specific cytotoxic T cell and NK cell responses. Other cytokines such as TGF β enhance fibrosis and scarring near tuberculous lesions and result in loss of functional pulmonary parenchyma.

These observations have led to the hypothesis that administration of endogenous IFN γ or IL-2 and other agents might augment immune responses in active TB, improve or accelerate clearance of tubercle bacilli, and improve clinical outcomes. The availability of highly purified recombinant cytokines, increasing rates of multidrug resistant tuberculosis, and successful experience with adjunctive therapy with human cytokines in cancer therapy and the treatment of other infectious diseases has led to strong interest in their possible role in the therapy of human mycobacterial diseases. Current approaches to the immunotherapy of tuberculosis center on promoting Th-1 responses by administration of Th-1 cytokines or immunomodulators, inhibition of macrophage-deactivating cytokines such as TGF β , and inhibition of pro-inflammatory cytokines by specific or general cytokine inhibitors such as corticosteroids, thalidomide or pentoxifylline.

The design of clinical trials to test these new treatments (both chemotherapy and immunotherapy) poses several unique challenges. Studies of adjuvant TB immunotherapy have, for the most part, been conducted using surrogate markers indicating relapse risk, and in patients with MDR-TB (in whom the outcome of standard treatment is poor). Delayed sputum culture conversion and reduced rate of decline in log sputum CFU counts during the first month of treatment are recognized indicators of increased relapse risk in tuberculosis (41,42).

IFN

IFN γ was first studied as adjunctive treatment in patients with non-tuberculous mycobacterial infections. In lepromatous leprosy, intradermal therapy with low dose IFN γ resulted in increased local T-cell and monocyte infiltration, HLA-DR (Ia) antigen expression and decreased bacillary load (43). In another study, twice or thrice weekly therapy with 25 to 50 $\mu\text{g}/\text{m}^2$ of subcutaneous IFN γ was administered to 7 HIV-non-infected patients with disseminated *M. avium* complex infection who had failed to respond to antibiotic therapy (44). Within 8 weeks of beginning IFN treatment, all 7 patients had significant and sustained clinical improvement. However, a similar study in patients with advanced AIDS revealed no benefit (45).

High-dose systemic therapy with IFN γ is associated with frequent side effects including fatigue, myalgias, and malaise. Treatment with aerosolized IFN γ has been studied in an attempt to decrease these systemic side effects and deliver therapy directly to the site of disease in the lung. An uncontrolled trial of therapeutic IFN γ in patients with MDR-TB tuberculosis without overt disorders of IFN γ production or responsiveness was reported by Condos in 1997 (46). In this study, 5 patients with MDR TB were administered 500 μg IFN γ 3 times per week by aerosol for 1 month in addition

to their previous chemotherapy. Sputum smears became negative in 4 of 5 patients after 1 month of IFN γ treatment and the time until positive culture in automated detection systems (MGIT) increased. Smears reverted to positive within one month after treatment was stopped, however.

Interferon- α is an immunomodulatory cytokine produced by mononuclear phagocytes stimulated by bacteria and viruses. IFN α modulates differentiation of T cells towards the Th-1 phenotype, induces production of IFN γ and IL-2 and inhibits proliferation of Th-2 cells. Two small studies have examined a possible role for IFN α in TB treatment. A randomized open-label trial in 20 HIV-seronegative TB patients in Italy studied the effects of aerosolized IFN α 3 million unit thrice weekly during the first 2 months of TB treatment (47). Patients treated with IFN α had earlier improvement in fever, sputum bacillary burden by quantitative microscopy after one week of treatment and pulmonary consolidation after 2 months than patients receiving placebo. In another pilot study, IFN α 2b (3 million units weekly) was administered subcutaneously for 3 months as an adjunct to chemotherapy to 5 patients with chronic MDR TB (48). Two of the 5 patients became consistently sputum culture negative over a 30 month follow-up period. However, other studies have not confirmed this modest measure of success (Table 1) (49-51).

The largest, most rigorous trial of IFN in MDR TB to date was initiated by Intermune in 2000 (52). It was designed as a randomized, placebo controlled, multicenter trial of inhaled adjunctive IFN γ for patients with chronic MDR TB. The trial was halted prematurely due to lack of efficacy, after review by an independent safety monitoring board. Unfortunately, its findings have never been published.

A study of adjunctive aerolized or subcutaneous (SC) IFN γ (200 μ g aerosol or SC 3x/wk for 4 months vs. standard short course chemotherapy) in patients with drug susceptible, cavitary pulmonary TB was recently initiated in South Africa. Preliminary data reported from this ongoing study showed that sputum AFB smears became negative in all treatment groups by 12 weeks and that patients treated with IFN γ converted their sputum earlier (53).

Recent basic research on the potential therapeutic role IFN in TB has indicated that IFN γ -induced genes such as IP-10 and iNOS are already upregulated in the lung in patients with tuberculosis, and that therapeutic aerosol IFN γ has relatively little additional effect (54). These findings would appear to indicate that the modest mycobactericidal capacity of lung macrophages cannot be effectively augmented by therapeutic IFN.

Interleukin-2

Early clinical trials with IL-2 in patients with leprosy and leishmaniasis, and other serious infections due to intracellular pathogens, demonstrated that IL-2 immunotherapy may be useful in controlling these infections (55). In leprosy patients, IL-2 administration led to enhanced local cell mediated immune responses and resulted in more rapid and extensive reduction in *M. leprae* bacilli compared to multidrug chemotherapy alone (56). IL-2 at low doses of 10 μ g (180,000 IU) twice a day for 8 days led to body-wide infiltration of CD4 + T cells, monocytes, and Langerhans' cells in the skin and a decline in the total body burden of *M. leprae* (57). The presumed mechanism of this anti-bacterial effect is via the destruction of oxidatively incompetent dermal macrophages

and the extracellular liberation of bacilli and their subsequent uptake and destruction by newly emigrated and oxidatively competent monocytes from the circulation.

Several clinical trials have examined IL-2 as an adjunct to TB treatment. A pilot study of IL-2 was performed in 20 TB patients in Bangladesh and South Africa to evaluate its safety, and microbiologic and immunologic activities (58). The patient population was diverse, and included new, partially treated, and chronic MDR cases. Patients received 30 days of twice daily intradermal injections of 12.5 µg (225,000 IU) of IL-2 in addition to combination chemotherapy. Patients in all 3 groups showed improvement of clinical symptoms during the 30 day treatment period. Results of direct sputum smears for acid fast bacilli (AFB) demonstrated conversion to negative following IL-2 and chemotherapy in all of the newly diagnosed patients and in 5 of 7 patients with MDR TB. Patients receiving IL-2 did not experience clinical deterioration or any significant side effects.

A randomized clinical trial of 35 patients with MDR TB in South Africa compared daily or pulse IL-2 therapy with placebo (59). Patients received the best available combination chemotherapy based on individual drug susceptibility testing results. Twelve patients received 12.5 µg (225,000 IU) IL-2 intradermally twice daily. Nine patients received pulse IL-2 therapy [twice daily intradermal injection of 25 µg (450,000 IU) IL-2 daily for 5 days, followed by nine days off IL-2 treatment, for 3 cycles] and 14 subjects received placebo. Immunotherapy or placebo was given in conjunction with combination chemotherapy during the first 30 days of the study. The total dose of IL-2 in both active treatment groups was identical. Pulse IL-2 therapy did not appear to have any microbiologic effect. However, 5 of 8 patients receiving daily IL-2 treatment who were smear positive on entry had reduced or cleared sputum mycobacterial load compared to 2 of 7 subjects receiving pulse IL-2 and 3 of 9 subjects in the placebo group. Chest X-ray improvement after 6 weeks of anti-TB treatment was present in 7 of 12 patients receiving daily IL-2 compared to 2 of 9 patients on pulse IL-2 treatment and 5 of 12 patients receiving placebo. The number of circulating CD25+ (low affinity IL-2 receptor bearing T cells) and CD56+ (NK) cells was significantly increased in patients receiving daily IL-2 but not in the pulse IL-2 or placebo arms. No significant side effects related to IL-2 treatment were observed. One patient developed mild flu-like symptoms during 2 cycles of pulse IL-2 treatment. Patients receiving IL-2 developed mild self-limited local induration and pruritus at injection sites. All patients receiving IL-2 treatment completed the study. The results of these studies suggest that IL-2 administration in combination with conventional combination chemotherapy is safe in patients with tuberculosis and may potentiate the antimicrobial cellular immune response to TB. Results from another trial of adjunctive IL-2 treatment in 203 previously treated patients from China showed improved significantly improved sputum culture conversion after one and two months of treatment and improved radiographic resolution at the end of TB treatment (60).

One study of IL-2 has been conducted in newly diagnosed, non-MDR TB cases. This randomized, double blind, placebo-controlled trial of the effect of IL-2 on sputum culture conversion was conducted by the CWRU TB Research Unit in 110 HIV-uninfected Ugandan adults with fully drug-susceptible, newly diagnosed smear positive pulmonary TB (61). IL-2 or placebo were administered at the same dose and schedule as the daily treatment group in the South Africa trial. Although IL-2 was well tolerated, it

did not increase the rate of sputum culture conversion after one and two months of treatment, the primary study endpoints. Instead, time to culture conversion to negative was prolonged and quantitative sputum colony forming units during the first month of treatment were greater in patients receiving IL-2 (figure 2). This was not due to lack of biologic activity of IL-2, as treated subjects had a greater proportion of CD4 cells expressing the IL-2 receptor CD25 as had occurred in previous trials.

Together, these studies suggest that adjunctive IL-2 is safe in patients with pulmonary tuberculosis, but appears to accelerate the microbiologic response to chemotherapy only in patients with MDR disease, in whom chemotherapy is otherwise sub-optimal. In patients with drug sensitive disease, the observed antagonism with strongly bactericidal therapy is consistent with IL-2 promoting bacterial sequestration and dormancy by granulomas. The potential role of adjunctive anti-granuloma therapy in tuberculosis patients with drug-sensitive disease is further discussed below.

GM-CSF

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a growth factor that increases the number of circulating white blood cells and enhances neutrophil and monocyte function. It is widely used in oncology in the management of patients with leukopenia and bone marrow failure. Early studies demonstrated that GM-CSF stimulated the killing of several intracellular pathogens including *Leishmania* species, *T. cruzi*, *C. albicans* and *M. avium* complex (6,62-64). Recombinant human GM-CSF also was shown to decrease the in vitro replication of *M. tuberculosis* in human monocyte macrophages (65). These observations led to interest in its potential use in patients with tuberculosis. In a randomized, placebo-controlled phase 2 trial assessing the safety and activity of one month of twice weekly subcutaneous GM-CSF 125 µg/m² in 31 patients with newly diagnosed pulmonary TB conducted in Brazil, a trend towards faster bacillary clearance in the sputum was observed during the first 8 weeks of treatment in patients receiving GM-CSF in addition to standard chemotherapy (66). No patients had to discontinue GM-CSF treatment. Mild local skin reactions and leukocytosis, which resolved within 3 days, were the most frequent side effects in patients receiving GM-CSF. Fever and increased pulmonary necrosis on chest X-ray were not observed. The results of this small phase 2 study suggest that adjunctive GM-CSF is reasonably well-tolerated by patients with TB and warrants further study, possibly in patients with drug resistant or MDR-TB.

Interleukin 12

Interleukin 12 is a pivotal cytokine that enhances host responses to intracellular pathogens by inducing IFN γ production and Th1 responses. Patients with congenital abnormalities of IL-12 receptors are highly susceptible to serious mycobacterial and salmonella infections (67,68). Administration of IL-12 to SCID or CD4+ T cell-depleted mice infected with *M. avium* enhances IFN γ production and had modest activity against *M. avium* (69). Recombinant IL-12 also has been shown to upregulate *M. tuberculosis*-induced IFN γ responses in human peripheral blood mononuclear cells and alveolar macrophages (70,71). Due to these properties, interest in a possible role for IL-12 immunotherapy in tuberculosis has been balanced by concerns about its non-specific

mechanism of action and potential toxicity. A trial of IL-12 immunotherapy in tuberculosis in the Gambia has been completed, but its findings have not yet been reported.

Thalidomide

Thalidomide (α -N-phthalimidoglutarimide) is a synthetic derivative of glutamic acid which was initially released as a sedative in Europe in 1957 but withdrawn from most countries 4 years later after recognition of its serious teratogenic effects. In 1965 an Israeli dermatologist prescribed thalidomide as a sedative for 6 patients with lepromatous leprosy and erythema nodosum leprosum (ENL) (72). ENL is a serious reaction characterized by painful nodules, fever, malaise, wasting, vasculitis, and peripheral neuritis. All 6 patients improved within hours. This observation spurred a series of studies by other researchers to investigate its underlying mechanisms.

It is now recognized that thalidomide has complex anti-inflammatory, immunologic and metabolic effects. Its activity has been attributed, at least in part, to its ability to inhibit TNF α synthesis in vitro and in vivo (73,74). Thalidomide also inhibits neutrophil phagocytosis, monocyte chemotaxis and angiogenesis, and, to a lesser degree, inhibits lymphocyte proliferation to antigenic and mitogenic stimuli (75-77). Thalidomide inhibits HIV-1 replication in the U-1 monocytoid cells and PBMC from patients with advanced AIDS (78,79). These studies indicate potential clinical roles of thalidomide to limit TNF-related clinical toxicities and to reduce cytokine-related HIV expression.

The side effect profile of thalidomide varies considerably among different patient groups. Aside from its teratogenic effects, the major toxicity of thalidomide is a peripheral polyneuropathy that occurs in 20 to 50% of patients. It is predominantly sensory, and can be irreversible. Other side effects include sedation, orthostatic hypotension, xerostomia, and rash. Thalidomide was approved in 1998 for use in the U.S. for the treatment of severe erythema nodosum leprosum and more recently for use in combination with dexamethasone for the treatment of newly diagnosed multiple myeloma. Due to its teratogenicity and neurologic toxicity, its use has been reserved for conditions refractory to other medical therapy and is strictly regulated in women of child-bearing age. Patients on chronic therapy must be followed closely for neurologic toxicity.

Adjunctive immunotherapy with thalidomide was studied in a double-blind placebo-controlled trial of 39 HIV-infected adults with and without active tuberculosis (80). Patients with active TB treated with thalidomide had decreased plasma TNF α and HIV-1 viral levels and greater weight gain than patients in the placebo group.

Thalidomide also has been evaluated as adjunctive therapy for TB meningitis, a severe form of tuberculosis that often has serious sequelae. The inflammation in the subarachnoid space is believed to play a central pathophysiologic role in the cerebral edema, vasculitis, and infarction typically seen in this form of tuberculosis. Levels of TNF α and other inflammatory cytokines are increased in the cerebrospinal fluid in patients with tuberculous meningitis and correlated with disease progression and brain injury in an animal model of tuberculous meningitis (81). Rabbits treated with the combination of thalidomide and anti-TB drugs are protected from death compared to animals treated only with anti-TB drugs (82).

Based on these promising pre-clinical data, a randomized, placebo-controlled trial of adjunctive thalidomide in HIV-non-infected children with tuberculous meningitis was initiated in children with severe TB meningitis. Following promising results in a pilot study in children with TB meningitis (83), a double-blind, placebo-controlled randomized clinical trial of high dose thalidomide (24 mg/kg/day orally for one month) was initiated in South Africa in children with severe (stage 2 and 3) tuberculous meningitis receiving standard chemotherapy plus corticosteroids (84). Enrollment in this trial was stopped after 47 patients were enrolled after all adverse events and all 4 deaths occurred in patients in the thalidomide group. Frequent side effects included rash, hepatitis and thrombocytopenia; two patients had severe neurologic deterioration. Motor function and mean IQ 6 months after treatment did not differ between patients receiving adjunctive thalidomide or placebo. TNF levels in CSF and blood were not affected by thalidomide treatment. Based on these results the investigators recommended that adjunctive high dose thalidomide not be used in tuberculous meningitis.

Anti-granuloma strategies

TNF is essential for the formation and maintenance of granulomas. Neutralization of TNF in experimental animals interferes with the early recruitment of inflammatory cells to the site of *M. tuberculosis* infection and inhibits the orderly formation of granulomas (85). In addition, TNF blockade also reduces the microbicidal activity of macrophages and NK cells. As a result, animals deficient in TNF are highly susceptible to granulomatous infections (86). Recent studies also indicate that the risk of tuberculosis is increased several fold in individuals with polymorphisms in TNF promoter regions (87), and is substantially increased in patients treated with TNF antagonists for chronic inflammatory conditions such as rheumatoid arthritis (88).

These observations indicate that adjunctive anti-TNF therapy in tuberculosis may have several beneficial effects. Blockade of the pro-inflammatory effects of TNF may reduce inflammation at the site of infection and promote resolution of symptoms. Disruption of granulomas may facilitate tissue sterilization, by eliminating dormancy and promoting drug penetration. Lastly, in HIV/TB co-infection, TNF blockade may prevent cytokine-driven HIV expression and T cell apoptosis and sequestration.

Two controlled clinical trials have examined the effects of potent anti-TNF therapies on microbiologic outcomes in TB. Both were conducted in HIV-1-infected cases with relatively preserved TB immune responses (based on the presence of high CD4 counts and cavitary lung disease). Their main objective was to examine the role of TNF in the acceleration of HIV disease progression due to tuberculosis; as such, their main endpoints were CD4 cell count and plasma HIV RNA load. However, all three studies prospectively collected data on microbiologic and clinical endpoints reached during TB treatment as an indicator of safety.

Etanercept (soluble TNF receptor). A phase I study examined the response to treatment in 16 subjects given adjunctive etanercept 25 mg subcutaneously twice weekly for 8 doses, beginning on day 4 of TB treatment (89). Responses were compared to 42 CD4-matched controls. Sputum culture conversion occurred a median of 7 days earlier in the etanercept arm ($P=.04$) (inverted triangles, figure 3). Etanercept was well tolerated. There were no serious opportunistic infections. CD4 cell counts rose

by 96 cells / μ l after one month of etanercept treatment ($P=.1$ compared to controls). This effect may have been due to inhibition of apoptosis, or to the release of sequestered T cells from lymph nodes or other sites (90). The etanercept arm also showed trends toward superior resolution of lung infiltrates, closure of lung cavities, improvement in performance score, and weight gain; these approached statistical significance despite the small number of treated subjects. There were no TB relapses in either treatment arm. No effect on HIV RNA was apparent, indicating factors other than TNF may drive HIV expression in AIDS/TB.

High dose methylprednisolone. A substantially greater microbiologic effect was observed in a phase II placebo-controlled study in 189 subjects of prednisolone 2.75 mg/kg/day for the first month of standard TB chemotherapy (91). This daily dose had been selected based on a phase I study indicating that required to reduce TB-stimulated TNF production by half. The dose was tapered to zero during the second month; the average subject received a cumulative dose of over 6500 mg methylprednisolone. Although there is extensive experience in the use of corticosteroids to ameliorate symptoms in TB, no previous studies have examined the microbiologic effects of doses of this magnitude. Fifty percent of prednisolone-treated subjects converted to sputum culture negative after 1 month vs. 10% in the placebo arm (upright triangles figure 3, $P=0.001$). The magnitude of this effect is greater than has been reported in any other studies of adjunctive TB immunotherapy. No serious opportunistic infections occurred. However, early serious adverse events, consisting of expected gluco- and mineralocorticoid toxicities (hypertension, edema, hyperglycemia, and one death due to hypertensive crisis) occurred significantly more often in the prednisolone arm. Two other prospective randomized trials of adjunctive corticosteroids given at lower doses have observed similar, albeit smaller, effects on the kinetics of sputum culture conversion (92,93). Several studies of AIDS/TB support the hypothesis that chemotherapy may be more effective in the absence of a strong granulomatous host response. These are reviewed in reference (22).

Together, these findings appear to indicate a substantial potential benefit for anti-TNF therapy in tuberculosis. However, it also appears that for corticosteroids to be effective in this context, they must be given in very high doses, and that these doses are not well tolerated. Additional studies of anti-granuloma adjunctive immunotherapy, such as infliximab (anti-TNF antibody) or other targeted therapies, are warranted.

Therapeutic Vaccines

In 1890 Koch demonstrated that intradermal injection of tuberculous guinea pigs with old tuberculin led to rapid necrosis and sloughing of tuberculous lesions – the “Koch phenomenon”. Nonetheless, immunotherapy with tuberculin was subsequently administered to TB patients with mixed results. Interest in therapeutic vaccines declined following the development of modern anti-TB chemotherapy; however, recognition of the limitations of current combination chemotherapy such as its relatively long 6 month duration and increasing rates of MDR TB, led to renewed work in this area. Two types of vaccines have been studied in this context: environmental mycobacteria and DNA vaccines.

Heat-killed *Mycobacterium vaccae*

Mycobacterium vaccae is a rapidly growing environmental mycobacterium which has low pathogenicity for humans (94). *M. vaccae* was originally isolated from the soil in an area of Uganda where BCG vaccination had been shown to be protective against leprosy. Heat-killed preparations of *M. vaccae* have been studied as an adjunct to standard anti-TB drug therapy for over a decade. *M. vaccae* expresses antigens common to many mycobacteria (95). Heat-killed *M. vaccae* preparations have been hypothesized to work in tuberculosis by restoring host recognition of shared mycobacterial antigens, and by promoting Th1 responses important to host defenses against intracellular pathogens. However, such mechanisms have generally not been evident in clinical trials, even in those in which a beneficial effect on sputum microbiology was observed (96). In recent work in a murine model of allergic airway disease *M. vaccae* has been shown to activate regulatory (suppressive) T cells (Treg) that act via production of IL-10 and TGF β (97). In that model, the *M. vaccae*-induced Treg cells suppressed deleterious allergic Th2 responses. Modulation of Th1 or Th2 responses by *M. vaccae*-induced Treg in tuberculosis has not yet been reported.

Because heat-killed *M. vaccae* is inexpensive, simple to administer, and could potentially be implemented by TB control programs in developing countries, there has been great interest in performing controlled trials to evaluate its potential role in TB treatment. In most trials, *M. vaccae* has been administered as an intradermal injection of an autoclaved preparation given within the first few days to first month after the initiation of standard chemotherapy. The heat-killed vaccine has been demonstrated to be safe in HIV-infected and HIV-non-infected adults. Side effects due to *M. vaccae* have been mild and infrequent. Forty per cent of subjects in an earlier trial developed a local scar similar to a BCG vaccination scar (98).

In early studies, heat-killed preparations of *M. vaccae* showed activity as an adjunct to anti-TB chemotherapy. In studies from the Gambia and Vietnam the proportion of TB cases cured was increased and mortality decreased among those treated with heat-killed *M. vaccae* immunotherapeutic agent (99). Other studies in Nigeria, Romania and Iran also suggested activity in TB patients with drug-susceptible and drug-resistant tuberculosis (100-103). These studies suffered from methodological problems including insufficient sample sizes, non-random treatment allocation, high losses to follow-up and the use of various TB drug treatment regimens, as noted in a Cochrane review (104).

Subsequent randomized, double-blind, placebo controlled clinical trials did not confirm the earlier results. Three studies in Malawi, South Africa, Uganda and Zambia examined the role of immunotherapy with heat-killed *M. vaccae* in a rigorous fashion. HIV-infected and -uninfected adults with smear positive pulmonary TB received one dose of *M. vaccae* or placebo early after beginning standard anti-TB treatment. Treatment with *M. vaccae* immunotherapy did not affect mortality or consistently affect sputum culture conversion after 2 months of treatment, radiographic clearance of disease, closure of cavities, weight gain, improvement in clinical symptoms or the outcome of anti-TB treatment (96,105-107). The data from these 3 rigorous trials in over 1500 patients showed no consistent benefit from immunotherapy with *M. vaccae* administered early during treatment to patients with drug susceptible pulmonary TB (104).

The use of adjunctive immunotherapy with *M. vaccae* also has been studied in patients with MDR-TB where treatment options are limited. In a randomized clinical trial from China in patients with MDR TB, sputum conversion, cavity closure and relapse were significantly better in patients treated with multiple doses of *M. vaccae* administered every 3 to 4 weeks for 6 months in addition to susceptibility-directed anti-TB chemotherapy (108). The vaccine administered in this study was prepared locally. These results are interesting but require confirmation.

Other Therapeutic Vaccines

Recent studies using a plasmid DNA encoding the *M. leprae* 65 kD heat shock protein (hsp65) as an adjunct to combination chemotherapy in mice infected intracheally with H37Rv or MDR TB accelerated bacillary clearance and was effective in disease due to MDR strains (109,110). Interestingly, corticosteroid administration after combined treatment with drugs and the DNA-hsp65 vaccine did not result in regrowth of H37Rv growth and reactivation of TB suggesting that the adjunctive DNA vaccine may prevent the development or improve the clearance of slowly metabolizing, persistent bacilli – properties desirable of an immunotherapeutic agent that might allow shortening of the duration of anti-TB treatment.

Conclusions

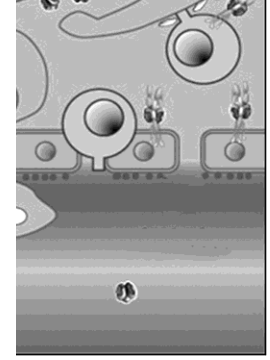
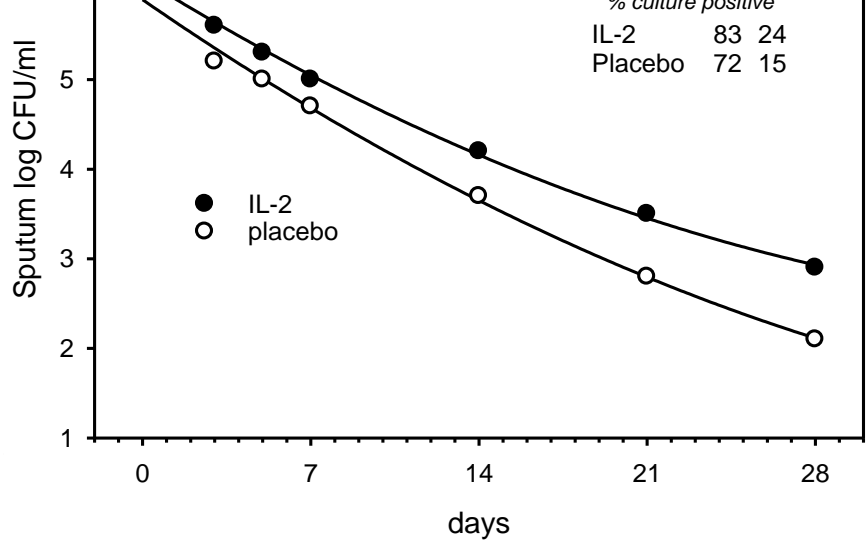
The evolution of *Mycobacterium tuberculosis* as an intracellular pathogen has led to a complex relationship between it and its host, the human mononuclear phagocyte. The products of *M. tuberculosis*-specific T lymphocytes, particularly IFN γ , are essential for macrophage activation for intracellular mycobacterial killing and/or sequestration of viable mycobacteria in granulomas. However, some cytokines, including products of both lymphocytes and phagocytic cells, may contribute to disease pathogenesis, by enhancing mycobacterial survival and by causing many of the pathologic features of the disease. In HIV-associated mycobacterial infections, cytokines may mediate accelerated progression of HIV disease.

The objectives of adjunctive immunotherapy for tuberculosis therefore are complex. In some situations, such as multi-drug resistant disease, clearance of bacilli may be enhanced by administration of IL-2, IL-12, IFN γ , or possibly by using inhibitors of the deactivating cytokines TGF β and IL-10. In other circumstances, it may be desirable to reduce the non-specific inflammatory response using inhibitors of TNF α such as pentoxifylline, prednisone, or soluble TNF receptor. In MDR TB, immunotherapy may play an important role in preventing the subsequent emergence of resistance to less active second-line anti-TB drugs. Further clinical trials are needed to define the role for immunotherapy of tuberculosis and other mycobacterial infections.

Tables

Citation	Study population	N	Regimen	Outcome
Condos (46)	MDR-TB	5	500 µg IFN γ 3x/wk by aerosol nebulizer for 1 month	Sputum smears became negative in 4 of 5 patients after 1 month of IFN γ treatment and time to positive culture decreased. Sputum smears became positive in 4 of 5 patients one month after adjunctive IFN γ was stopped.
Palmero (119)	MDR-TB	5	3MI IFN α 2b SC once wkly for 3 months	2 of 5 patients became smear and culture negative long-term; one patient became smear negative but culture positive; two patients showed no improvement.
Giosuè (49)	MDR-TB	7	3 MU IFN α 3x/wk by aerosol for 2 months	Transient improvement in sputum smears, minimal effect on CFU counts
Suarez-Mendez (50)	Drug resistant [n = 4; resistant to HS (2) and H (2)] and MDR (n = 4) TB	8	1 MU IFN γ IM daily, then 3x/wk IM for 6 months	Sputum smears and cultures became negative in all patients after 3 months of treatment and remained negative after 6 months. Results difficult to interpret due to simultaneous change in chemotherapy
Koh (51)	MDR-TB	6	2 MU IFN γ 3x/wk by aerosol for 6 months	Sputum smears remained positive in all subjects. Cultures were negative after 4 months in 2 subjects but became positive again after 6 months of IFN γ therapy. Five patients had radiological improvement.

Table 1. Clinical trials of adjunctive IFN for treatment of drug resistant and multidrug-resistant (MDR) pulmonary tuberculosis.



multinucleated giant
concentric rings of
bacilli of neutrophils,

Figure 2. Deleterious effect of IL-2 on sputum culture conversion in drug-sensitive pulmonary tuberculosis ($N=110$). Adapted from reference (61).

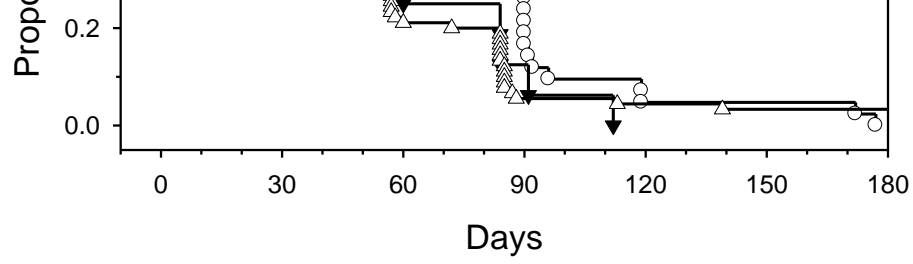


Figure 3. Acceleration of sputum culture conversion by etanercept (soluble TNF receptor) and high dose methylprednisolone (2.75 mg/kg/d) in pulmonary tuberculosis. Each symbol represents an individual subject. Both treatments differed from control subjects by Kaplan-Meier analysis ($P=.04$ and $.001$, respectively). From reference (22,91).

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