Reconsidering Adjuvant Immunotherapy for Tuberculosis

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Background. Shortened regimens for treatment of pulmonary tuberculosis (TB) are urgently needed to facilitate its global eradication. Prolonged treatment is presently required to prevent relapse, which is thought to arise from persisting foci of semidormant infection contained within granulomas.

Methods. The medical literature was reviewed to identify clinical trials of adjuvant TB immunotherapy, as well as other studies of the relationship between immune status and TB relapse or reactivation.

Results. Four studies of therapeutic interferon indicated its inability to effectively augment the mycobactericidal capacity of lung macrophages. One randomized, placebo-controlled trial of therapeutic interleukin-2 found that it delayed the microbiologic response to treatment, whereas 2 controlled trials of anti–tumor necrosis factor (TNF) therapies (high-dose prednisolone and etanercept [a soluble TNF receptor]) found that these interventions significantly accelerated the response to treatment. Four retrospective studies were identified in which the response to TB therapy was accelerated and/or the relapse risk was reduced in persons with human immunodeficiency virus coinfection; one study reported that immune reconstitution syndrome due to use of antiretroviral therapy was associated with increased risk of relapse. Several studies indicated that granulomas may be efficiently targeted and disrupted by the anti-TNF antibody infliximab, apparently because of its ability to bind to cell-surface TNF and to induce apoptosis in TNF-expressing cells.

Conclusions. These findings support the hypothesis that the granulomatous host response to TB may paradoxically protect sequestered mycobacteria from administered anti-TB therapy and that treatment may be improved by therapeutic disruption of granulomas. Clinical trials to test this hypothesis are warranted.

Granulomas, the hallmark of the host response to mycobacterial infection, represent a strategy to physically contain infections that cannot otherwise be eradicated by host defenses (figure 1). The sequential recruitment of cells to the site of *Mycobacterium tuberculosis* infection forms a physical barrier to mycobacterial dissemination and creates a hostile microenvironment in which oxygen tension, pH, and micronutrient supply are all likely reduced. Faced with this environment, mycobacteria undergo profound alterations in metabolism, biosynthesis, and replication. This adaptation forms the basis of clinical latency in tuberculosis. Although the elucidation of the biology of these sequestered, semidormant bacilli has become a critical area of research in tuberculosis, their paucity makes direct study in vivo problematic. Therefore, most research on this question has been performed using in vitro models, such as oxygen deprivation or intracellular growth in macrophages [1–9]. These models have identified metabolic pathways (e.g., the glyoxylate shunt pathway) and 2-component regulatory systems (Rv3133c/DosR) required for mycobacterial persistence.

Karakousis et al. [10] recently reported a study of the biology of dormancy using artificial granulomas that comprised hollow, *M. tuberculosis*–containing, semipermeable microfibers implanted subcutaneously in mice. The fibers, which permit diffusion of nutrients and soluble mediators but exclude intact cells, rapidly become surrounded by macrophages and lymphocytes. The model requires intact cellular host immune responses; it is of particular interest, because its extracellular bacillary localization may mirror that of human tuberculosis. Karakousis and colleagues found that the mycobacteria contained within these lesions quickly adapted to the altered environment, showing stationary colony-forming unit (CFU) counts, decreased metabolism, altered gene-expression profiles (including activation of Rv3133c/DosR), and decreased susceptibility to the bactericidal effects of isoniazid. Isoniazid is thought to mainly kill metabolically active, replicating bacilli, during the early phase of therapy. As a consequence, it has little sterilizing effect, which has been defined in clinical trials as the ability to shorten therapy and prevent relapse. This is therefore the first experimental model in which granulomas interfered with sterilization.

Treatment of active tuberculosis presently requires 6 months of multidrug treatment. Relapses occur if patient adherence is inadequate. Even after optimal

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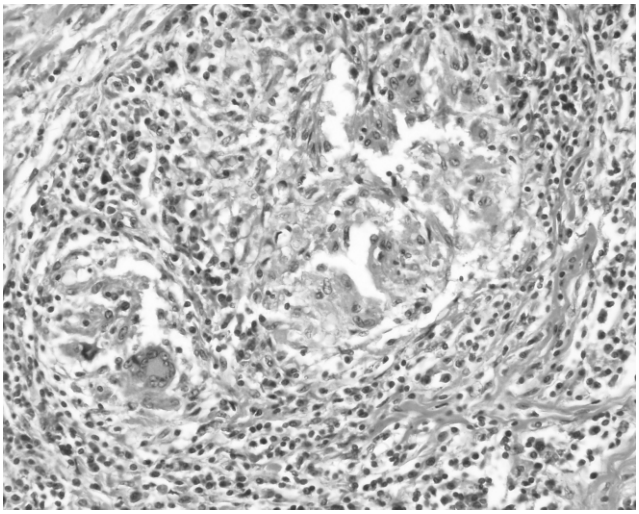

Figure 1. Tuberculous lung granuloma. The central region of multinucleated giant cells, mycobacteria, and necrotic debris are surrounded by concentric rings of tightly apposed epithelioid cells and lymphocytes, with smaller numbers of neutrophils, plasma cells, and fibroblasts.

treatment, relapses occur in ~5% of cases. It is believed that the requirement for prolonged treatment reflects the relative inactivity of current drugs against semidormant organisms contained in granulomas; however, this hypothesis has never explicitly been tested. The long duration of treatment poses a major obstacle to global tuberculosis eradication. For this reason, the development of new treatments capable of shortening the duration of tuberculosis treatment is recognized as a major objective of tuberculosis drug discovery [11].

The design of clinical trials to test new treatments poses several challenges. The existence of effective but inefficient standard "short-course" therapy makes conventional trials prolonged, large, and costly. As a result, most studies of adjuvant tuberculosis immunotherapy have adopted an alternative strategy, testing new treatments in patients with multidrug resistant (MDR) disease or substituting surrogate markers that indicate relapse risk (such as 2-month sputum culture status or time to conversion of sputum culture results) as the main outcome measure. Delayed sputum culture conversion and a reduced rate of decline in log sputum counts (in CFUs) during the first month of treatment are recognized indicators of increased relapse risk in patients with tuberculosis [12–14]. However, the use of these end points has the potential disadvantage that they require that the experimental treatment be administered during the initial phase of tuberculosis therapy.

CLINICAL TRIALS OF ADJUNCTIVE IMMUNOTHERAPY

IFN-γ. IFN-γ is essential for antimycobacterial host defenses [15]. In mice, IFN-γ increases the mycobactericidal capacity of macrophages by promoting the production of reactive nitrogen intermediates, such as nitric oxide [16, 17]. The first trial of therapeutic IFN-γ in patients with tuberculosis without overt defects of IFN-γ production or responsiveness was reported in 1997 by Condos et al. [18]. In this uncontrolled study, 500 μg of IFN-γ was administered 3 times per week by aerosol to 5 patients with MDR tuberculosis in addition to their previous therapy. The study found that sputum smear results became negative and that the number of CFUs tended to decrease. Three similar subsequent studies by other investigators that differed in IFN type, dose, and route of administration failed to meet even this modest measure of success (table 1) [19–21]. The only randomized, placebo-controlled, multicenter trial of inhaled adjunctive IFN-γ for MDR tuberculosis was initiated by InterMune in 2000 [22]. The trial was halted prematurely because of a lack of efficacy; its findings have never been published. Subsequent research 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 [23]. These findings indicate that the modest mycobactericidal capacity of lung macrophages cannot effectively be augmented by therapeutic IFN.

IL-2. IL-2 promotes T cell replication and is essential for cellular immune function and granuloma formation. In 1997, a small, unblinded study of 2 low-dose IL-2 regimens (daily or in 5-day "pulses") in patients with MDR tuberculosis found that the daily regimen appeared to decrease sputum acid-fast bacilli counts [24]. On the basis of this preliminary observation, a randomized, double-blind, placebo-controlled trial of the effect of IL-2 on conversion of sputum culture results was conducted by the Case Western Reserve University Tuberculosis Research Unit (Cleveland, OH) with 110 Ugandan, HIV-uninfected patients with drug-susceptible tuberculosis [25]. IL-2 or placebo was administered twice daily for the first month of standard therapy. Contrary to expectations, the study found significant delays in clearance of viable *M. tuberculosis* CFU and conversion of sputum culture results in the IL-2 treatment arm (figure 2). This report was the first clinical indication of possible antagonism during combined chemotherapy and immunotherapy for tuberculosis.

TNF. TNF, like IFN-γ, is essential for host defenses against tuberculosis. TNF is a potent proinflammatory cytokine that is expressed by macrophages and T cells, first as a cell-surface–associated cytokine, and then, after cleavage of membrane-anchor-

Table 1. Clinical trials of adjunctive IFN for treatment of multidrug-resistant pulmonary tuberculosis.

Reference	No. of subjects	Regimen	Outcome
Condos et al. [18]	5	500 μg of IFN-γ 3 times per week by aerosol for 1 month	Sputum smear results became negative; trend toward reduced numbers of CFUs
Giosuè et al. [19]	7	3 MU of IFN-α 3 times per week by aerosol for 2 months	Transient improvement in sputum smear results; minimal effect on the numbers of CFUs
Suarez-Mendez et al. [20]	5	1 MU of IFN-γ daily, then 3 times per week im for 6 months	Not evaluable because of simultaneous changes in chemotherapy
Koh et al. [21]	6	2 MU of IFN-γ 3 times per week by aerosol for 6 months	No microbiologic effect

NOTE. CFU, colony-forming unit.

ing domains, as a soluble homotrimer [26, 27]. TNF stimulates the release of inflammatory cytokines, endothelial adhesion molecules, and chemokines.

TNF plays a central role in the host response to *M. tuberculosis*. Monocytes express TNF after phagocytosis of mycobacteria or after stimulation by mycobacterial proteins or glycolipids [26, 28–30]. TNF is expressed at the site of disease in patients with newly diagnosed tuberculosis [31–33]. TNF levels increase shortly after initiation of antituberculosis therapy [33], possibly because of a release of microbial constituents that stimulate TNF production [34–36]. Levels subsequently decrease as the bacillary burden is reduced by treatment [31].

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 [37, 38]. In addition, TNF blockade also reduces the microbicidal activity of macrophages and NK cells [39, 40]. As a result, animals deficient in TNF are highly susceptible to granulomatous infections [41]. Recent studies also indicate that the risk of tuberculosis is increased several-fold in individuals with polymorphisms in TNF-promoter regions [42], and the risk may increase by as much as 20-fold in patients treated with TNF antagonists (see "Can Granulomas Be Targeted Efficiently?," below).

Two controlled clinical trials have examined the effects of potent immunosuppressive and/or anti-TNF therapies on microbiologic outcomes in tuberculosis. Both were conducted with HIV-1–infected patients who had relatively preserved tuberculosis immune responses (based on the presence of high CD4 cell counts and cavitary lung disease). The trials shared a single placebo control arm (for tuberculosis therapy only). Their main objective was to examine the role of TNF in the acceleration of HIV disease progression due to tuberculosis; as such, their main end points were CD4 cell count and plasma HIV RNA load. However, both studies prospectively collected clinical and microbiologic data as indicators of safety.

Etanercept (soluble TNF receptor). A phase I study examined the effects of etanercept (25 mg sc twice weekly for 8 doses) given to 16 subjects, starting on day 4 of tuberculosis treatment [43]. Responses were compared with those for 42 CD4 cell count–matched control subjects. Conversion of sputum culture results occurred a median of 7 days earlier in the etanercept arm ($P = .04$) (figure 3). Etanercept was well tolerated. There were no serious opportunistic infections. CD4 cell counts increased by 96 cells/μL after 1 month of etanercept treatment ($P = .1$, for comparison with control subjects); this effect may have been associated with inhibition of apoptosis [44, 45]. The etanercept arm also showed trends toward superior resolution of lung infiltrates, closure of lung cavities, improvement in performance score, and weight gain; these findings approached statistical significance, despite the small number of treated subjects.

High-dose methylprednisolone. A substantially greater microbiologic effect

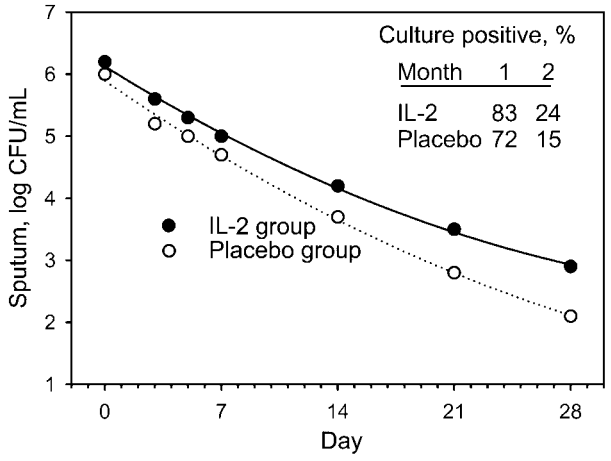

Figure 2. Deleterious effect of IL-2 on sputum microbiologic end points in a study of 110 subjects with pulmonary tuberculosis. Adapted from [25]. CFU, colony-forming units.

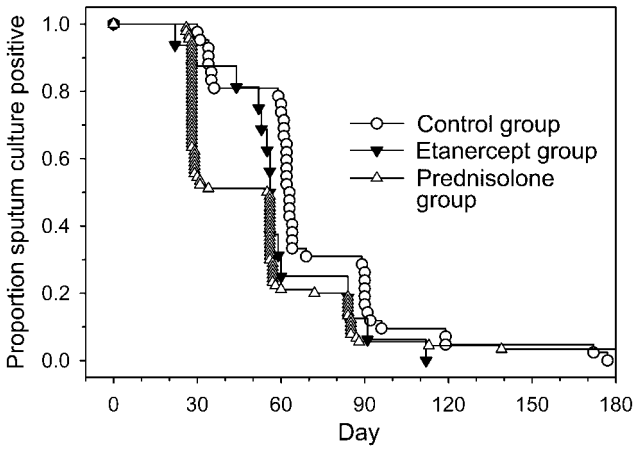

Figure 3. Acceleration of conversion of sputum culture results associated with receipt of etanercept and high-dose methylprednisolone (2.75 mg/kg/day) in patients with pulmonary tuberculosis. Each symbol represents an individual subject. Results for etanercept and high-dose methylprednisolone treatments differed from those for control subjects by Kaplan-Meier analysis ($P = .04$ and .001, respectively). Adapted from [43, 46].

was observed a phase II study reported by Mayanja-Kizza et al. [46] in which 189 subjects who were treated with prednisolone (2.75 mg/kg/day) or placebo during the first month of standard tuberculosis therapy. The prednisolone dosage had been selected on the basis of a phase I study indicating that it reduced the rate of tuberculosis-stimulated TNF production ex vivo by one-half. The daily dose was tapered to 0 mg/kg during the second month; the average subject received a cumulative dose of >6500 mg. Although there is extensive experience with the use of corticosteroids to ameliorate symptoms of tuberculosis, no previous studies have examined the microbiologic effects of doses of this magnitude. Unexpectedly, one-half of prednisolone-treated subjects had conversion of sputum culture results to negative after 1 month of treatment, compared with 10% of subjects in the placebo arm ($P = .001$) (figure 3). This effect was of greater magnitude than that observed in the landmark study in which the addition of rifampin to a 6-month regimen of streptomycin and isoniazid reduced the relapse rate from 29% to 2% and to increase the 2-month sputum culture conversion rate from 49% to 69% [47]. The effect of prednisolone therapy was not due to reduced sputum production, which decreased similarly during treatment in both study arms. There were no serious opportunistic infections. However, prednisolone-treated subjects were more likely to experience other early serious adverse events, including edema, hyperglycemia, electrolyte disturbances, and severe hypertension.

Two other prospective, randomized trials of adjunctive corticosteroids administered at lower doses have observed similar, albeit smaller, effects on the kinetics of conversion of sputum culture results [48, 49]. A third trial found no effect [50]. There have been no reports of deleterious effects of corticosteroids on microbiologic outcomes in patients with tuberculosis.

DO GRANULOMAS INTERFERE WITH STERILIZATION?

The incidence of tuberculosis is elevated among patients who are treated with prednisone or etanercept [51, 52], indicating that these treatments interfere with granuloma formation and/or maintenance in humans, as they do in experimental animals. Yet, paradoxically, when administered to patients with active tuberculosis, these treatments accelerate the microbiologic response to therapy, an effect otherwise associated with decreased risk of relapse. Can these trials then be broadly interpreted as indicating a potential therapeutic benefit conferred by adjunctive antigranuloma therapy in tuberculosis?

Several caveats are appropriate before this question can be answered definitively. No surrogate markers have yet been fully validated as predictors of tuberculosis relapse or indicators of the effectiveness of new tuberculosis therapies. Moreover, experience with these markers during conventional antituberculosis therapy may not necessarily predict the experience during immunotherapy. In mice, neither prior bacille Calmette-Guérin (BCG) vaccination nor concurrent corticosteroid therapy significantly affected the response to antituberculosis therapy [53, 54]. The mouse may be a poor model in which to study the therapeutic effects of granuloma disruption, however, because murine tuberculosis granulomas are loose collections of activated and epithelioid macrophages and lymphocytes that lack the multinucleated giant cells, necrosis, caseation, and cavitation characteristic of human pathology [55, 56]. In humans, cavitary disease is associated with delayed sputum culture conversion and increased risk of subsequent relapse [14].

Tuberculosis granulomas in the guinea pig and rabbit more closely resemble those of humans. Prior vaccination with BCG reduces the bactericidal activity of isoniazid in the guinea pig, whereas it does not in the mouse [53]. No analogous studies have yet examined the effects of corticosteroids or TNF blockers during tuberculosis therapy in the guinea pig. Some TNF blockers (including infliximab) are highly specific for human TNF [57]. The lack of highly potent, specific TNF antagonists for the guinea pig or rabbit limits the present role of these animal models in addressing this question.

AIDS, like high-dose corticosteroid therapy, profoundly inhibits the granulomatous response to mycobacterial infection. Unlike iatrogenic immunosuppres-

sion, immune dysfunction due to AIDS may either worsen because of HIV disease progression or improve after initiation of antiretroviral therapy, thus complicating the analysis of its effects on antituberculosis therapy. Only 1 prospective study of the influence of HIV infection on tuberculosis relapse in the absence of antiretroviral therapy has been reported [58]. In it, Sonnenberg and colleagues studied recurrent tuberculosis in a cohort of 326 South African miners with and without HIV infection, using restriction fragment–length polymorphism analysis to distinguish true relapses from disease due to reinfection. Although the total rate of recurrence was increased by HIV-1 infection, strain typing revealed that these cases were primarily due to reinfection (the rate of which increased 18-fold) rather than true relapse (the rate of which was reduced by one-half).

Several additional studies using surrogate markers support the observation that HIV infection may improve the effectiveness of antituberculous therapy. A combined analysis of Tuberculosis Trials Consortium Studies 22 and 23 indicates that 2-month sputum culture conversion rates for HIV-infected patients were superior to those for noninfected patients (94% vs. 78%; $P < .05$) [59]. Two small treatment trials using other sputum surrogate markers concur with this observation [13, 60]. Lastly, a large, multicenter study of tuberculosis chemotherapy for HIV-infected persons initiated jointly by AIDS Clinical Trials Group and the Terry Beirn Community Programs for Clinical Research on AIDS in the pre-HAART era found that >95% of subjects had conversion of sputum culture results to negative by week 8 of the study and that the main end point (relapse) could not be assessed because rates were unexpectedly low in all treatment arms [61].

Some patients who begin receiving combined treatment for AIDS and tuberculosis experience a particularly rapid recovery of tuberculosis-specific immune functions. This syndrome, which has been termed "immune reconstitution inflammatory syndrome" (IRIS), occurs most often in patients with far-advanced AIDS who have both pulmonary and extrapulmonary tuberculosis. Patients with IRIS typically are anergic prior to starting antiretroviral therapy but rapidly have conversion to strongly positive tuberculin skin test results as the syndrome evolves. They experience "paradoxical" reactions to treatment (i.e., worsened fever, pulmonary infiltrates, lymphadenopathy, etc.) due to immune reconstitution. One study by Narita et al. [62] found a 6-fold increased risk of subsequent tuberculosis relapse in patients who experienced IRIS during early tuberculosis treatment.

CAN GRANULOMAS BE TARGETED EFFICIENTLY?

Formal testing of the hypothesis that granuloma disruption accelerates the response to tuberculosis therapy will require an efficient means to target and disrupt human granulomas. Experience with prednisolone indicates that corticosteroids may not be the best intervention with which to test this hypothesis, because the high doses that appear to be required to produce a therapeutic effect are associated with a substantial risk of early serious adverse events [46]. This safety profile likely will prevent high-dose prednisolone therapy from becoming a first-line treatment, even if other aspects of therapeutic efficacy in tuberculosis could be established.

The treatment of other chronic inflammatory conditions, such as rheumatoid arthritis, has been transformed in the past decade by the development of targeted therapies that block specific aspects of the inflammatory cascade, such as TNF, while avoiding the recognized toxicities of high-dose corticosteroid therapy. Several studies indicate that infliximab (anti-TNF antibody), may be highly effective in disrupting established granulomas, because reactivation of latent granulomatous infections has been one of its most frequently reported serious adverse effects. Tuberculosis occurs at a rate of 95 cases per 100,000 person-years during the first 90 days of infliximab therapy, compared with 11 cases per 100,000 person-years for etanercept therapy and 5 cases per 100,000 person-years in the United States as a whole [63, 64]. The risk posed by infliximab subsequently decreases (consistent with reactivation of preexisting latent infection), whereas that of etanercept does not (consistent with the inability to contain incident tuberculosis infection) [52]. Granulomatous infections other than tuberculosis are similarly more common among infliximab-treated patients [65–67].

Corresponding differences between infliximab and etanercept exist in their therapeutic indications. Only infliximab is effective for treatment of chronic granulomatous inflammatory diseases, such as Crohn disease, sarcoidosis, or Wegener granulomatosis [68–71]. In patients with Crohn disease, a single dose of infliximab can induce long-lasting remissions and closure of chronic enteric fistulas [72]. This effect is not readily explained by the pharmacokinetics of its binding to soluble TNF, but may rather reflect its capacity to induce apoptosis in activated monocytes and lymphocytes by binding to cell-surface TNF [73–76]. This mechanism, which presumably kills TNF-expressing cells that constitute granulomas, is presently the subject of ongoing research.

Infliximab has not yet been studied as adjunctive therapy for tuberculosis. For such a study to be performed safely and ethically, its design must ensure that subjects are not exposed to an undue risk of disseminated or uncontrolled mycobacterial infection. Such a study could only be conducted with fully drug-susceptible cases; indeed, in MDR cases, containment remains a viable alternative to eradication. The trials of etanercept and methylprednisolone described earlier indicate that other potent anti-inflammatory and anti-TNF treatments do not per se pose a significant risk to patients with pulmonary tuberculosis, even to those with HIV coinfection. This risk may be further reduced

by delaying infliximab therapy until after the first week of treatment (at which time sputum CFU counts have decreased by nearly 2 log), initially testing subtherapeutic doses, monitoring blood cultures and clinical outcomes closely, and adding moxifloxacin to the treatment regimen. Unlike isoniazid and rifampin, the long serum half-life of moxifloxacin ensures that its levels remain greater than the MIC for *M. tuberculosis* throughout the dosing interval.

Other risks may also be considered. IRIS has been reported in infliximab-associated tuberculosis cases after withdrawal of infliximab [77]. Like patients with AIDS-associated IRIS, all patients with infliximab-associated IRIS had extrapulmonary and/or disseminated tuberculosis at the time of diagnosis; therefore, IRIS may be unlikely in patients with limited pulmonary disease. Nonetheless, monitoring for IRIS, with supplemental low-dose corticosteroids (or repeated infliximab administration), may be appropriate. These measures will reduce the anticipated risks posed by study participation to an acceptable level.

SUMMARY

Although granulomas are an essential part of host defenses against mycobacterial infection, they appear, paradoxically, to protect *M. tuberculosis* bacilli during tuberculosis therapy. Additional studies of targeted disruption of granulomas are warranted to better understand granuloma biology, to inform drug discovery, and to test a new therapeutic approach in tuberculosis.

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