Randomized Trial of Adjunctive Interleukin-2 in Adults with Pulmonary Tuberculosis

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Interleukin (IL)-2 has a central role in regulating T cell responses to *Mycobacterium tuberculosis*. Adjunctive immunotherapy with recombinant human IL-2 was studied in a randomized, placebo-controlled, double-blinded trial in 110 human immunodeficiency virus–seronegative adults in whom smear-positive, drug-susceptible pulmonary tuberculosis was newly diagnosed. Patients were randomly assigned to receive twice-daily injections of 225, 000 IU of IL-2 or placebo for the first 30 days of treatment in addition to standard chemotherapy. Subjects were followed for 1 year. The primary endpoint was the proportion of patients with sputum culture conversion after 1 and 2 months of treatment. After 1 month, the proportion of patients for whom sputum culture converted to negative was 17% for the IL-2 group compared with 30% in the control group ($p = 0.14$; χ^2). After 2 months, 77% in the IL-2 group were culture negative compared with 85% of those receiving placebo ($p = 0.29$, χ^2). Results were similar when patients with isoniazid monoresistance were included in the analysis. There were no differences in weight gain and no improvement in fever, cough, and chest pain between groups. One patient in each arm relapsed. IL-2 did not enhance bacillary clearance or improvement in symptoms in human immunodeficiency virus–seronegative adults with drug-susceptible tuberculosis.

Keywords: tuberculosis, pulmonary; antitubercular agents; immunotherapy; interleukin-2

Tuberculosis (TB) is a major global health problem. Up to one-third of the world's population is infected with *Mycobacterium tuberculosis*. The World Health Organization estimates that 8 million new TB cases and 1.9 million deaths due to TB occurred worldwide in 1997 (1). In addition, drug resistance to standard anti-TB drugs has increased in many areas during the past decade (2). New modalities for the prevention and treatment of TB are clearly needed.

Recovery from TB depends, in part, on the generation of an effective cell-mediated immune response against the pathogen. Effective T cell function is key in controlling *M. tuberculosis* infection. Interleukin (IL)-2, a cytokine produced by activated T lymphocytes, has a central role in the activation and expansion of T cells. In murine models of *Mycobacterium lepraemurium*, *Mycobacterium avium* and *Mycobacterium bovis* bacillus Calmette–Guérin infection, IL-2 has been shown to limit mycobacterial replication, possibly by macrophage activation via interferon-mediated pathways or directly by the development of cytotoxic T lymphocytes recognizing mycobacterial antigens (3–5). Patients with TB frequently have deficient IL-2–induced cell proliferation and decreased IL-2 receptor generation (6). These observations form the basis for studies of recombinant IL-2 as adjunctive immunotherapy against mycobacterial diseases in humans.

Early clinical trials with IL-2 in patients with leprosy, leishmaniasis, TB and other serious infections due to intracellular pathogens demonstrated that IL-2 immunotherapy may be useful in controlling these infections (7–14). In patients with leprosy, IL-2 administration led to enhanced local cell-mediated immune responses and resulted in more rapid and extensive reduction in bacilli compared with multidrug chemotherapy alone (11, 12).

In a pilot study from Bangladesh and South Africa, treatment of patients in whom drug-sensitive and chronic multidrug-resistant (MDR) TB was newly diagnosed with 12.5 μg (225, 000 IU) of intradermal IL-2 twice daily during the first month of TB therapy resulted in rapid sputum conversion (13). A later randomized trial in South Africa comparing daily and pulsed IL-2 with placebo in MDR TB found improved sputum clearance with daily treatment (14). These results suggested a potential role for IL-2 in TB treatment.

To further study this issue, we conducted a randomized, double-blinded, placebo-controlled Phase II clinical trial to evaluate the safety and microbiologic and immunologic effects of IL-2 in human immunodeficiency virus–seronegative adults with initial episodes of smear-positive, drug-susceptible pulmonary TB. We hypothesized that adjunctive treatment with IL-2 would enhance cell-mediated immune responses in TB and increase the rate of killing of tubercle bacilli and that these effects may be most evident during early treatment in patients with drug-susceptible TB.

Some of the results of this trial have been previously reported in the form of an abstract (15).

METHODS

Patients

Ambulatory patients, aged 18 to 50 years, with suspected pulmonary TB were referred from the main outpatient clinic of the National TB Treatment Centre, Mulago Hospital, Kampala, Uganda for possible study participation. Human immunodeficiency virus–seronegative patients in whom initial episodes of smear-positive, culture-confirmed TB was newly diagnosed and who had moderately advanced or far-advanced

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TB on chest X-ray (16), a Karnofsky performance scale score greater than 50% (17), and were not pregnant or lactating were eligible for the study. Persons previously treated for TB, patients with asthma, untreated thyroid disease, or other serious medical conditions, those with a hemoglobin less than 80 g/L, total white blood cell count less than 3,000/mm^3, serum aspartate aminotransferase greater than 100 IU/L or serum creatinine more than 177 μM/L or limited respiratory reserve, and persons on chronic corticosteroid or immunosuppressive drugs were excluded. Patients found to have initial drug resistance to isoniazid (INH), rifampin, ethambutol or pyrazinamide were excluded from the study when susceptibility testing results became available and their TB treatment was adjusted accordingly.

The study was approved by the institutional review boards at University Hospitals of Cleveland and Case Western Reserve University and the Ugandan National AIDS Research Subcommittee. All participants gave informed consent.

Treatment Allocation and Masking

After screening, eligible subjects were admitted to hospital for the first month of anti-TB treatment. Patients were randomly assigned to treatment with standard short-course chemotherapy (2 months of daily INH, rifampin, pyrazinamide, and ethambutol followed by 4 months of daily INH and rifampin) plus twice-daily intradermal injections of 225,000 IU recombinant human IL-2 (Proleukin, aldesleukin—kindly donated by Chiron, Emeryville, CA) or sterile 5% dextrose for injection, United States Pharmacopeia (placebo). Injections were administered intradermally twice daily into the skin of the back during the first 30 days of anti-TB treatment. The dosage, route, and schedule for IL-2 administration were identical to the earlier trial done in South Africa (14).

A computer-generated randomization sequence with a block size of 10 was used to assign subjects to study treatment.

Clinical and laboratory staff were masked to treatment assignment. Separate clinical assessors were used to assess local and systemic adverse experiences. Treatment assignments were not revealed to any investigator or subject during the trial. Additional details are available in the online supplement.

Baseline Measurements

Purified protein derivative skin testing. Skin testing was performed with 5 tuberculin units of purified protein derivative (PPD) (Tubersol, Aventis Pasteur) by the Mantoux method (18).

Bacteriology. Early morning sputum specimens were collected for qualitative acid-fast bacilli smears and culture in BACTEC liquid media and Middlebrook 7H-10 agar plates. Quantitative cultures were performed after homogenization with *N*-acetyl-L-cysteine/sodium citrate and decontamination with 2% sodium hydroxide using previously published methods (19). Susceptibility testing against INH, rifampin, ethambutol, and pyrazinamide was performed using standard BACTEC methods (20).

Immunologic measurements. Immunologic measurements including PPD skin test responses, expression of IL-2 receptor by CD4 and CD8 T cells, determination of frequencies of natural killer (NK) cells (exhibiting the surface marker profile CD3$^-$/CD16$^+$/CD56$^+$), and assessment of soluble IL-2 receptor (sIL-2R) immunoreactivity in serum were performed at baseline and during study drug treatment to demonstrate systemic activity of the IL-2 study drug. The proportion of peripheral blood T cells expressing the activation marker CD25$^+$ (IL-2 receptor) and frequencies of NK cells were assessed by three-color flow cytometry (FACScan; Becton-Dickinson, San Jose, CA) using commercial reagents. Serum sIL-2R was measured using a commercially available ELISA kit (BioSource, Camarillo, CA) according to the manufacturer's instructions. The sensitivity of the kit was less than 0.016 ng/ml.

Sera were stored before beginning treatment and after 2, 4, and 6 weeks of anti-TB treatment for measurement of anti–IL-2 antibodies. Detailed procedures for the microbiologic and immunologic assays are included in the online supplement.

Anti-TB Chemotherapy

Treatment was administered for TB under direct supervision during the initial 30-day hospitalization and was then self-administered on an ambulatory basis. The dosage of standard anti-TB drugs was adjusted for body weight. After the first 30 days, drugs were dispensed monthly during the remainder of treatment. Adherence was measured by self-report, dispensing records, and at least monthly urine INH metabolite testing.

Follow-up Measurements

History, physical examination, and adverse event surveys were performed thrice weekly during study drug treatment. Injection sites were inspected twice daily. Complete blood counts, blood chemistries, and urinalysis were repeated weekly during study drug treatment and at 12 months. Serum thyroid-stimulating hormone and thyroxine levels were checked after 1 month. Chest X-rays were repeated after 1, 2, 6, and 12 months and graded as normal, minimal, moderately advanced, or far-advanced disease using a standardized scheme (16). PPD skin testing was repeated after 4 weeks.

Sputum was collected for acid-fast bacilli smear and culture after 2 and 4 days of treatment, then weekly from weeks 1 through 4, after 6 weeks, and then monthly during the remainder of treatment as long as the subject was able to produce sputum. Immunologic measurements were repeated after 2 weeks and 6 weeks of anti-TB treatment.

Subjects were followed for 1 year after the onset of anti-TB treatment.

Statistical Analysis

The primary study endpoints were the rate of sputum culture conversion on solid media after 1 and 2 months of anti-TB treatment and the safety and tolerability of intradermal IL-2 as measured by the proportion of subjects with local and systemic reactions. Sputum culture conversion after 1 and 2 months was defined as having all sputum cultures negative at that time point and no positive cultures subsequently during treatment. Subjects who were unable to produce sputum at a follow-up time point and subjects for whom all sputum cultures were overgrown by bacteria or yeast at a follow-up time point were considered unevaluable at that time point. Secondary clinical endpoints included improvement in cough and chest pain, defervescence, weight gain, and Karnofsky performance scale score. Time on treatment until culture conversion was a secondary microbiologic endpoint. Immunologic endpoints included changes in PPD skin test responses; the proportions of circulating CD25$^+$, CD4, and CD8 T cells; frequencies of NK cells; and levels of immunoreactive IL-2R in serum.

The total sample size (110) was calculated to have 80% power (α = 0.05 [one-tailed]) to detect a minimum difference of 19% in sputum culture conversion rates after 1 and 2 months of anti-TB treatment comparing the IL-2 and placebo groups, assuming a 75% culture conversion rate after 2 months of treatment with standard chemotherapy alone (21, 22). A treatment effect of this magnitude on 2-month culture conversion is similar to the effect adding rifampin to anti-TB treatment (23–25). The total sample size was adjusted for an estimated 5% mortality, 8% prevalence of primary drug resistance, and 10% loss to follow-up.

All statistical analyses were performed using SAS software (SAS, version 6.12, SAS Institute, Cary, NC). Significant univariate differences between the two study arms were determined using χ^2 contingency tables and *t* tests for means of continuous data. Equivalent nonparametric tests were used when data were not normally distributed. The date of sputum culture conversion was defined as the first date of continuous culture negativity. Analyses were performed on an intention-to-treat basis.

Studies of the British Medical Research Council with rifampin-containing short-course chemotherapy regimens suggested that INH monoresistance or resistance to INH plus streptomycin had little effect on sputum culture conversion after 2 months of treatment and relapse (26). We therefore also analyzed our data combining those subjects with INH monoresistance with those with fully drug-susceptible TB.

RESULTS

Study Population

Five hundred fifty-four adult volunteers with suspected initial episodes of pulmonary TB were evaluated for study participation. One hundred and ten patients were enrolled and randomized to study treatment (Figure 1). Fifty-five patients received IL-2 immunotherapy and 55 patients received placebo.

Table 1 shows the characteristics of all subjects at the time of randomization. Patients randomized to IL-2 were less likely

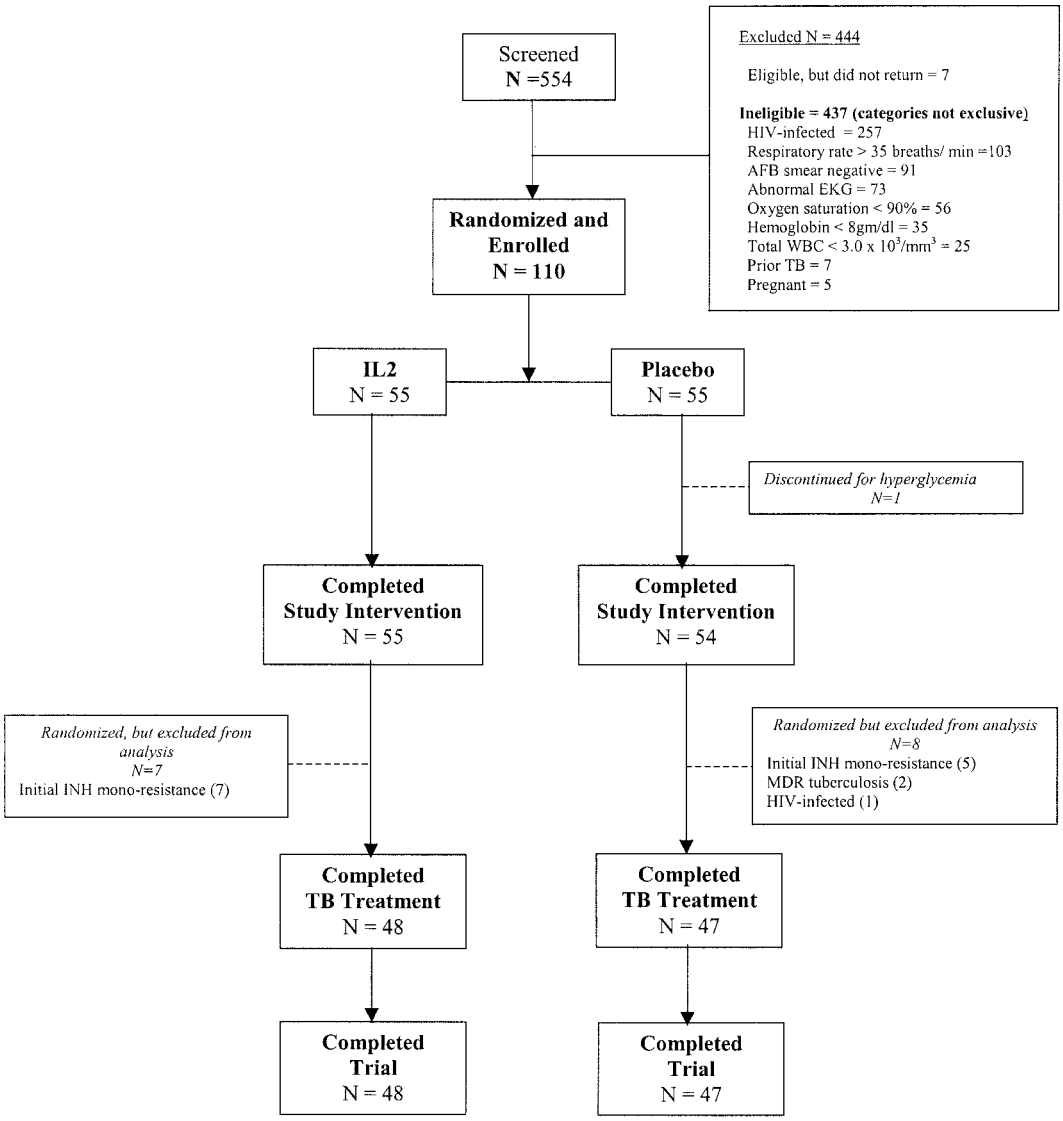


Figure 1. Profile of the randomized clinical trial. AFB = acid-fast bacilli; HIV = human immunodeficiency virus; INH = isoniazid; TB = tuberculosis.

to have a bacillus Calmette–Guérin scar and report chest pain or night sweats. These variables were explored for potential confounding and interaction; no strong evidence for either was found. Twenty-five percent of all subjects had moderately severe TB on chest X-ray and 75% had far-advanced disease. Ninety-six percent had cavitary disease. Ninety-two percent of all subjects had grade 3+ or 4+ sputum acid-fast bacilli smears at entry.

One hundred nine subjects received all 60 injections of study drug treatment. Injections were stopped early (after 32 injections) in one subject in the placebo arm who was found to have diabetes mellitus and was transferred from the TB ward to the medical ward for diabetic control.

Fifteen enrolled subjects were later terminated from the study or declared ineligible after review of initial drug susceptibility testing results—12 due to INH monoresistance (seven subjects randomized to the IL-2 arm and five in the placebo arm), two subjects in the placebo arm with INH and rifampin resistance, and one subject in the placebo arm who was found to be human immunodeficiency virus–seropositive after enrollment. As specified in the study protocol, these subjects were followed but were excluded from the primary clinical, radiographic, microbiologic, and immunologic analyses. None of these reasons for exclusion differed significantly between treatment groups. All enrolled subjects were included in the safety analysis. The number of patients completing each phase of the study is shown in Figure 1.

Compliance with Standard Short-Course Chemotherapy

Patient compliance with standard anti-TB chemotherapy was excellent and comparable in both treatment arms at each time point. Ninety-five percent of 384 urine INH metabolite tests performed during TB treatment were positive in the IL-2 group compared with 94% of 377 tests done in the placebo arm. Additional data on compliance are included in Table E2 in the online supplement.

Microbiologic Outcomes

The primary study endpoint was the proportion of subjects in each treatment arm who had converted their sputum cultures to negative on solid media after 1 and 2 months of anti-TB treatment (Figure 2). After Month 1, 8 (17%) of the subjects in the IL-2 arm had converted their sputum culture to negative compared with 14 (30%) of the subjects in the placebo group ($p = 0.14$, χ^2). After 2 months of treatment, 36 (77%) and 40 (85%) of the subjects in the IL-2 and control arms, respectively, were sputum culture negative ($p = 0.29$, χ^2). When considering

TABLE 1. BASELINE CHARACTERISTICS OF STUDY SUBJECTS IN EACH TREATMENT GROUP

Characteristic	IL-2 (*n* = 55)	Placebo (*n* = 55)	p Value*
Age, yr; mean ± SD	26.7 ± 7	27.4 ± 7	0.60
Male, n (%)	34 (61%)	41 (75%)	0.15
Weight, kg; mean ± SD	52.7 ± 7	51.8 ± 7	0.47
BCG scar, n (%)	12 (22%)	26 (47%)	0.01
Fever, n (%)	14 (26%)	20 (36%)	0.22
Cough, n (%)	55 (100%)	55 (100%)	—
Hemoptysis, n (%)	2 (4%)	3 (6%)	1.0
Chest pain, n (%)	19 (39%)	38 (69%)	0.003
Night sweats, n (%)	22 (44%)	38 (69%)	0.01
Body temperature, °C; mean ± SD	37.4 ± 0.8	37.2 ± 0.8	0.17
Sputum smear grade, n (%)			
1+ and 2+	3 (5%)	7 (13%)	0.33
3+	10 (18%)	12 (22%)	
4+	42 (76%)	36 (65%)	
White blood cell count, $\times 10^3/mm^3$; mean ± SD	8.7 ± 3	9.0 ± 3	0.56
Hemoglobin, gm/dl; mean ± SD	12.1 ± 2	11.9 ± 2	0.67
PPD, mm induration; mean ± SD	17 ± 5	16 ± 6	0.18
Extent of disease on chest X-ray, n (%)			
Moderately advanced	17 (31%)	10 (18%)	0.12
Far advanced	38 (69%)	45 (82%)	
Cavitary disease, n (%)	54 (98%)	52 (95%)	0.62

Definition of abbreviations: BCG = bacillus Calmette–Guérin; IL-2 = interleukin-2; PPD = purified protein derivative.

* *t* test, χ^2, or χ^2 for trend comparing IL-2 and placebo arms.

both subjects with drug-susceptible and INH-monoresistant TB (n = 107), the proportion of subjects who had converted their sputum cultures to negative after 1 month of treatment was 17% for the IL-2 arm and 28% for the placebo arm (p = 0.15; χ^2). After 2 months, the sputum culture conversion rate was 76 and 85% in the IL-2 and placebo arms, respectively (p = 0.24, χ^2). In a Kaplan–Meier analysis, time until sputum culture conversion after the onset of anti-TB treatment was longer among subjects in the IL-2 arm (p = 0.05, Wilcoxon, data not shown).

Quantitative sputum colony counts also were performed during anti-TB treatment. At several time intervals after initiation of treatment, the sputum bacillary load was lower in subjects receiving placebo compared with IL-2 (p = 0.04 after 4 days, p = 0.02 after 3 weeks, and p = 0.03 after 4 weeks; *t* test, Figure 3).

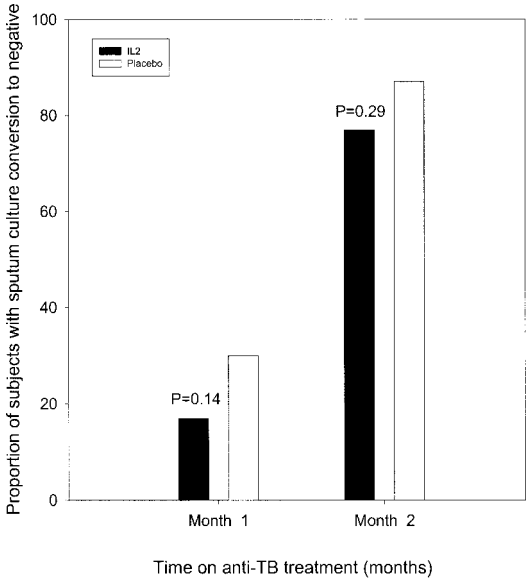


Figure 2. Sputum culture conversion after 1 and 2 months of antituberculosis treatment for subjects with drug-susceptible tuberculosis.

Clinical and Radiographic Outcomes

No treatment failures or deaths occurred. One bacteriologically confirmed relapse occurred in each treatment group. For both subjects who relapsed, the sputum *M. tuberculosis* isolate obtained at the time of relapse was identical to the patient's baseline isolate when compared by IS6110 DNA genotyping (27). One subject who relapsed had initial resistance to INH. Neither subject who relapsed had acquired drug resistance.

There were no significant differences in the rate of weight gain, rate of defervescence, improvement in Karnofsky performance scale score, and improvement in self-reported cough and chest pain during TB treatment between groups (data not shown). Radiographic improvement in the extent of disease by one or more severity grades (16) comparing baseline and follow-up chest X-rays after 1, 2, 6, and 12 months of TB treatment did not differ between treatment arms (data not shown). These results were unchanged when patients with INH monoresistance were included in the analysis. Data describing radiographic changes during treatment are included in Table E3 in the online supplement.

Immunologic Changes during Anti-TB Treatment

There was no difference in the mean PPD size comparing study arms after 1 month of treatment (19.6 ± 2 mm for the IL-2 group and 19.5 ± 3 mm for the placebo arm [p = 0.49, *t* test]). The mean change in PPD response from baseline to Month 1 was 2.1 ± 6 mm in the IL-2 arm compared with 4.0 ± 6 mm in the placebo arm (p = 0.18, *t* test). These results were comparable when subjects with INH resistance were included in the analysis.

Flow cytometric analysis of peripheral blood cells stained

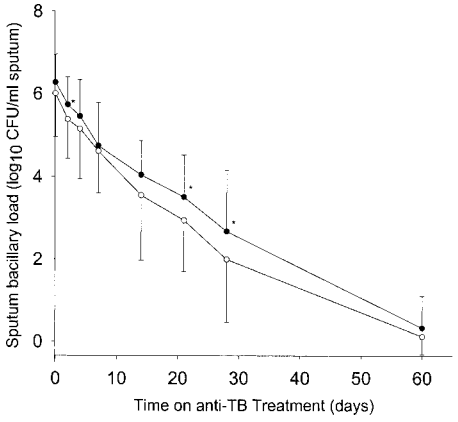


Figure 3. Sputum bacillary load during antituberculosis treatment for subjects with drug-susceptible tuberculosis. Data are expressed as mean ± SD log_{10} cfus per milliliter of sputum. *Filled circles* represent the IL-2 arm and *open circles* represent the placebo arm. *Values significantly different (p < 0.05, *t* test) between treatment groups. The number of subjects in each treatment arm with evaluable sputum specimens at each time point is (*1*) baseline—IL-2 47 subjects, placebo 46 subjects; (*2*) at Day 2—IL-2 47 subjects, placebo 46 subjects; (*3*) at Day 4—IL-2 46 subjects, placebo 44 subjects; (*4*) at Day 7—IL-2 47 subjects, placebo 45 subjects; (*5*) at Day 14—IL-2 47 subjects, placebo 45 subjects; (*6*) at Day 21—IL-2 47 subjects, placebo 43 subjects; (*7*) at Day 28—IL-2 48 subjects, placebo 44 subjects; and (*8*) at Day 60—IL-2 43 subjects, placebo 42 subjects.

with a combination of monoclonal antibodies to CD3, CD4 or CD8, and CD25 (to assess activation of T cell subsets) or CD3, CD16, and CD56 (to determine frequencies of NK cells) were performed at baseline and after 2 and 6 weeks of anti-TB treatment. The 6-week time point was chosen to assess ongoing immune activation 2 weeks after the end of study drug treatment. The median percentage of $CD4^+/CD25^+$ T lymphocytes was greater after 2 and 6 weeks of anti-TB treatment in subjects receiving IL-2 than placebo ($p = 0.05$ and $p = 0.08$, Mann–Whitney *U* test, Figure 4). By contrast, the percentage of $CD8^+/CD25^+$ cells was not increased with IL-2 treatment (data not shown). Systemic activity of IL-2 at Week 2 of study was further corroborated by increased levels of sIL-2R (by ELISA) in serum from patients in the IL-2 compared with the placebo arm ($p = 0.004$, Mann–Whitney *U* test, Figure 4). In contrast to previously published results (14), the percentage of $CD3^-/CD16^+/CD56^+$ NK cells was not increased among peripheral blood mononuclear cells from subjects in the IL-2 arm compared with the placebo arm (data not shown).

The results for the above immunologic measurements were comparable when patients with initial INH resistance were included in the analysis.

Safety

Immunotherapy with recombinant human IL-2 was safe and generally well tolerated. Adverse events were usually mild to moderate in severity and of limited duration. No subject required dose reduction or discontinuation of IL-2 immunotherapy due to study drug–related side effects. Local pain, tenderness on palpation, erythema, ecchymoses, and temporary hyperpigmentation at the injection site occurred more frequently in subjects receiving IL-2 (Table 2). Systemic adverse events did not differ between the treatment arms except transient mild lymphadenopathy that occurred more frequently in patients treated with IL-2. Thirteen subjects in the IL-2 group had transient mild lymphadenopathy compared with two patients in the placebo group. Additional information on systemic adverse events is included in Table E4 in the online supplement. Hypothyroidism or hyperthyroidism did not occur in any subject. Changes in serum chemical and hematologic parameters were similar between the two treatment arms. The proportion of subjects with detectable serum anti–IL-2 antibodies after 4 weeks of study drug treatment was greater among subjects in the IL-2 arm than in those receiving placebo (35% IL-2 arm vs. 6% placebo arm; $p < 0.001$, Fisher's exact test); however, there was no difference between groups at 6 weeks.

DISCUSSION

In this randomized placebo-controlled clinical trial, we found that adjunctive immunotherapy with recombinant human IL-2 did not enhance sputum bacillary clearance or improvement in important clinical symptoms in human immunodeficiency virus–seronegative adults with drug-susceptible pulmonary TB. Intradermal therapy with IL-2 was generally safe and well tolerated. The study popula-

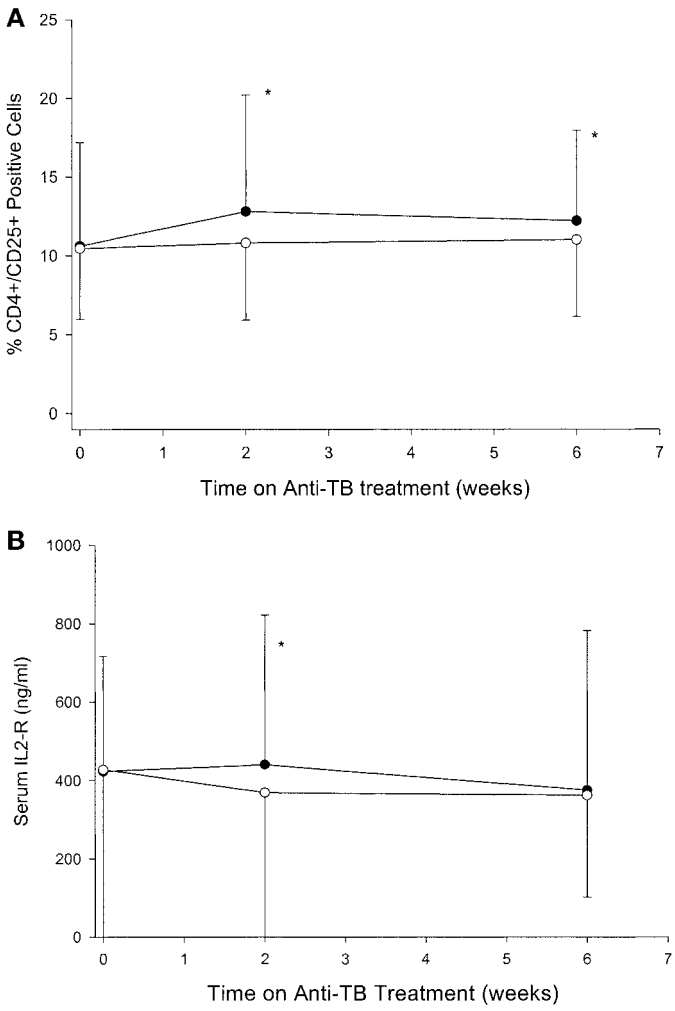


Figure 4. Expression of peripheral blood $CD4^+/CD25^+$ surface markers (*A*) and concentration of serum sIL-2R (*B*) during antituberculosis treatment. Data are expressed as the median ± interquartile range. *Filled circles* represent the interleukin (IL)-2 arm (n = 48) and *open circles* represent the placebo arm (n = 47). *Values significantly different ($p < 0.05$, Mann–Whitney *U* test) between treatment groups.

TABLE 2. NUMBER OF LOCAL ADVERSE EVENTS BY TREATMENT ARM

Local Adverse Event Description	IL-2 (n = 55) *n* (%)	Placebo (n = 55) *n* (%)	p Value*
Pain at injection site	27 (49)	1 (2)	<0.0001
Tenderness on palpation	48 (87)	2 (4)	<0.0001
Erythema	53 (96)	1 (2)	<0.0001
Ecchymosis	10 (18)	1 (2)	0.008
Hyperpigmentation	55 (100)	1 (2)	<0.0001
Axillary pain or lymphadenopathy	1 (2)	0	1.0

Definition of abbreviation: IL-2 = interleukin-2.
* Fisher's exact test comparing IL-2 and placebo arms.

tion included primarily patients with advanced cavitary TB and high sputum bacillary loads where an effect of adjunctive immunotherapy would most likely be evident.

Two earlier trials of adjunctive IL-2 in pulmonary TB have been reported (13, 14). In Bangladesh and South Africa, 20 patients who were partially treated, had MDR TB, or were newly diagnosed for TB received 30 days of twice-daily intradermal injections of 225,000 IU (12.5 μg) of IL-2 in addition to chemotherapy (13). All patients in whom TB was newly diagnosed and five of seven patients with MDR TB converted their sputum smears to negative. An increase in PPD skin test size and enhanced T cell responses were seen in patients with drug-susceptible TB.

A clinical trial in 35 patients with MDR TB from South Africa compared daily or pulsed IL-2 therapy with placebo (14). Patients received susceptibility-directed chemotherapy and were randomized to receive daily (225,000 IU IL-2 intradermally twice daily), pulsed (three cycles of 450,000 IU IL-2 twice daily for 5 days, followed by 9 days off IL-2), or placebo during the first 30 days of TB treatment. Among smear-positive patients, five of eight patients receiving daily IL-2 had reduced or negative sputum smears compared with two of seven subjects receiving pulsed IL-2 and three of nine subjects in the placebo group. The numbers of IL-2 receptor–positive T cells and of NK cells were increased in patients receiving daily IL-2 but not in the pulsed IL-2 or placebo arms. Chest X-ray improvement after 6 weeks of TB treatment also was more frequent in patients receiving daily IL-2. No significant side effects of IL-2 treatment were observed.

We were unable to confirm these earlier reports of a positive effect of adjunctive immunotherapy with IL-2 on clinical, bacteriologic, and radiographic responses in patients who were partially treated, had MDR TB, and were newly diagnosed for TB. The IL-2 treatment regimen used in our study, 225,000 IU intradermally twice daily for the first 30 days of TB treatment, was identical to that used in the randomized clinical trial performed in patients with MDR TB in South Africa by Johnson and colleagues, where IL-2 enhanced sputum smear clearance and radiographic improvement (14). In that study, IL-2 treatment was associated with an increase in peripheral blood of IL-2 receptor–bearing T cells and NK cells. Like Johnson and colleagues, we were able to demonstrate a modest systemic effect of IL-2 as evidenced by an increase in the percentage of CD25-positive CD4 T cells and an increase of levels of sIL-2R and IL-2 in serum in our trial; however, the effect was transient. Thus, the lack of a clinical response to IL-2 therapy may be the result of the transient nature of its effect on immune parameters. However, recent evidence from the literature indicates that a successful anti–*M. tuberculosis* immune response involves both CD4 and CD8 T cells (28–30). Interestingly, IL-2 therapy resulted in expansion and excess activation of CD4 but not CD8 T cells in the current study. Therefore, it is possible that the lack of a positive effect of adjunctive immunotherapy with IL-2 on clinical, bacteriologic, and radiographic responses is due, at least in part, to its inability to elicit CD8 T cell responses necessary for effective host defense against *M. tuberculosis*.

The reasons underlying the differing results between our study and the earlier South African and Bangladesh trials are unclear but may be due to differences in the populations studied and the bacteriologic methods. All the studies involved patients with severe forms of pulmonary TB. The earlier South African and Bangladesh studies were small, and only the South African trial in patients with MDR TB was a randomized trial. The South African and Bangladesh studies relied on sputum smear results, whereas the current trial assessed changes in sputum bacillary load by both qualitative and quantitative cultures performed at frequent intervals. The most notable difference between the earlier and current studies is the inclusion of patients with MDR TB in the South African and Bangladesh trials. MDR TB is more difficult to treat with currently available chemotherapy, and the effect of immunotherapy might be more evident in patients with drug-resistant disease. Nonetheless, despite careful quantitative microbiologic surveillance, we were unable to demonstrate any positive impact of IL-2 immunotherapy on bacillary clearance in patients with advanced cavitary TB.

Our study has several important limitations. First, the intradermal IL-2 injections produce recognizable stigmata, such as warmth and pruritus, compared with placebo. Patients and examiners may have been able to determine treatment assignment by inspecting injection site, thus introducing ascertainment bias. Observation bias was minimized during the study by the use of dedicated nurse-injectors, who were not responsible for other patient assessments, to administer the test article. In addition, sputum smears and cultures, immunologic assessments, and chest X-ray interpretations were performed without knowledge of treatment assignment. Second, the trial was a Phase II study focusing on preliminary evidence of microbiologic and immunologic activity of IL-2 immunotherapy in patients in whom drug-susceptible TB was newly diagnosed. Our sample size estimate was calculated to have 80% power to detect a 19% improvement in sputum culture conversion after 1 and 2 months of anti-TB therapy, a difference similar to that of adding rifampin to combination chemotherapy. Our study had lower power to detect smaller improvements in bacteriologic responses to treatment that might be beneficial for some patients. The trial also was not powered to detect significant differences in final TB treatment outcomes such as relapse between the immunotherapy and control arms. Finally, we analyzed patients with drug-susceptible and INH-monoresistant TB and cannot, therefore, exclude the possibility of an effect in patients with highly drug resistant TB. We studied only one dosing schedule of adjunctive IL-2; however, the regimen used in the current trial was selected on the basis of positive results with this regimen in published studies and the best information available at the time the trial protocol was designed.

Our data from a double-blind, placebo-controlled clinical trial in patients with advanced, drug-susceptible pulmonary TB showed that, despite evidence of a transient systemic effect of adjunctive IL-2, immunotherapy with 450,000 IU of intradermal IL-2 daily during the first month of TB treatment did not enhance bacillary clearance or improvement in symptoms in human immunodeficiency virus–seronegative adults with drug-susceptible TB. Although IL-2 might potentially be of benefit in patients with MDR TB where drug treatment options and responses are sub optimal, our data suggest that adjunctive IL-2 immunotherapy is unlikely to improve results with current rifampin-containing short-course chemotherapy regimens in drug-sensitive TB.

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